11281

# UNIVERSIDAD NACIONAL AUTONOMA DE MEXICO

37

## FACULTAD DE MEDICINA

## POSGRADO EN CIENCIAS BIOMÉDICAS

# "LIPOPROTEÍNA Lp(a) Y RIESGO CARDIOVASCULAR: DETECCIÓN DE ISOFORMAS PATÓGENAS DE Lp(a) POR UN NUEVO MÉTODO DE UNIÓN A LA FIBRINA"

# T E S I S

QUE PARA OBTENER EL GRADO ACADÉMICO DE:

**DOCTORA EN CIENCIAS**

P R E S E N T A :

**AURORA DE LA PEÑA DÍAZ**

**DIRECTOR DE TESIS:**

**DR. EDMUNDO CHÁVEZ COSSIO**

**MÉXICO, D.F.** **2002**

VNIVERSIDAD NACIONAL AVFNOMA DE MEXICO

# DOCTORADO EN CIENCIAS BIOMEDICAS

FACULTAD DE MEDICINA

pdcb/grad/047Jur/2002

**ING. LEOPOLDO SILVA**
DIRECTOR GENERAL DE ADMINISTRACION
ESCOLAR DE LA UNAM.
PRESENTE.

Por medio del presente me permito informar a usted que en la reunión del Subcomité Académico de Doctorado en Ciencias Biomédicas que se llevo a cabo el día 9 de octubre del presente año, **se acordó designar el siguiente jurado para examen de Doctorado en Ciencias Biomédicas** de la M. en C. PEÑA DIAZ AURORA DE LA con número de expediente 91542 y número de cuenta 7152037-1 con la tesis titulada: "*LIPOPROTEÍNA Lp(a) Y RIESGO CARDIOVASCULAR: DETECCIÓN DE ISOFORMAS PATÓGENAS DE Lp(a) POR UN NUEVO MÉTODO DE UNIÓN A LA FIBRINA*", dirigida por el Dr. Edmundo Chávez Cossío.

| | |
|---|---|
| PRESIDENTE: | DR. GUSTAVO PASTELIN HERNANDEZ |
| SECRETARIO: | DR. EDMUNDO CHAVEZ COSSIO |
| VOCAL: | DR. MARCO ANTONIO JUAREZ OROPEZA |
| VOCAL: | DRA. BLANCA HAYDE RUIZ ORDAZ |
| VOCAL: | DRA. ADELA RODRÍGUEZ ROMERO |
| SUPLENTE: | DR. EDUARDO ANGLES-CANO |
| SUPLENTE: | DRA. GUADALUPE BAÑOS DE MACCARTHY |

Atentamente
"POR MI RAZA HABLARA EL ESPIRITU"
Cd. Universitaria, D.F., a 9 de octubre de 2002.

**Dr. Gabriel Roldán Roldán**
Responsable
Facultad de Medicina

**Dr. Abel Moreno Cárcamo**
Coordinador
Doctorado en Ciencias Biomédicas.

C.c.p. Biol. Francisco Javier Incera, Jefe de la Unidad de Administración del Posgrado.
Tutor(a) Dr. Edmundo Chávez Cossío.
Coordinación de Doctorado

# *LIPOPROTEÍNA Lp(a) Y RIESGO CARDIOVASCULAR: DETECCIÓN DE ISOFORMAS PATÓGENAS DE Lp(a) POR UN NUEVO MÉTODO DE UNIÓN A LA FIBRINA*

*AGRADECIMIENTOS*

Al Dr Eduardo Anglés-Cano

Al Dr Edmundo Chávez Cossio

Al Dr Salvador Uribe Carvajal

Al Dr Raúl Izaguirre Ávila

A la Dra Guadalupe Mac Carthy

# *LIPOPROTEINA (a), Lp(a) Y ENFERMEDAD ATEROTROMBÓTICA.*

Este trabajo de tesis se realizó con la colaboración de:

· El Instituto Nacional de la Salud y de la Investigación Médica (INSERM), Unidad 143 Trombosis y Biología Vascular, Hospital de Bicêtre y la Unidad 146 , Facultad de Medicina Xavier Bichat EPI 9907, Paris, Francia.

· El Instituto Nacional de Cardiología "Ignacio Chávez", (INC), México, D.F.

· El Instituto Nacional de Antropología e Historia, (INAH), México, D.F.

· El Centro de Ingeniería Genética y Biotecnología. División de Inmunotecnología y Diagnósticos. El Instituto Nacional de Angiología, La Habana, Cuba.

En el departamento de Hematología del INC, se identificó la inhibición competitiva del plasminógeno por la Lp(a), con esta finalidad se empleó un modelo "in vitro". El modelo permite ponderar la participación de la Lp(a) como inhibidor del plasminógeno y obtener un perfil de la inhibición fibrinolítica de un individuo por la presencia de Lp(a). La concentración de Lp(a) y las isoformas de apo(a) se identificaron en el departamento de Endocrinología del INC.

En el Centro de Ingeniería Genética y Biotecnología en la Habana, Cuba, se diseñó y obtuvo un anticuerpo monoclonal anti apo-(a).

El Instituto Nacional de Antropología e Historia identificó y facilitó el acceso para obtener las muestras de sangre de las comunidades mazahuas y mayas.

Recibimos también el apoyo de la Secretaría de Salud del Estado de Sinaloa para

obtener las muestras de la comunidad de mayos, poblados de Capomos y Tehuecos.

El Instituto Nacional de Neurología remitió, para su estudio, a un grupo de individuos con enfermedad vascular cerebral, que se incluyó dentro del grupo de estudio.

Durante el primer año de trabajo de tesis, del 15 de enero de 1997 al 15 de agosto de 1997, recibí una beca del sistema ECOS-ANUIES, para realizar una estancia de investigación en la U 143 del INSERM, Hospital de Bicetre, Francia. Durante el periodo mencionado se pusieron a punto las técnicas que permiten identificar la unión de apo(a) a la fibrina, que actualmente se aplican con éxito en el INC.

Del 15 de octubre al 15 de diciembre de 1999 recibí una beca de la Dirección General de Postgrado de la UNAM para realizar una segunda estancia de investigación en la U 143 del INSERM, Hospital de Bicetre, Francia.

## *RESUMEN*

***Este trabajo de tesis propone una nueva estrategia para explorar la capacidad trombogénica de la Lp(a), a través del estudio funcional de la unión de la apo(a) a la fibrina como indicador de la asociación de la Lp(a), al proceso aterotrombótico.***

***Con esta finalidad se puso a punto una técnica que permite evaluar la unión de apo(a) a la fibrina. Se estudiaron también 248 individuos, 105 con cardiopatía isquémica (CI) y 52 con enfermedad cerebrovascular (EVC) de origen trombótico y 91 controles.***

***El grupo de EVC tuvo una alta unión de apo(a) a la fibrina (0.268 ± 0.15 nmol/L) en comparación con el grupo de CI (0.155 ± 0.12 nmol/L), y con el grupo control (0.158±0.09 nmol/L) $p<0.0001$; lo que sugiere que existen diferencias particulares en los mecanismos de regulación anti-trombótica en los lechos arteriales cerebrales y coronarios.***

***Es también importante resaltar que existen diferencias en la concentración y en la presencia de distintas isoformas en poblaciones genéticamente diferentes.***

# SUMMARY

This thesis proposes a well-characterized model of a fibrin surface to develop a functional approach for the detection of pathogenic Lp(a). The assay is based on the competitive binding of Lp(a) and plasminogen for fibrin, and quantifies fibrin-bound Lp(a). High Lp(a) binding to fibrin is correlated with decreased plasmin formation. In a transversal case-control study we studied 248 individuals, 105 had a history of ischemic cardiopathy (IC), 52 had cerebro-vascular disease (CVD) of thrombotic origin, and 91 were controls.

The remarkably high apo(a) fibrin-binding in CVD (0.268±0.15 nmol/L) as compared to IC (0.155±0.12 nmol/L) suggests the existence of peculiar and poorly understood differences in pro- or anti-thrombotic mechanisms in either cerebral and/or coronary arteries.

Our results demonstrated that Lp(a) fibrin-binding and are associated with athero-thrombotic disease

# INTRODUCCIÓN

Las enfermedades aterotrombóticas, cardiovasculares o cerebrovasculares, son una de las principales causas de morbilidad y mortalidad en los países occidentales. En nuestro país, la Secretaría de Salud publicó en su página electrónica (http://www.salud.gob.mx) que en el año 2000 la enfermedad isquémica del corazón fue la segunda causa de muerte y que la enfermedad cerebrovascular la sexta causa, en la población general.

Tanto factores genéticos como ambientales dan origen a la aterosclerosis; algunos de ellos como la lipoproteína de baja densidad (LDL), la hipertensión arterial, la diabetes y el tabaquismo se han estudiado ampliamente y se puede, actualmente, conocer el riesgo que conlleva presentar alguno o varios de estos factores con el de padecer enfermedades aterotrombóticas.

Sin embargo, existen otros factores que actualmente se están conociendo y evaluando. A este grupo pertenece la lipoproteína (a) o Lp(a). La Lp(a) tiene una estructura muy parecida a la LDL, ambas poseen un núcleo de lípidos de composición similar; rodeado por la apolipoproteína B-100, la Lp(a) presenta además otra apolipoproteína, apo(a), que le confiere características antigénicas y fisiopatológicas diferentes, favorece el desarrollo de la placa aterosclerosa y favorece el desarrollo y permanencia de un trombo, por lo que se considera el punto común entre la aterosclerosis y la trombosis.

La presencia de una LDL en la estructura de Lp(a) se asocia al desarrollo acelerado de la aterosclerosis. La similitud entre la estructura de la apolipoproteína (a) y el plasminógeno, precursor de la plasmina, se asocia con una permanencia mayor de depósitos de fibrina en sitios de lesión endotelial y menor generación de plasmina, lo que acentúa las propiedades aterogénicas y trombogénicas de la Lp(a).

Bajo esta perspectiva, se realizaron numerosos estudios epidemiológicos que describen una asociación positiva entre la elevada concentración plasmática de Lp(a) y un incremento en las enfermedades cerebrovasculares, cardiovasculares, la reestenosis de puentes coronarios, la reoclusión de angioplastías y el desarrollo prematuro de aterosclerosis, en ocasiones asociada a altas concentraciones de LDL y/o a bajas concentraciones de lipoproteínas de alta densidad (HDL). La mayoría de estudios prospectivos confirman estos resultados, aún cuando no existe un acuerdo en el límite de corte para considerar una concentración plasmática normal; algunos autores consideran que es de 20 mg/dL y otros hasta 30 mg/dL. También se han empleado técnicas y diferentes anticuerpos en su detección, monoclonales o policlonales, lo que ha generado divergencias en los resultados.

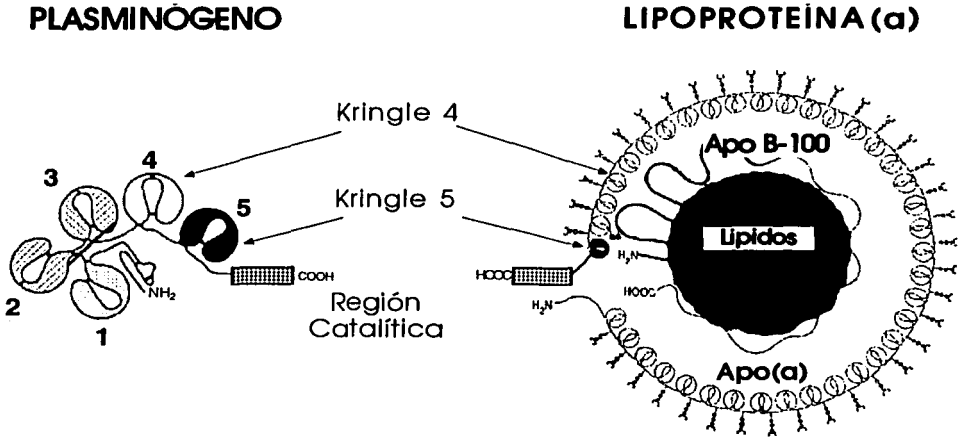
Sin embargo, otros estudios no encuentran asociación entre la Lp(a) y la enfermedad arterial coronaria.

Esta discrepancia en los resultados podría ser entre varios motivos, el reflejo de la gran heterogeneidad estructural de la molécula de apo(a), que se traduce en una

heterogeneidad funcional como inhibidor competitivo del plasminógeno, disminuyendo la formación de plasmina.

Desde 1963, Kåre Berg, identificó la presencia de Lp(a) en el plasma[1]; sin embargo, hasta 1987 Eaton y col[2] identificaron parcialmente la secuencia de la glicoproteína apo(a) que posteriormente clonaron [3], demostrando el gran parecido en su estructura terciaria entre uno de los componentes de la Lp(a), la glicoproteína apo(a), con el plasminógeno, el precursor de la plasmina, Figura 1.

*FIGURA 1.*




***Relación entre la estructura química de Lp(a) y del plasminogeno. En Lp(a) hay un número variable de kringles tipo 4, un kringle tipo 5, una región catalítica serina-proteasa que en Lp(a) no puede activarse.***

Este hallazgo despertó el interés de numerosos grupos de investigación que encontraron el punto común entre la aterosclerosis y la trombosis [4, 5, 6, 7, ,8, 9, 10].

No todos los organismos sintetizan apo(a). Su presencia se identifica en los humanos, en los primates del viejo mundo, en algunos primates del nuevo mundo [11].y en el puerco espín [12].

## FUNCIÓN

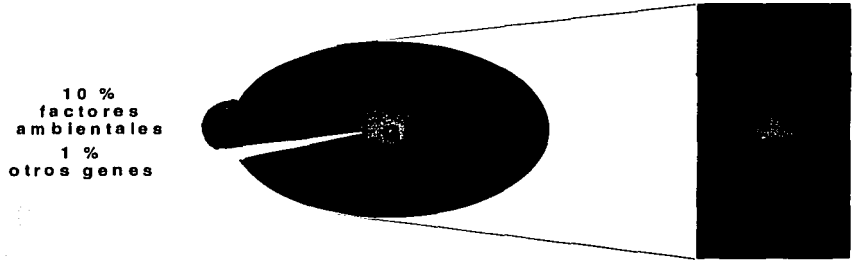
No se conoce con certeza cuál puede ser su función en el organismo. Aporta colesterol desde el hígado a los órganos que sintetizan hormonas esteroidales [13] y a las células de los tejidos en proceso de reparación[14]. Además, tampoco se conoce la función de la apo(a) que se encuentra en los testículos y en el cerebro de manera independiente, es decir, sin formar parte de la molécula de Lp(a) [15].

## CONCENTRACIÓN PLASMÁTICA

La concentración plasmática de Lp(a), que depende de su síntesis hepática [16], [17], varía de un individuo a otro en un límite aproximado de <10 mg/dL a > 100 mg/dL; Es independiente de otros factores como la dieta, el colesterol, la obesidad, el tabaquismo, y se mantiene con variaciones discretas a lo largo de la vida[18, 19], la Figura 2 muestra que el gene de apo(a) participa en casi el 90% en la regulación de la concentración plasmática de Lp(a).



FIGURA 2




**Regulación de los niveles plasmáticos de Lp(a)**
(Boerwinckle et al. J. Clin. Invest. 90: 52-60, 1992)

Generalmente, un individuo hereda de una manera autosómica codominante dos isoformas de apo(a) que pueden identificarse en el laboratorio por la técnica de SDS-PAGE y posteriormente por inmunotransferencia, empleando anticuerpos monoclonales o policlonales contra apo(a). Por este método se identifican cerca de 37 isoformas, que según la clasificación de Uterman se denominan F (faster), B (=apoB-100), S1(slow), S2 , S3, S4, dependiendo de su velocidad de migración en comparación con apoB-100 [20].

Las isoformas de apo(a) muestran una relación inversa entre el peso molecular y la concentración plasmática de Lp(a)[21], probablemente porque a medida que se incrementa el tamaño de apo(a) se incrementa la dificultad para secretarse de la célula, como ocurre en la línea celular HepG2 de hepatocarcinoma humano[22].

El método no permite distinguir diferencias entre las isoformas de pesos moleculares cercanos, aunque recientemente, se describe la posibilidad de identificar con esta técnica, isoformas en un rango muy amplio de pesos moleculares con la ayuda de una referencia que contiene recombinantes de apo(a) de diferentes pesos moleculares y que correlaciona con la técnica de electroforesis de campo pulsado (r= 0.97)[23] que se emplea para identificar los diferentes genotipos que codifican para la apo(a)[24]. Con la técnica de campo pulsado se describieron, en un estudio de población caucásica americana, 19 alelos diferentes[25].

Algunas hormonas pueden modificar su concentración plasmática; la hormona tiroidea[26], los estrógenos[27, 28, 29, 30] y esteroides anabólicos la reducen[31], y la

hormona de crecimiento la incrementa[32]; pero no puede modificarse por medicamentos hipolipemiantes o por la dieta[18, 33].

La concentración plasmática de la Lp(a) se incrementa en padecimientos como el síndrome nefrótico, la artritis reumatoide y transitoriamente después de un infarto al miocardio o de intervenciones quirúrgicas[34, 35, 36].

Existen variaciones en la concentración promedio y en la presencia de isoformas en poblaciones genéticamente diferentes[37, 38, 39, 40]. Los individuos negros tienen al menos, el triple de concentración plasmática de Lp(a) que los blancos[41, 42, 43, 44].

# CONCENTRACIÓN PLASMÁTICA DE LP(a) EN DIVERSAS POBLACIONES

Con la finalidad de identificar a las poblaciones en las que la Lp(a) represente un factor de morbilidad y mortalidad importante y resaltar su impacto en la salud pública, se han llevado a cabo numerosos estudios en poblaciones sanas. En la Tabla 1 se muestra algunos de estos estudios[45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66].

En el curso del trabajo de tesis analizamos los trabajos de poblaciones publicados en los últimos diez años, que aparecen en medline. Como criterio de búsqueda se empleó las siguientes palabras clave: Lp(a), ethnicity, population.

Encontramos que a pesar del gran interés que el tema ha cobrado en los últimos años no existe un criterio para el manejo pre-analítico de la muestra, ni estándar ni técnica de referencia, ni un consenso en el empleo de anticuerpos monoclonales o policlonales para determinar la concentración plasmática de Lp(a). Entre los

puntos que señalan la dificultad que existe para comparar los diferentes estudios, resalta también la ausencia de estudios en la población latinoamericana y china. En el mapa que se muestra en la Figura 3 se señalan los estudios resultantes de la búsqueda y que emplearon la técnica de Elisa, la más empleada para determinar la concentración plasmática de Lp(a). El diámetro de los círculos representa el número de sujetos estudiados. El círculo que representa a la población mexicana corresponde a nuestro estudio realizado en el grupo de mestizos, mazahuas, mayas y mayos.

TABLA 1

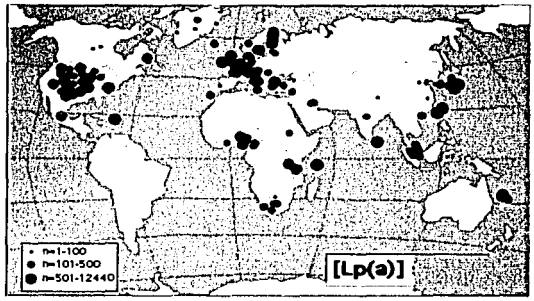
| Ref | Población | Concentración de Lp(a) |
|---|---|---|
| 46 | USA Blancos y negros. | Negros tienen en promedio, el doble de la concentración que los blancos. |
| 47 | Húngaros | No existe diferencia de concentración promedio entre hombres y mujeres. Las mujeres tienen una distribución bimodal. Sólo 9.4% de la población en general supera 30 mg/dL. |
| 48 | Italianos | 75% de la población tiene una concentración promedio menor a 10 mg/dL. |
| 49 | Alemanes, Gahananos, Chinos | La concentración promedio de los gahaneses es el doble que la de los alemanes y 1.65 veces mayor que la de chinos. Las diferencias interétnicas se asocian a las isoformas de apo(a) de alto PM. |

| | | |
|---|---|---|
| 50 | Tibetanos, Coreanos, Chinos, Nigerianos, Belgas. | Los nigerianos tienen una concentración 2.7 veces más alta que los tibetanos. Sin correlación con otras variables estudiadas. |
| 51 | Tiroleses, Alemanes, Galeses, Israelitas, Chinos de Singapur, Indués de Singapur. Grupo enfermos coronarios | Los enfermos tienen mayor concentración que los controles, excepto en los israelitas. En los alemanes no se midió concentración. El gen de apo(a) es un factor determinante en la concentración de Lp(a) y confirma su papel como factor de riesgo en CI. |
| 52 | Japoneses y Chinos | Correlación inversa entre la concentración y el tamaño de la isoforma de apo(a) en ambas poblaciones. |
| 53 | Españoles | 38% de la población estudiada con concentración mayor a 30 mg/dL |
| 54 | Negros de Sychelles, Blancos Suizos | La concentración promedio de Lp(a) es el doble en los negros que en los blancos. Correlación positiva con colesterol total, LDL colesterol y Apo B. Sin correlación con alcohol, tabaco o IMC. |
| 55 | Caucásicas y afro caribeñas en el 3er trimestre embarazo | Las mujeres afro caribeñas tienen mayor concentración de Lp(a) que las mujeres caucásicas |
| 56 | Mexicanos | Correlación inversa entre la concentración y el |

| | | |
|---|---|---|
| | residentes en EUA | tamaño de la isoformas de apo(a). |
| 57 | Caucásicos y japoneses | Diferencias significativas de los subtipos de alelos entre las poblaciones estudiadas y en pacientes con IAM. |
| 58 | Esquimales y Daneses caucásicos | Correlación inversa entre la concentración y el tamaño de las isoformas de apo(a) en las 3 poblaciones. |
| 59 | Chinos e hindúes residentes en Singapur. Recién nacidos | Hindúes tienen mayor concentración que los chinos, dato que concuerda con la tasa de mortandad de sus respectivas poblaciones adultas en Singapur. |
| 60 | Niños españoles | 15.1 % de la población estudiada tiene concentración arriba de 30 mg/dL. |
| 61 | Griegos | El percentil 77 de los hombres y 66 de las mujeres, tiene menos de 30 mg/dL de Lp(a). |
| 62 | Pigmeos y Bantús | 41% de los hombres pigmeos y 52% de las mujeres pigmeas tiene una concentración mayor a 30 mg/dL. En los bantús fue de 47% y 55% en hombres y mujeres, respectivamente. No hay diferencias significativas entre las 2 poblaciones. Sin correlación con otras variables estudiadas. |
| 63 | Mexicanos | Análisis segregacional sugiere la existencia de otro |

| | | |
|---|---|---|
| | residentes en EUA | gen que participa con otro 41% en el control de la concentración. |
| 64 | Negros Sudafricanos | Sin correlación entre el tamaño de la isoforma de apo(a) y la concentración de Lp(a). |
| 65 | Sicilianos | Resultados similares a los de poblaciones caucásicas. |
| 66 | Estonianos y Rusos | Estonianos mayor concentración de Lp(a) que los rusos. |
| 67 | Taiwaneses | Correlación positiva con la edad, LDL, fibrinógeno. Correlación negativa con TG, bajo HDL, IMC, resistencia insulina en hombres. Menopausia no cambia la concentración de Lp(a)?, anticonceptivos la disminuyen. |

FIGURA 3




***Distribución geográfica de los estudios de la concentración plasmática de Lp(a)***

# ESTRUCTURA QUÍMICA

La composición de la Lp(a) es parecida a la de lipoproteínas de baja densidad o LDL. Ambas lipoproteínas contienen colesterol, triacilgliceroles y fosfolípidos, que pueden disolverse y transportarse en el plasma, gracias a la presencia de una proteína, apoB-100, que rodea al grupo de lípidos y colesterol [67] .

TABLA 2

| Propiedades | Lp(a) | LDL |
|---|---|---|
| Diámetro A° | 269 ± 12 | 206 ± 29 |
| Movilidad electroforética | pre beta | Beta |
| Punto isoeléctrico | 4.9 | 5.6 |
| Peso molecular ($x10^6$) | 3.08 | 2.93 |
| Densidad hidratada | 1.055 - 1.12 | 1.02 - 1.063 |
| Apolipoproteínas | Apo(a), Apo B-100 | Apo B-100 |
| Proteínas (%) | 27- 30.9 | 22.4 |
| Colesterol (%) | 7.9 | 8.5 |
| Éster de colesterol (%) | 37.1 | 40.7 |
| Triacilgliceroles (%) | 19 | 21.3 |
| Fosfolípidos (%) | 5 | 7.1 |

La diferencia principal entre la molécula de LDL y de Lp(a) radica en la presencia de apo(a) que se une a través de un puente disulfuro con apoB-100 entre Cys en posición 69 del KIV-9 de apo(a) y Cys 3734 de apoB-100; la unión se estabiliza

por puentes de hidrógeno e interacciones van der Waals en otras regiones de ambas proteínas [68, 69, 70]. La apoB-100 [71] tiene la misma estructura y conformación en la molécula de Lp(a) y en LDL. La proporción de apo(a):apoB-100 es de 1:1 [72], pero considerando sus parámetros fisicoquímicos podrían existir entidades en proporción 2:1[69].

Como es frecuente en las lipoproteínas plasmáticas, Lp(a) puede tener diferentes tamaños, con pesos entre 800 y 1300 kDa y por lo tanto diferente densidad; estas diferencias reflejan, en menor medida, la composición del núcleo de lípidos y, principalmente, del polimorfismo estructural de apo(a)[73] .

Existen dificultades técnicas para obtener la forma nativa de apo(a) y las predicciones de su estructura secundaria sugieren ausencia de α-hélice [74]. Sin embargo, después de reducir los enlaces sulfhidrilo y por la técnica de dicroísmo circular, se observó 8 % de α-hélice, 21% de hoja β y 71% de arreglo al azar [75].

La apo(a) pertenece a la familia de serina proteasas, al igual que el plasminógeno, la protrombina, el activador tisular del plasminógeno, el activador del plasminógeno tipo urocinasa y el factor XII. Estas proteínas derivan de un gen ancestral común a todas ellas.

La apo(a) es muy parecida al plasminógeno (Figura 1). Los genes que codifican para ambas proteínas se encuentran muy cercanos, en el cromosoma 6, banda q26-27 [20]. Ambos genes se orientan cabeza con cabeza a 50 kb de distancia [76], 56 en la región terminal 5', que en el caso de apo(a) presenta un polimorfismo que puede expresarse modificando la eficacia en la trascripción y da lugar a

diferencias en la concentración plasmática de Lp(a) no sólo entre individuos sino, como ya se ha mencionado, entre diferentes grupos étnicos.

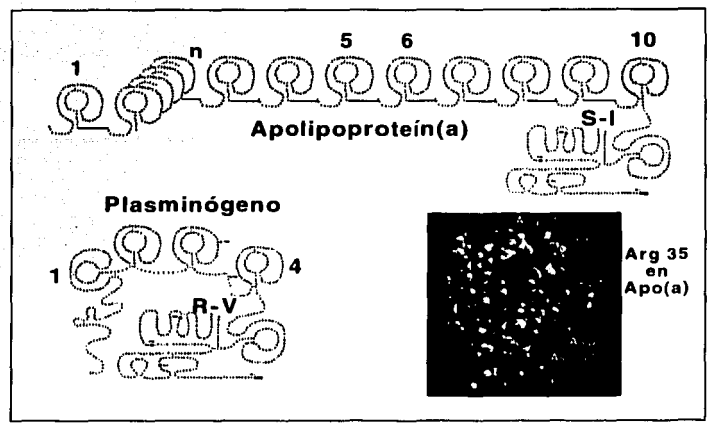
El gen de la apo(a) puede tener diferentes tamaños, cada uno corresponde al número de veces que se presenta una secuencia de 5.5 kb cuyo número puede variar entre 12 a 51. La región promotora del gen de apo(a) tiene, rio arriba, un fragmento de 1 kb de tamaño cuya secuencia presenta varios sitios potenciales de interacciones. Con la interleucina 6 (IL6) presenta siete sitios, hecho que permite explicar el incremento pasajero en la concentración plasmática de Lp(a) durante estados de inflamación aguda y tres sitios con elementos específicos de trascripción hepática (HNF-1, CEBP, y LF-A1).

La apo(a) y el plasminógeno tienen una región catalítica con una similitud de 94%, pero que en el caso de apo(a) carece de la capacidad de activarse y de tener una función enzimática a causa de la presencia de arginina en lugar de serina en el sitio de activación.

El plasminógeno y la apo(a), tienen también, diferente número de módulos llamados kringles, Figura 4. A través de los kringles se reconocen y se unen a otras macromoléculas y/o a sitios específicos de la membrana celular [77].

Los kringles se unen entre ellos por regiones interkringles, que son segmentos de 26 a 36 aminoácidos ricos en serina, prolina y/o treonina; cada región interkringle tiene 6 sitios potenciales para O-glicosilaciones .

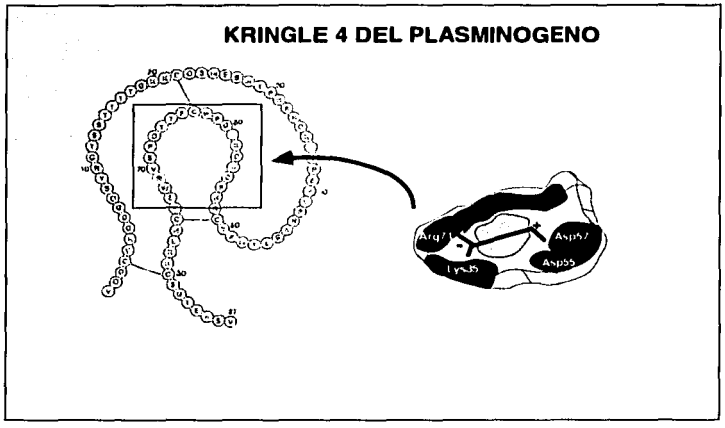
FIGURA 4




***Estructura de plasminógeno y de Apo(a). R-V indica el sitio de hidrólisis del plasminógeno en Apo(a) el sitio S-I no se hidroliza; n indica el número de KIV tipo 2.***

En la función de reconocimiento participa una estructura globular que se genera en el interior del kringle y que rodea a una región hidrófoba formada por varios aminoácidos aromáticos que se estabilizan a través de puentes de hidrógeno y separan a un grupo catiónico de un grupo aniónico [78]. Esta región se conoce como el sitio de unión a la lisina, o LBS Figura 5, por sus siglas del término en inglés lysine-binding-site. Sus características estructurales generan una geometría relativamente rígida a la que selectivamente tienen acceso y pueden unirse ligandos alifáticos o aromáticos de 6.8 Å, del tipo de ácidos ω-amino-carboxílicos como es el ácido ε-aminohexanóico o compuestos análogos .

FIGURA 5




***Representación esquemática del sitio LBS del kringle 4 del plasminógeno.***

El plasminógeno tiene 5 tipos de kringles muy similares entre sí, pero con pequeñas diferencias en el sitio de unión a la lisina, que modifican el grado de afinidad por diferentes ligandos. El K1 del plasminógeno tiene un LBS de gran afinidad; el polo catiónico se forma con Arg 35 y Arg-71, el polo aniónico se forma con Asp-55 y Asp-57[79] .

El LBS del K4 del plasminógeno tiene una afinidad intermedia; es una región hidrófoba en forma de V que genera una topografía en la que se alinean los anillos aromáticos de Phe-64, Trp-62 y Trp-72 que separan al grupo aniónico, formado por Asp-55 y Asp-57 del catiónico, formado por Lys-35 y Arg-71[80].

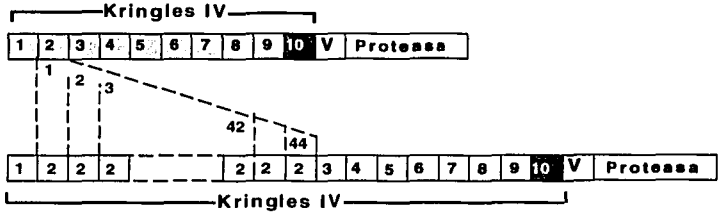
# POLIMORFISMO DE APOLIPOPROTEINA (a)

La apo(a) comparte con el plasminógeno el kringle V y un número variable de kringles IV, Figura 6.

No todos los kringles IV de apo(a) son iguales entre sí; se clasifican en 10 subtipos diferentes[81];

FIGURA 6

## Polimorfismo Estructural de la Apo(a)




***Posibles variaciones en el tamaño de Apo(a) dependiendo del número de kringles IV tipo 2***

En la molécula de apo(a) cada uno está presente una sola vez y únicamente el kringle IV-2 se presenta un número diferente de veces, lo que da origen a una heterogeneidad estructural y a isoformas de diferente tamaño con pesos moleculares entre 280 y 800 kDa. De todos ellos, el kringle IV-10 es el más parecido al kringle 4 del plasminógeno; el sitio de unión de alta afinidad a la lisina se forma por un polo aniónico Asp-55 y Asp-57 y por un polo catiónico Arg-71 y Arg-35; entre ambos polos hay un microambiente hidrófobo que se forma por la presencia de tres aminoácidos aromáticos Trp-62, Phe-64 y Trp-72. El KIV-10 de la apo(a) difiere del K-4 del plasminógeno, por la presencia de Arg en lugar de Lys en la posición 35. Este kringle tiene un papel preponderante en la unión de Lp(a) a la lisina presente en la fibrina[82], de esta forma impide que el plasminógeno tenga

acceso a las redes de fibrina y se active por el activador tisular del plasminógeno[83, 84, 85, 86], generándose una insuficiencia fibrinolítica que favorece la aterosclerosis y la trombosis .

Otra fuente de polimorfismo de apo(a) son las glicosilaciones; cada kIV de apo(a) tiene un sitio potencial para una N-glicosilación y, considerando las glicosilaciones interkringles, 30% de cada mol de apo(a) corresponde a carbohidratos: manosa, galactosa, galactosamina, glucosamina y ácido siálico, en relaciones aproximadas de 3:7:5:4:7, respectivamente[75] .

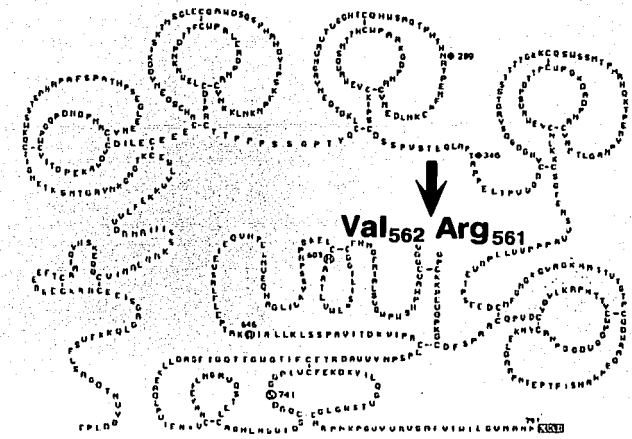
# FIBRINOLISIS

En el interior de los vasos sanguíneos la fibrina se deposita para consolidar las reacciones hemostáticas. Es un proceso benéfico que permite reparar y devolver sus funciones al endotelio vascular después de una lesión; sin embargo, cuando la permanencia de la fibrina no es autolimitada, favorece el desarrollo de la aterosclerosis.

El sistema fibrinolítico destruye los depósitos de fibrina en el interior de los vasos sanguíneos, tanto los remanentes de la actividad hemostática como los que se van formando y acumulando durante la evolución de la placa aterosclerosa. Gracias a un balance entre los activadores del plasminógeno y los diferentes tipos de inhibidores de estos activadores, su respuesta se inclina entre el reposo y la actividad fibrinolítica.

Como resultado de la activación fibrinolítica se genera plasmina, Figura 7.

## FIGURA 7

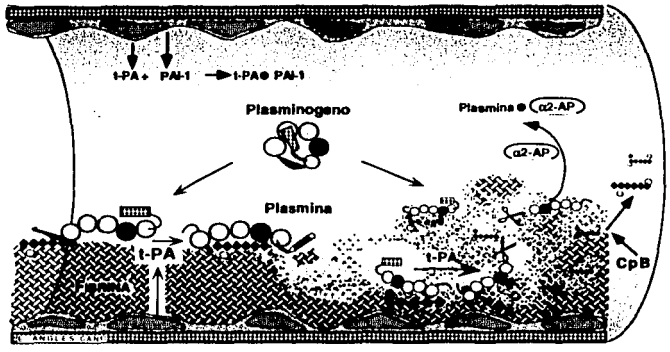



*La flecha indica el sitio de escisión del plasminógeno para generar plasmina*

La plasmina es una enzima proteolítica que puede ejercer su efecto sobre numerosos substratos plasmáticos. Esto no ocurre así, gracias a que es la propia fibrina la que localiza y señala el sitio de la activación fibrinolítica. Entre sus redes se une el plasminógeno, precisamente en el sitio de alta afinidad o LBS y permite y orienta adecuadamente la unión de su activador tisular (t-PA). Así se genera plasmina en el interior del depósito de fibrina, que actúa escindiéndola y a la vez poniendo al descubierto otros sitios de afinidad, residuos de lisina carboxi-terminales, para amplificar su respuesta. Una vez que se desintegra la fibrina, el mecanismo se detiene, el endotelio vascular no libera más activador. En este punto los remanentes de plasmina y de activador tisular, que alcanzan la

circulación, se encuentran con sus inhibidores respectivos, la alfa2-antiplasmina y el inhibidor del activador tisular del plasminógeno tipo 1 (PAI-1). Figura 8.

FIGURA 8



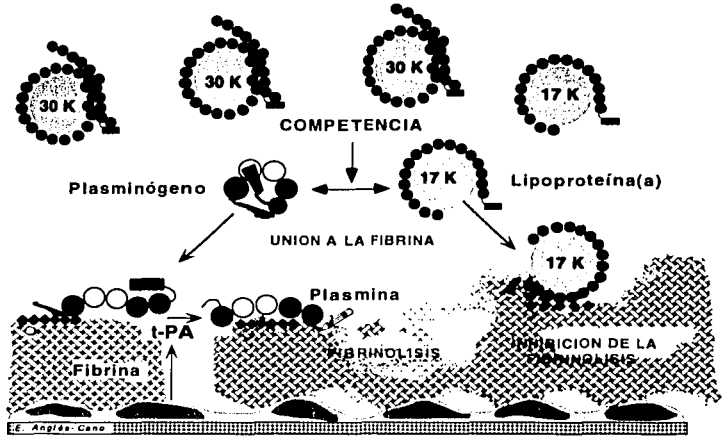

*Representación esquemática de la fibrinolisis*

# Lp(a) INHIBIDOR COMPETITIVO DEL PLASMINÓGENO

La semejanza entre el plasminógeno y la apolipoproteína (a) permite que las diferentes isoformas de apo(a) compitan por los sitios de afinidad (LBS) de la fibrina con el plasminógeno.

Sin embargo, no todas las isoformas de apo(a) muestran la misma afinidad. Esta depende del tamaño de la isoforma y de su concentración plasmática [87, 88] Figura 9.

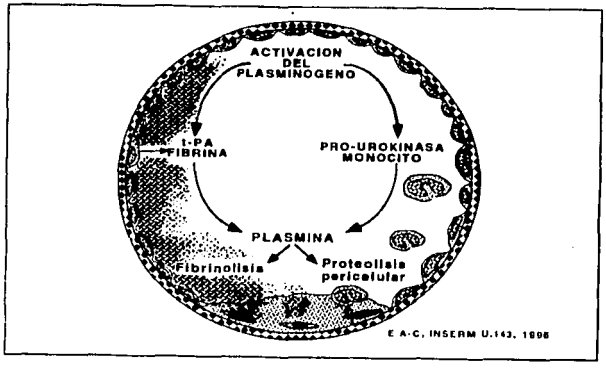
FIGURA 9




***Mecanismo de acción de la lipoproteína Lp(a). El polimorfismo estructural de la apolipoproteína apo(a) influye en el efecto antifibrinolítico de la Lp(a). Las isoformas de talla más pequeña poseen mayor afinidad por la fibrina y producen un efecto más pronunciado.***

El plasminógeno y las diferentes isoformas de Lp(a) [89], compiten también por los residuos de lisina en los receptores de la superficie de las células endoteliales [90], en los monocitos U937 [91], en las plaquetas[92], en las células mononucleares y en modelos *in vitro* de matrices que simulan la membrana extracelular [93]. La Figura 10 muestra esquemáticamente la formación de plasmina, tanto sobre la superficie de fibrina como sobre los monocitos.

FIGURA 10




**El plasminógeno se activa tanto sobre la superficie de fibrina como sobre la superficie celulares.**

Los dos activadores del plasminógeno: activador tipo tisular activa la fibrinolisis y el activador tipo urocinasa activa la proteolisis pericelular.

Otro mecanismo más que altera el balance del sistema fibrinolítico, es la disminución de la síntesis del activador tisular del plasminógeno (t-PA) y el incremento de la síntesis del inhibidor del activador tisular del plasminógeno (PAI-1) [94] que se observa en cultivos de células endoteliales expuestas a Lp(a).

## Lp(a) y ATEROGÉNESIS.

La aterosclerosis es un proceso multifactorial y crónico [95]. Las arterias más afectadas son las de mediano o gran calibre, particularmente en los sitios que están expuestos a las fuerzas de cizalla. Las alteraciones en el metabolismo de los lípidos, las lesiones o alteraciones en el funcionamiento de las células endoteliales y la pérdida de equilibrio entre el depósito de fibrina y la fibrinolisis,

son los principales factores que se identifican como precursores de la placa ateromatosa. Los dos primeros favorecen el inicio [96] mientras que la insuficiencia fibrinolítica interviene principalmente en el crecimiento de la placa ateromatosa[97].

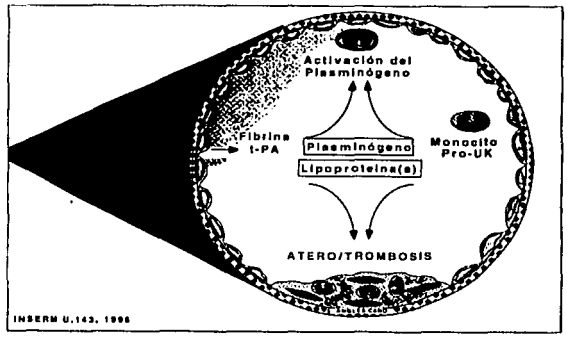
Las lesiones de la íntima favorecen la migración y la proliferación de células, la acumulación de lípidos, la aceleración en la síntesis de la matriz extracelular y el desequilibrio en la síntesis de factores quimiotácticos y de crecimiento. Todas estas acciones originan las lesiones ateromatosas que se clasifican en tres categorías:[98]

La lesión precoz, que se caracteriza por un depósito en la íntima de lípidos (principalmente de baja densidad o LDL), presencia de macrófagos y de células espumosas. En la lesión intermedia existe ya proliferación celular de macrófagos, de células espumosas, de células musculares lisas que provienen de la media y que éstas últimas adquieren la capacidad de sintetizar ciertas proteínas de la matriz extracelular. En las lesiones avanzadas se pueden identificar deformaciones de la pared arterial en las que hay depósitos de lípidos que se rodean de una capa fibrosa formada por proteoglicanos, células espumosas y células musculares lisas. Una fisura en este sitio permite el contacto del factor tisular con el factor VII de la coagulación y se inician los mecanismos pro coagulantes que culminan con la formación de un trombo que puede o no obstruir completamente el flujo sanguíneo, y dar un curso acelerado y en ocasiones fatales a estos padecimientos.

La Lp(a) favorece la aterogénesis a través de varios mecanismos; los macrófagos fagocitan a la Lp(a) [99] y se depositan en el subendotelio, se transforman en células

espumosas y dan lugar a deformaciones que disminuyen la luz de los vasos sanguíneos.

FIGURA 11

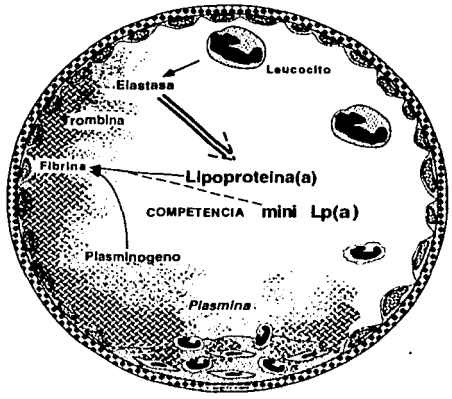



**La Lp(a) favorece la aterotrombosis y disminuye la fibrinolisis.**

En las células endoteliales de arteria coronaria en cultivo, se observa que la Lp(a) estimula la expresión de moléculas de adhesión vascular 1 (VCAM-1) y de selectina-E, proceso que desencadena la atracción de los macrófagos[100] . Otro mecanismo propuesto se relaciona con la disminución de la generación de plasmina que tiene las siguientes consecuencias: a) permanencia prolongada de depósitos de fibrina , con el consecuente incremento en el depósito de colesterol y formación de la placa aterosclerosa y b) disminución de la activación, por hidrólisis parcial con plasmina, del factor de crecimiento transformante ß (Transforming Growth Factor o TGF-ß), factor que limita el crecimiento de las células del músculo liso vascular.

Se ha propuesto[101] que durante los episodios inflamatorios los leucocitos, macrófagos y células T, secretan enzimas proteolíticas. Los fragmentos de Lp(a)/Apo(a) que se forman bajo estas circunstancias, pueden tener una mayor capacidad aterosclerosa como lo representa la figura 12

Figura 12




***Por la acción de enzimas proteolíticas se forman partículas de Lp(a) con mayor actividad aterosclerótica.***

# Lp(a) INHIBE LA FIBRINOLISIS

La elevada concentración plasmática de Lp(a) no siempre interfiere con la fibrinolisis normal. Las isoformas de apo(a) muestran diferente actividad antifibrinolítica, por lo que se debe subrayar la importancia de considerar la actividad antifibrinolítica de las isoformas en la predicción de la enfermedad cardiovascular [102].

Se han llevado a cabo diversas estrategias de estudio *in vitro*. Empleando un modelo de fibrina en fase sólida [103, 104, 105, 106, 107], ha sido posible identificar de manera sensible y específica la inhibición de la fibrinolisis por las isoformas de apo(a).

Estos estudios han permitido concluir el mecanismo y las diferentes variables que intervienen en la inhibición competitiva del plasminógeno, por diferentes isoformas nativas de Lp(a) o por formas recombinantes de apo(a) .Estos trabajos muestran que:[108]

1) La afinidad de Lp(a) por la fibrina muestra una relación inversa con el tamaño de las isoformas de apo(a), que pueden tener constantes de disociación (Kd) entre 50 y 500 nmol/L [87]

2) Tanto el plasminógeno como la Lp(a) compiten por los mismos sitios de unión, lo que corresponde a un mecanismo de inhibición competitiva saturable.

3) El potencial antifibrinolítico de Lp(a) depende de la afinidad y de la concentración de cada una de las 2 isoformas de apo(a) presentes en el plasma108.

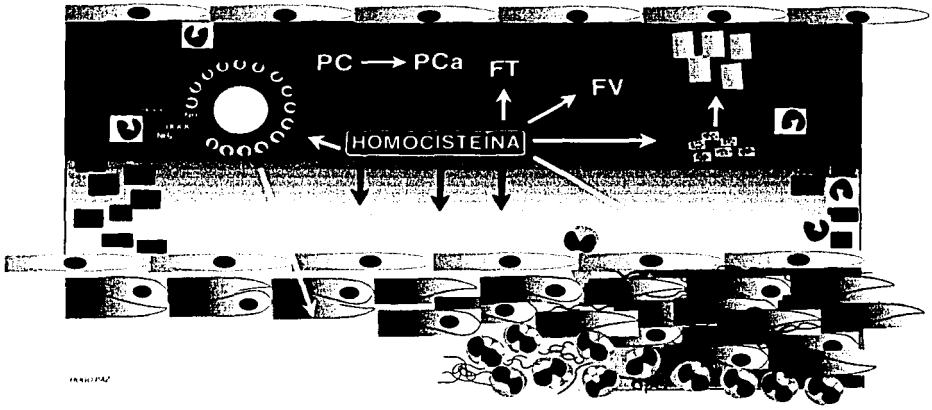
## Lp(a) Y HOMOCISTEINEMIA

La hiperhomocisteinemia se asocia con un incremento de las enfermedades trombóticas y aterosclerosas[109]. La trombosis se favorece porque altera varios mecanismos de regulación antitrombótica de la hemostasia.

Los mecanismos fisiológicos que regulan la hemostasia permiten, junto con el sistema fibrinolítico, que la actividad pro- y anti-coagulante mantenga un equilibrio.

Entre los mecanismos anticoagulantes naturales se encuentra: la antitrombina III, que inhibe directamente a los factores enzimáticos de la coagulación activados (IIa, IXa, Xa) ; el sistema de la Proteína C y su cofactor la proteína S, que inhibe a los cofactores activados de la coagulación (Va, VIIIa),.y el inhibidor del factor tisular.

Figura 13




***PC, proteína C; PCa, proteína C activada; FT, factor tisular; FV, cofactor V de la coagulación. $PGI_2$, prostaciclina o prostaglandina $I_2$, NO, óxido nítrico; Tm, trombomodulina.***

La homocisteína incrementa la actividad del factor tisular, disminuye la expresión y la actividad de la trombomodulina que es indispensable para la activación de la proteína C, disminuye la actividad anticoagulante de la antitrombina III así como la unión del activador tisular del plasminógeno a su receptor en la superficie de las celulas (anexina II)[110] y también el incremento de la concentración de homocisteína plasmática, se refleja como un incremento en la unión de Lp(a) a la fibrina [111]., Figura 13

# Lp(a) e INHIBIDOR DEL FACTOR TISULAR

Otro mecanismo recientemente propuesto[112] por el que Lp(a) favorece la trombosis intravascular, contempla la unión directa, dependiente de la concentración, al inhibidor del factor tisular. El inhibidor del factor tisular (IFT) modula la actividad catalítica del factor VII de la coagulación y es reconocido como un mecanismo fisiológico anticoagulante. El inhibidor del factor tisular se localiza en las células endoteliales y la interacción Lp(a)/IFT sugiere que existe un mecanismo procoagulante que se localiza en la placa aterosclerosa.

# ESTUDIOS CLíNICOS

Bajo esta perspectiva, se realizaron numerosos estudios epidemiológicos, que describen una asociación positiva entre la elevada concentración plasmática de Lp(a) y un incremento en las enfermedades cerebrovasculares [113], cardiovasculares, la reestenosis de puentes coronarios, la reoclusión de angioplastías y el desarrollo prematuro de aterosclerosis asociada a altas concentraciones de LDL y/o a bajas concentraciones de HDL[114, 115]. La mayoría de estudios prospectivos confirman estos resultados [116, 117, 118, 119, 120, 121, 122], aún cuando no existe un acuerdo en el límite de corte para considerar una concentración plasmática normal; algunos autores consideran que es de 20 mg/dL[121], otros hasta 30 mg/dL 116 y que el empleo de anticuerpos diferentes, monoclonales o policlonales, genera divergencias en los resultados[5].

Sin embargo, otros estudios no encuentran asociación entre la Lp(a) y la enfermedad arterial coronaria [123, 124]. Esta discrepancia en los resultados podría

ser el reflejo de la gran heterogeneidad estructural de la molécula de apo(a), que se traduce en una heterogeneidad funcional como inhibidor competitivo del plasminógeno por la fibrina, disminuyendo la formación de plasmina.[7], [125].

Craig y col [126] hicieron un meta-análisis de los diferentes estudios prospectivos que estudian a la Lp(a) como factor de riesgo de la enfermedad arterial coronaria en población caucásica, poniendo especial atención en los criterios de selección de los trabajos a fin de que los resultados de los diferentes estudios fueran comparables. La tabla 3 muestra que, los individuos que desarrollaron enfermedad arterial coronaria tenían una mayor concentración de Lp(a) que los controles[116, 117, 118, 119, 124, 127, 128, 129, 130, 131, 132, 133]. Sugieren que la variabilidad de los resultados puede ser efecto del manejo pre-analítico de la muestra, en especial a la temperatura que se almacenó la muestra. Concluyen que la Lp(a) es un factor prospectivo de riesgo independiente en la enfermedad isquémica coronaria.

## TABLA 3

| Ref | País | N Casos | N Controles | Cociente [Lp(a)] mg/L Casos/Controles | ES |
|---|---|---|---|---|---|
| 128 | Finlandia | | | | |
| | Hombres | 97 | 148 | 0.68 | 0.050 |
| | Mujeres | 97 | 121 | 1.24 | 0.049 |
| 118 | Alemania | 107 | 5124 | 2 | 0.038 |
| 129 | Alemania | 33 | 828 | 1.8 | 0.084 |
| 130 | Reino Unido | 49 | 192 | 1.4 | 0.045 |
| 131 | Finlandia | 138 | 130 | 1.18 | 0.044 |
| 132 | Dinamarca | 74 | 190 | 1.32 | 0.078 |
| 120 | EUA | 233 | 390 | 1.22 | 0.021 |
| 125 | EUA | 296 | 296 | 1 | 0.018 |
| 134 | EUA | | | | |
| | Hombres | 90 | 90 | 1.97 | 0.132 |
| | Mujeres | 44 | 44 | 1.34 | 0.179 |
| 117 | Suiza | 26 | 109 | 1.61 | 0.070 |
| 133 | Islandia | 104 | 1228 | 1.35 | 0.034 |
| 119 | Reino Unido | 229 | 1145 | 1.54 | 0.033 |

Craig et al. Clinical Chemistry 1998; 44: 2301-2306

# JUSTIFICACION DEL ESTUDIO

Por la inconsistencia en los resultados entre los diferentes estudios prospectivos para señalar a la Lp(a) como un factor de riesgo en la enfermedad arterial vascular, es importante evaluar el impacto de la Lp(a) como factor de riesgo a través de su capacidad para inhibir la fibrinolisis. Por esta razón es necesario el empleo de un método funcional que identifique la unión de Lp(a) a la fibrina en los diferentes sujetos de estudio.

# HIPÓTESIS

La cantidad de apo(a) unida a la fibrina, las isoformas de Lp(a) de bajo peso molecular, se encuentran aumentadas en las muestras plasmáticas de sujetos con enfermedad arterial vascular en comparación con sujetos sin ésta.

# OBJETIVO DEL ESTUDIO

1.-Determinar cuál es la cantidad de apo(a) unida *in vitro* a la fibrina, en las muestras plasmáticas de sujetos con enfermedad arterial vascular, en comparación con sujetos sin ella.

2.- Analizar qué tipo de isoformas de Lp(a) se unen en mayor proporción a la fibrina en las muestras plasmáticas de sujetos con enfermedad arterial vascular, en comparación con sujetos sin ella .

3.- Determinar si existe una relación entre la concentración plasmática de Lp(a) y la cantidad de apo(a) unida *in vitro* a la fibrina, en las muestras plasmáticas de sujetos con enfermedad arterial vascular, en comparación con sujetos sin ésta.

# DISEÑO DEL ESTUDIO

# TRANSVERSAL ANALITICO

Por el control de la maniobra: observación.

Por la captación de la información: prolectivo.

Por la medición de la maniobra en el tiempo: transversal.

Por la presencia de un grupo control: comparativo.

Por la asignación de sujetos al grupo de estudio y control : no aleatorio.

Por la capacidad en la aplicación y evaluación de las maniobras: abierto y ciego simple.

## POBLACIÓN DEL ESTUDIO

Individuos con enfermedad isquémica coronaria que acudieron a la consulta externa del Instituto Nacional de Cardiología, que cumplieron con los criterios de selección y consintieron, por escrito, en participar en el estudio.

Individuos con enfermedad vascular cerebral que acudieron a la consulta externa del Instituto Nacional de Neurología y fueron remitidos al Instituto Nacional de Cardiología, cumplieron con los criterios de selección y consintieron, por escrito, en participar en el estudio.

Población sin antecentes de enfermedad isquémica que acudieron a donar sangre al Instituto Nacional de Cardiología.

# CASOS

### *Criterios de inclusión:*

- Antecedente de enfermedad vascular coronaria, infarto agudo del miocardio, angina estable o inestable.
- Antecedente de infarto cerebral de origen trombótico.
- En caso de ser necesario, los enfermos estaban bajo tratamiento con fármacos hipolipemiantes, antihipertensivos, hipoglucemiantes o antiagregantes plaquetarios.

### *Criterios de no inclusión:*

- ► Evento trombótico en el mes anterior al estudio.
- ► Hipertensión sin control.
- ► Déficit neurológico con demencia
- ► Embolia cerebral de origen cardiaco o consecuente con una angiografía coronaria.
- ► Enfermedad hepática aguda o crónica.
- ► Enfermedad renal aguda o crónica.
- ► Enfermedad hemorrágica.
- ► Procedimientos quirúrgicos, 3 meses previos al estudio.
- ► Procesos infecciosos graves, 2 meses previos al estudio.

# CONTROLES

Donadores de sangre sin antecedentes de enfermedad isquémica cerebral o coronaria.

### *Definición operativa de los criterios de exclusión*

1) Enfermedad hepática

Incremento en: bilirrubina directa > 1.5 mg/dL

bilirrubina indirecta > 1.0 mg/dL

fosfatasa alcalina > 70 U/l

transaminasa oxalacética > 25 U/l

transaminasa pirúvica > 20 U/l

Tiempo de protrombina prolongado >20 %

Cuando esté consignado en el expediente por médico del Instituto Nacional de Cardiología.

2) Enfermedad renal

Creatinina > 1.5 mg/dL

Cuando esté consignado en el expediente por un médico del Instituto Nacional de Cardiología.

3) Enfermedad hemorrágica

Tiempo de protrombina prolongado > 20 %

Tiempo de tromboplastina parcial prolongado > 20 %

Fibrinógeno < 1.5 g/L

Cuando esté consignado en el expediente por un médico del Instituto Nacional de Cardiología.

4) Trombosis

Dímeros D-D positivos. Cuando esté consignado en el expediente por un médico del Instituto Nacional de Cardiología.

***DEFINICIÓN OPERATIVA DE VARIABLES***

***Variables independientes***

Enfermedad aterotrombótica, variable nominal.

***Categorías:***

1 Enfermedad isquémica coronaria (CI). Diagnóstico clínico, electrocardiográfico o por angiografía coronaria. Se considera positivo cuando esté consignado en el expediente por un cardiólogo del Instituto Nacional de Cardiología.

2 Enfermedad vascular cerebral (EVC). Diagnóstico clínico, o por estudio doppler o tomografía computarizada. Se considera positivo cuando esté consignado en el expediente por un neurólogo del Instituto Nacional de Neurología y se remita para inclusión al estudio en el Instituto Nacional de Cardiología.

***Variables dependientes***

1 Concentración plasmática de Lp(a). Se estimó en mg/dL. Es una variable continua con escala de medición de proporción.

2 Lp(a) unida a la fibrina. Se estimó la cantidad de apo(a) unida a las placas de fibrina extrapolando cada resultado a una curva de calibración que se construye con un estándar de recombinantes apo(a) desde 10 a 34 kringles. Variable continua con escala de medición de proporción.

3 Isoformas de Lp(a). Se reconocieron comparando su migración contra un estándar de recombinantes apo(a) desde 10 a 34 kringles. Variable discreta en escala de medición nominal.

***Variables de confusión***

1 Colesterol total, triacilgliceroles, lípidos de baja densidad, lípidos de alta densidad, variables continuas en escala de medición de proporción, (mg/dL).

2 Antecedentes heredo-familiares, variable nominal.

3 El fibrinógeno, el factor VII de la coagulación, el volumen de sedimentación globular, los leucocitos, son variables continuas con escala de medición de proporción.

Variables modificadoras, variables continuas en escala de medición de proporción.

1 Presión arterial. Se registrará en mm Hg con el individuo en posición sedente, después de 20 min. de reposo.

2 Circunferencia de la cintura. Se medirá con cinta métrica , se registrará en cm.

3 Circunferencia de la cadera. Se medirá con cinta métrica , se registrará en cm.

4 Índice de masa corporal. Se calculará dividiendo el peso entre el cuadrado de la talla ($Kg/m^2$).

Variables universales, variables continuas, escala de medición de proporción.

1) La edad se registrará en años.

2) El peso se registrará en Kg

3) La talla se medirá de pie y sin calzado con una cinta métrica colocada en la pared. Se registrará en cm .

# DESCRIPCION OPERATIVA DEL ESTUDIO

Se revisarán los expedientes un día previo a su consulta y a los candidatos se les informará del estudio y se les invitará a participar previa carta de consentimiento informado.

Los enfermos que aceptaron ingresar al estudio se sometieron a exámenes de escrutinio (si no se han practicado en el transcurso del último año), para descartar enfermedades hepática, renal o trastornos hematológicos.

A los enfermos que cumplieron con los criterios de selección se les aplicó un cuestionario que incluyó una ficha de identificación, antecedentes familiares, antecedentes personales.

# ASPECTOS ÉTICOS

Ya que los participantes en el estudio se sometieron a una punción venosa adicional a su manejo habitual, implicando un riesgo mayor al mínimo, se les informó sobre las características y objetivos del estudio.

# MATERIAL, MÉTODOS Y PROCEDIMIENTOS

## GRUPO DE ESTUDIO

Estudiamos 248 individuos, 157 enfermos y 91 controles. 105 enfermos con cardiopatía isquémica, infarto del miocardio, o con angina estable/inestable. 52 enfermos con enfermedad vascular cerebral de origen trombótico, Tabla 4

***TABLA 4***

| Categorias | Número Individuos | Porcentaje |
|---|---|---|
| EVC | 52 | 21 |
| CI | 105 | 42.3 |
| Infarto del miocardio | 70 | 28.2 |
| Angina estable/inestable | 35 | 14.1 |
| Controles | 91 | 36.6 |
| Total | 248 | 100 |

# MUESTRA DE SANGRE

Se obtuvo de cada individuo con 12 horas de ayuno, después de permanecer sentado 20 minutos, una muestra de 15 mL de sangre por punción venosa con una jeringa estéril desechable. La muestra de sangre se dividió en 3 alícuotas iguales. La primera se colocó en un tubo A de plástico que contenía citrato de sodio (3.8%) en una relación 1:9 como anticoagulante, para determinar los

tiempos de coagulación, fibrinógeno y dímeros D-D. La segunda se colocó en un tubo B de vidrio, sin anticoagulante para la determinación de química sanguínea. La tercera se colocó en un tubo de plástico C que contenía EDTA 3 mM como anticoagulante para determinar concentración de Lp(a), isoformas, grado de unión a la fibrina. Los tubos se centrifugan a 2000 x g durante 20 min. a 4°C.

El plasma del tubo C se separó en varias alícuotas. A las alícuotas destinadas al estudio de Lp(a) se adicionaron inhibidores de proteasas (Trasylol®, Bayer Farma, Puteaux, Francia). Las muestras se congelaron a -70°C hasta efectuar las pruebas, máximo 6 meses. Se analizaron en bloques con el fin de disminuir la variabilidad ínter ensayo.

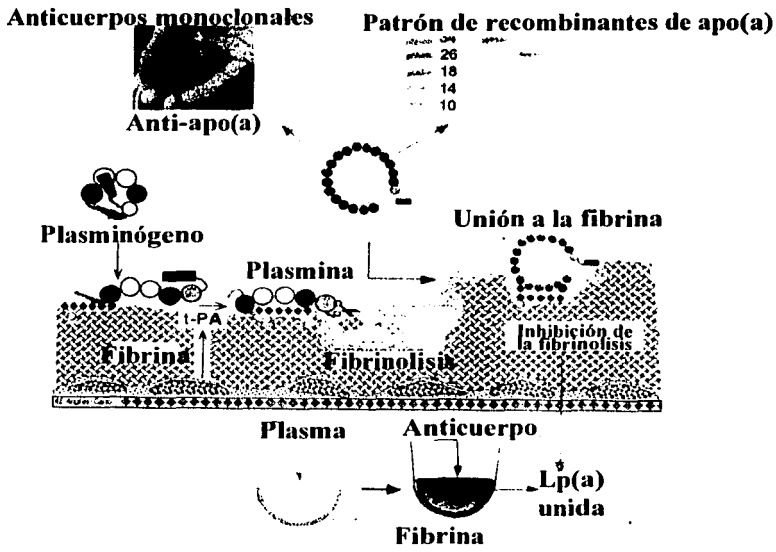
## UNIÓN DE APO(a) A LA FIBRINA

Se pusieron a punto las técnicas para obtener un modelo experimental que permite identificar *in vitro* la unión de la Lp(a) a la fibrina.

El modelo distingue la inhibición competitiva entre la Lp(a) y el plasminógeno como se muestra en la Figura 14, que se traduce en una incompetencia fibrinolítica.

La malla de fibrina que se forma en los pozos de la placa presenta sitios expuestos de lisina de 6.8 Å, a los que selectivamente tienen acceso y pueden unirse moléculas que presenten una estructura globular que se genera en el interior de los kringles.

FIGURA 14




***Representación esquemática del método para identificar la unión de apo(a) a la fibrina***

Este modelo tiene las siguientes características:

Superficie de fibrina 121 $mm^2$ = 410 fmol de fibrina/$cm^2$. La superficie que se cubre con un volumen de 50 µl corresponde a 88 $mm^2$.

El fibrinógeno no se desprende de la placa de PVC gracias a la presencia de polímeros de glutaraldehído que lo fijan y le proporcionan una flexibilidad adecuada para permitir el acceso de las proteínas afines.

La presencia de fibrina en las placas se puso en evidencia con un anticuerpo monoclonal, Y18, cuyo epítopo se sitúa en el fragmento 1-51 de la cadena Aα del fibrinógeno; la reactividad del anticuerpo disminuye a medida qure se incrementa el tiempo de reacción entre el fibrinógeno y la trombina. Con la ayuda del anticuerpo FDP-14, (colaboración con el Dr Nieuwenhuizen, Gaubius Instiitute, Leyden, Holanda), se identificó el fragmento E, producto de fragmentación del fibrinógeno[134] y el anticuerpo DD3B6/22 identificó los dimeros D-D, producto de fragmentación de la fibrina, (proporcionado por el Dr Rylatt, Agen Biomedical LTD, Brisbane, Australia).

Para hacer las determinaciones de unión de apo (a) a la fibrina, se lavaron las placas de fibrina tratadas con plasmina con amortiguador A (0.05 mmol/L de fosfato de sodio, pH 7.4, 0.08 nmol/L cloruro de sodio, 0.01 % thymerosal, 0.01% Tween 20, 2mg/mL albúmina bovina).

Se depositó por duplicado en cada pozo 50 µl de plasma (dilución 1:16 en amortiguador A + 40 mg/mL albúmina bovina)

Se incubó durante 12 h a 4°C con la finalidad de permitir el equilibrio entre la unión de plasminógeno/apo(a) a la fibrina.

Se retiraron las proteínas que no se unieron lavando la placa con amortiguador A.

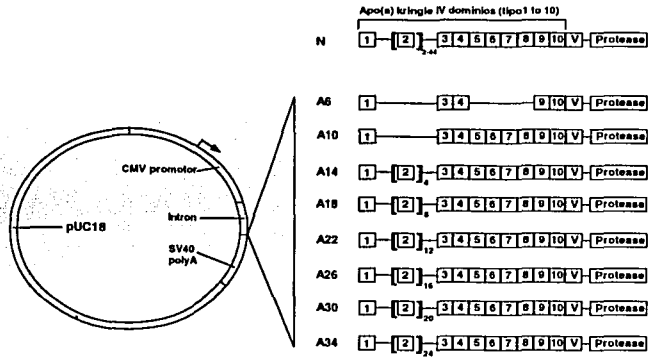
Se adicionó 50 µl de un anticuerpo monoclonal anti-apo(a)[135] conjugado con peroxidasa.

Se incubó la placa 2 h a 37°C

Se lavó la placa con amortiguador A y se adicionan 50 µl de una solución de 1 mg/mL de ácido 2,2'-azino-bis(3-etilbenzotiazolina)-6 sulfónico (ABTS, Boehringer Mannheim) para revelar la coloración.

El cambio en absorbencia (▲ A 405nm/min) se midió en un lector de placas (MR 500, Dynatech). La cantidad de apo(a) unida a la fibrina se obtuvo extrapolando los valores obtenidos a una curva patrón elaborada con un calibrador de referencia que contenía cantidades equimoleculares de isoformas recombinantes de apo(a) de 10 a 34 kringles23, Figura 15.

FIGURA 15




**Representación esquemática de las isoformas de apo(a)**

# CONCENTRACIÓN PLASMÁTICA DE Lp(a)

Método inmunonefelométrico, reactivos y equipo de Beckman Co, Palo Alto Ca. EUA.

# ISOFORMAS DE Apo(a)

Método de inmunoelectroforesis con transferencia en papel de nitrocelulosa que comprende los siguientes puntos:

- ► Reducción de los enlaces sulfhidrilo que unen a la apolipoproteína (a) con el resto del núcleo lipoproteíco que conforma a la lipoproteína (a).
- ► Depósito y migración de las muestras en gel de acrilamida.
- ► Transferencia de las proteínas del gel a papel de nitrocelulosa.
- ► Las isoformas de apo(a) se localizan con un anticuerpo monoclonal dirigido contra apo(a) marcado a la peroxidasa cuya presencia se revela con 4 cloronaftol.

Las proteínas presentes en el plasma son identificadas tomando como referencia un estándar que contiene diferentes isoformas recombinantes de apo(a) de masa molecular conocida[23], Figura 13.

Cada imagen de inmunotransferencia se digitalizó (Molecular Analyst TM/PC) y se analizó la densidad de la banda (Densitometric scanning Bio Rad GS-670).

Cada uno de los datos densitométricos de las isoformas ( una o dos) se sumó y consideró como el 100%.

# ANÁLISIS ESTADÍSTICO

Los promedios, medias, desviaciones estándar y rangos intercuartiles se calcularon para todas las variables.

Los límites de los cuartiles se calcularon tomando en consideración a toda la población (CI, EVC y controles). Las diferencias en la proporción de enfermos y

controles en cada cuartil se analizó con la prueba de chi-cuadrada para variables discretas.

Para las variables continuas, las comparaciones entre los grupos fueron evaluadas con análisis paramétrico o no paramétrico de acuerdo con la distribución de la variable.

Para las variables no paramétricas se empleó la prueba de LSD (least significant difference test) o la prueba de U de Mann-Whitney.

Para eliminar la posible interferencia de las diferentes edades entre los grupos se empleó el análisis de covarianza.

Los resultados de la distribución de la concentración de Lp(a) y de la unión de Lp(a) a la fibrina, se compararon con la prueba de Kolmogorov-Smirnov.

Para determinar la asociación entre la concentración de Lp(a), la unión de Lp(a) a la fibrina y los fenotipos de las isoformas de apo(a) con la enfermedad isquémica aterotrombótica, se comparó el número de individuos entre el primero y el último cuartil. Las diferencias se estimaron por tablas de contingencia, razones de momios e intervalos de confianza 95%.

Todas las pruebas estadísticas se hicieron empleando el programa SPSS-10.1 (EUA).

# RESULTADOS

El grupo de pacientes con evidencia clínica de enfermedad isquémica, no exhibe diferencias significativas con el grupo control respecto al género y al índice de masa corporal Tabla 4 . Sin embargo la edad difiere significativamente en ambos grupos de enfermos con respecto al control, motivo por el cual, aún cuando la concentración plasmática de Lp(a) no se modifica con la edad, los resultados que se muestran en la Tabla 5 se ajustaron a esta variable (análisis ANCOVA).

TABLA 5

Características demográficas de la población estudiada

| | CONTROL | EVC | CI |
|---|---|---|---|
| EDAD (años) | 40.2±13.1 | 53.4±10.5* | 62.5±10.6* |
| IMC $Kg/m^2$ | 26.3±5.6 | 27.0±3.5 | 27.2±3.4 |
| Cintura/Cadera | 0.88±0.069 | 0.93±0.056 | 0.94±0.44 |

**ANOVA $p < 0.001$, IMC=índice de masa corporal*

TABLA 6

Concentración plasmática de Lp(a) y Unión de apo(a) a la fibrina

| Variable | Control | EVC | CI |
|---|---|---|---|
| Concentración de Lp(a) mg/dL | 16.3±18 | 20.3±22.8 | 20.5±25.29 |
| Unión de apo(a) a la fibrina nM | 0.158±0.09 | 0.268±0.15* | 0.155±0.12 |

**ANCOVA $p < 0.0001$ contra grupo control y CI*

La unión de apo(a) a la fibrina en el grupo de EVC se incrementó de manera muy significativa ($p<0.001$) comparada tanto con el resultado del grupo control como con el del grupo de CI.

La Tabla 7 ilustra la distribución expresada en porcentaje tanto en enfermos como en los controles en cada cuartil. Las variables que se muestran son: la concentración plasmática de Lp(a), la unión de apo(a) a la fibrina y los fenotipos de apo(a).

En el grupo control tanto la concentración de Lp(a) como la unión de apo(a) a la fibrina, tienen una distribución no paramétrica, el mayor porcentaje de individuos se encuentra en los primeros cuartiles.

El grupo de CI muestra una distribución bimodal, el mayor porcentaje de individuos se encuentra en los dos primeros cuartiles y no disminuye gradualmente como en el caso del grupo control.

El grupo de EVC difiere notablemente tanto del grupo de CI como del grupo control, el mayor porcentaje de individuos se encuentra en los últimos cuartiles tanto para la concentración de Lp(a) como para la unión de apo(a) a la fibrina.

## TABLA 7

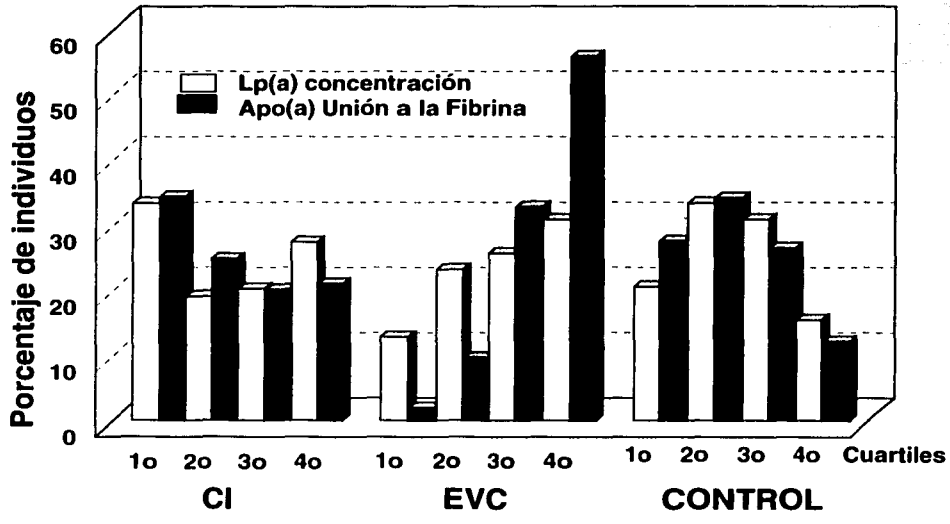
Porcentaje de individuos en cada cuartil para las variables de concentración plasmática de Lp(a), Unión de apo(a) a la fibrina y fenotipos de apo(a). El fenotipo 1 es la isoforma con mayor concentración en los sujetos heterocigotos.

| Cuartil | 1 | 2 | 3 | 4 | P* |
|---|---|---|---|---|---|
| Concentración Lp(a) mg/dL | <6.1 | 6.1-13.8 | 13.9-22.45 | >22.45 | |
| CI (%) | 33.3 | 19 | 20.2 | 27.4 | 0.074 |
| EVC (%) | 12.8 | 23.1 | 25.6 | 38.5 | <0.001 |
| CONTROL (%) | 20.5 | 33.3 | 30.8 | 15.4 | 0.009 |
| Unión de Apo (a) (nM) | <0.098 | 0.098-0.162 | 0.163-0.231 | >0.231 | |
| CI (%) | 34.3 | 24.8 | 20 | 21 | 0.089 |
| EVC (%) | 1.9 | 9.6 | 32.7 | 55.8 | <0.001 |
| CONTROL (%) | 27.5 | 34.1 | 26.4 | 12.1 | 0.003 |
| Apo(a) fenotipo 1 (K4) | <20 | 20-23 | 23.1-26 | >26 | |
| CI (%) | 20.8 | 35.1 | 28.6 | 15.6 | 0.006 |
| EVC (%) | 28.9 | 18.4 | 21.1 | 31.6 | 0.102 |
| CONTROL(%) | 23.1 | 18.5 | 32.3 | 26.2 | 0.187 |
| Apo(a) fenotipo2 (K4) | <16.5 | 16.6-19 | 19.1-21 | >21 | |
| CI (%) | 28.3 | 28.2 | 17.4 | 26.1 | 0.217 |
| EVC (%) | 24.1 | 17.2 | 31 | 27.6 | 0.118 |
| CONTROL (%) | 20.6 | 20.6 | 29.4 | 29.4 | 0.332 |

*P* <0.05 prueba de chi-cuadrada*

La Figura 16 muestra gráficamente los datos de la Tabla 7

FIGURA 16




La Tabla 8 muestra los resultados de la prueba de razón de momios y los intervalos de confianza 95. Las variables de concentración plasmática de Lp(a), unión de apo(a) a la fibrina y fenotipos de apo(a) en los tres grupos de estudio CI, EVC y control.

## TABLA 8

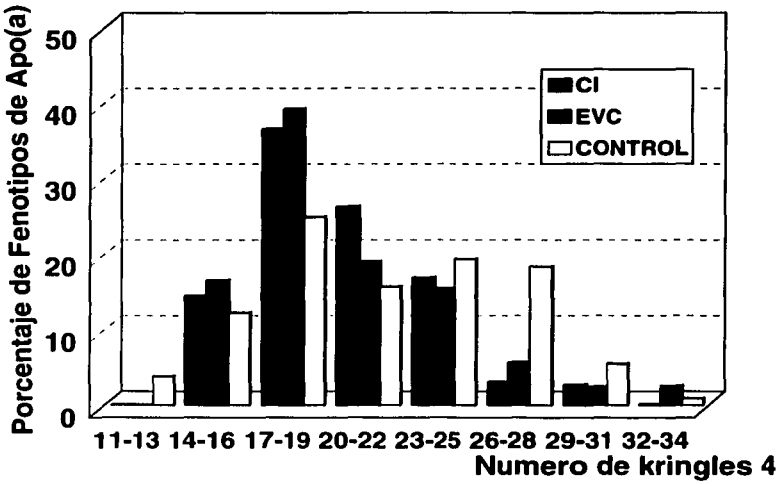
Razón de momios e intervalos de confianza 95% para las variables de concentración plasmática de Lp(a), Unión de apo(a) a la fibrina y Fenotipos de apo(a)

| | Cuarto Cuartil | Primer Cuartil | RM (95 % IC) |
|---|---|---|---|
| | | Lp(a) (mg/dL) | |
| CI (%) | 27.4 | 33.3 | 1.10 (0.43-2.78) |
| EVC (%) | 38.5 | 12.8 | 4 (1.13-14.09) |
| CONTROL (%) | 15.4 | 20.5 | |
| | | Unión de Apo(a) (nM) | |
| CI (%) | 21.0 | 34.3 | 1.39 (0.57-3.37) |
| EVC (%) | 55.8 | 1.9 | 65.9 (7.95-546.8) |
| CONTROL (%) | 12.1 | 27.5 | |
| | | Apo(a) Fenotipo 1 | |
| CI (%) | 15.6 | 20.8 | 0.66 (0.24-1.84) |
| CONTROL (%) | 26.2 | 23.1 | |
| EVC (%) | 31.6 | 28.9 | 0.96 (0.33-2.84) |
| | | Apo(a) Fenotipo 2 | |
| CI (%) | 26.1 | 28.3 | 0.64 (0.19-2.24) |
| EVC (%) | 27.6 | 24.1 | 0.80 (0.20-3.25) |
| CONTROL (%) | 29.4 | 20.6 | |

En el grupo de enfermos estudiados no se observa una asociación significativa entre la cardiopatía isquémica y la concentración de Lp(a) o de la unión de apo(a) a la fibrina. En contraste, el grupo de EVC muestra diferencias muy significativas tanto de la concentración de Lp(a) como de la unión de apo(a) a la fibrina. También se distingue una tendencia de asociación entre las enfermedades isquémicas con las isoformas de bajo peso molecular (aunque por el número de individuos, la observación no es significativa).

Empleando un método de análisis que relaciona la contribución de cada uno de los fenotipos a la concentración total de Lp(a), se aprecia que las isoformas,( Figura 17), menores a K22 son más frecuentes en la enfermedad isquémica coronaria y en la enfermedad vascular cerebral, que en los controles (76.86 %, 74.32 % y 56.3 %, respectivamente). El análisis nos permitió distinguir que los fenotipos K17-K19 son los más frecuentes tanto en la enfermedad isquémica como en la población control.

FIGURA 17



# DISCUSIÓN Y CONCLUSIONES

La afinidad de Lp(a) por la fibrina muestra una relación inversa con el tamaño de las isoformas de apo(a), que pueden tener constantes de disociación (Kd) entre 50 y 500 nmol/L [87]; y con la concentración de Lp(a).

La relación entre la estructura y la actividad antifibrinolítica de la Lp(a) ha permitido identificar que cada isoforma tiene una afinidad diferente por la fibrina, muestra una relación inversa con el tamaño y por lo tanto, concentraciones totales similares pueden exhibir diferente potencial anti-fibrinolítico y atero-trombótico. En acuerdo con estos hallazgos, recientemente se publicó [136] que la presencia de isoformas de bajo peso molecular es un indicador importante para la evolución de la aterosclerosis estenótica.

Hasta la fecha ha sido difícil establecer el riesgo asociado entre la Lp(a) y la enfermedad isquémica coronaria y la enfermedad cerebral arterial. El motivo principal ha sido la ausencia de un método funcional que refleje la actividad anti-fibrinolítica y atero-trombótica, consecuencia de la presencia de los diversos fenotipos y de la concentración de cada uno de ellos.

Aunado a este inconveniente, los diferentes estudios epidemiológicos se han hecho midiendo la concentración plasmática de Lp(a) en ausencia de un método y de una referencia internacional, lo que no ha permitido obtener resultados comparables y las diferencias que provienen del polimorfismo genético han sido difíciles de identificar. Dos estudios prospectivos han sido particularmente

importantes en los que no fue posible distinguir diferencias significativas entre la población isquémica coronaria y la población control.

Sin embargo, a pesar de estos antecedentes, un meta-análisis en donde se analizaron 27 estudios prospectivos, contrastando los resultados de Lp(a) plasmática en el tercio superior contra los del tercio inferior, concluyó que el riesgo relativo de padecer una enfermedad coronaria si se tiene una concentración elevada de Lp(a) es de 1.6 (IC 95% 1.4-1.8, $p<0.0001$)[137]

Esta nueva estrategia para estudiar a la Lp(a) nos ha permitido identificar que no en todos los individuos representa el mismo riesgo patogénico. La originalidad del método reside en su capacidad para señalar a los sujetos en los cuáles la presencia de una determinada isoforma de apo(a) puede disminuir su capacidad fibrinolítica. Muestra un perfil fibrinolítico que depende de la presencia de Lp(a).

Bajo las condiciones del método el mecanismo antagonista competitivo de la Lp(a) sólo puede modificarse por la presencia de análogos de la lisina [87]

Nuestro sistema experimental simula el comportamiento del plasminógeno y de la Lp(a), cuando, en condiciones fisiológicas, se enfrentan a la fibrina.

Este trabajo de tesis aporta evidencia de: a) que el efecto antifibrinolítico de la Lp(a) tiende a asociarse (en nuestro trabajo sin significado estadístico) a las isoformas de bajo peso molecular, b) que los enfermos con enfermedad vascular cerebral tienen una alta asociación con la unión de apo(a) a la fibrina, 65.9 (7.95-546.8).

Este inesperado resultado, concuerda con la hipótesis propuesta por Jürgens y col[138] de que la Lp(a) es el factor de riesgo más importante para la enfermedad cerebrovascular.

Aún más, nuestros resultados sugieren que existen mecanismos antitrombóticos diferentes entre la arteria coronaria y la cerebral en los enfermos estudiados.

Recientemente, se propuso un modelo que aporta evidencias de las diferencias pro- y anti-coagulantes, entre las células endoteliales de un lecho vascular a otro[139]. Confirma la existencia de diferencias regionales en el balance hemostático. La trombomodulina es una proteína que está más involucrada en mantener el balance hemostático en los vasos de los pulmones y del corazón que en los vasos del hígado. Mientras que los activadores del plasminógeno, tipo tisular y tipo urocinasa son importantes para mantener el balance hemostático en los tres órganos.

En el cerebro, no se conocen cuáles son los mecanismos fisiológicos que tienen un mayor peso para mantener el balance hemostático. Los resultados de este trabajo de tesis, que mostraron una asociación muy grande entre la unión de la Lp(a) a la fibrina y la enfermedad cerebrovascular, podrían ser un factor que permita orientar estudios futuros para esclarecer los mecanismos fisiopatológicos de la aterotrombosis en este lecho vascular.

# PERSPECTIVAS

La mayoría de los diferentes estudios epidemiológicos resaltan la asociación entre la concentración plasmática de la lipoproteína (a) y el riesgo de padecer enfermedad cardiovascular y cerebrovascular, sobre todo cuando se asocian otros factores de riesgo como son el colesterol de lipoproteínas de baja densidad y el tabaquismo. Pero es importante resaltar que existen diferencias en la concentración y en la presencia de isoformas en poblaciones genéticamente diferentes. Identificar el comportamiento de inhibición de la fibrinolisis por las diferentes isoformas de Lp(a) representa una estrategia de estudio que podrá permitir identificar a los individuos y a los grupos étnicos con incompetencia fibrinolítica por la presencia de Lp(a).

# REFERENCIAS


1 Berg K. A new serum type system in man-the Lp system. Acta Pathol Microbiol Scand 1963; 59: 369-382

2 Eaton DL, Fless GM, Kohr WJ, Mc Lean JW Xu QT, Miller CG, Lawn RM, Scanu AM. Partial amino acid sequence of apolipoprotein (a) shows that is homologous to plasminogen. Proc Natl Acad Sci (USA) 1987; 84: 3224-3228

3 Mc Lean JW, Tomlinson JE, Kuang WJ, Eaton OL, Chen EY, Fless GM, Scanu AM, Lawn RM. cDNA sequence oh human apolipoprotein(a) is homologous to plasminogen. Nature 1987, 330: 132-137

4 Berg K. Lp(a) Lipoprotein: an overview. Chem Phys Lipids 1992; 67/68: 9-18.

5 Dahlén GH. Lp(a) Lipoprotein in cardiovascular disease. Atherosclerosis 1994; 108: 111-126

6 Brown MS, Goldstein JL. Plasma lipoproteins: teaching old dogmas new tricks. Nature 1987; 330: 113-114

7 Anglés-Cano E, Hervio L, Rouy D.Effects of lipoprotein(a) on the binding of plasminogen to fibrin and its activation by fibrin-bound tissue-type plasminogen activator. Chem Phys Lipids 1994; 67/68: 369-380

8 MBewu AD, Durrington PN. Lipoprotein(a):structure, properties and possible involvement in thrombogenesis and atherogenesis. Atherosclerosis 1990; 85: 1-14

9 Miles LA, Fless GM, Levin EG, Scanu AM, Plow EF. A potential basis for the thrombotic risks associated with lipoprotein (a). Nature 1989; 339; 301-303

10 Nachman RL. Thrombosis and atherogenesis:molecular conections. Blood 1992;79: 1897-1906

11 Guo HC, Michel JB, Blouquit Y, Chapman MJ. Lipoprotein (a) and apolipoprotein (a) in a new world monkey, the common Marmoset (Callithrix jacchus). Arterioscler. Thromb. 1991; 11: 1030-1041

12 Laplaud M, Beaubatie L, Rall SC, Luc G, Saboureau M. Lipoprotein (a) is the major apoB-containing lipoprotein in the plasma of a hibernator, the hedgehog (Erinaceus europaeus). J Lipid Res. 1988; 29: 1157-1170

13 Kostner GM. The physiological role of Lp(a). In Lipoprotein(a). Ed Scanu AM. Academic Press, Inc 1990, pag 183-203

14 Cardoso GC, Posadas C, Orvañanos OO, Peniche C, Zamora J, Aguilar R, Olguin JA, Raynaud S, Morriset JD, Guevara J Jr. Long distance runners and body-builders exhibit elevated plasma levels of lipoprotein (a). Chem Phys Lipids 1994; 67-68: 207-221

15 Lawn RM, Tomlinson JE, McLean JW, Eaton DL. Molecular biology of Lipoprotein(a) . In Lipoprotein(a). Ed Scanu AM. Academic Press, Inc 1990, pag 25-40

16 Kraft HG, Menzel HJ, Hoopichler F, Vogel W, Uterman G. Changes of genetic apolipoprotein phenotypes caused by liver transplantation. J Clin Invest 1989; 83: 137-142

17 Kostner GM, Wo X, Frank S, Kostner K, Zimmermann R, Steyrer E. Metabolism of Lp(a): assembly and excretion. Clin Genet 1997; 52: 347-354

18 Anglés-Cano E, Hervio L, Loyau S. Lipoprotéine, hypofibrinolyse et athérothrombose, quo vadis?. Sang Thrombose Vaisseaux, 1995; 7: 315

19 Aznar J, Estelles A. Lipoproteína (a) y trombosis. Revista Iberoamericana de Trombosis y Hemostasia. 1993; 2: 123-125

20 Utermann G. The mysteries of lipoprotein (a). Science, 1989:246: 904-910

21 Utermann G, Menzel HJ, Kraft G, Duba HC, Kemmler HG, Seitz C. Lp(a) glycoprotein phenotypes: inheritance and relation to Lp(a)-lipoprotein concentations in plasma. J Clin Invest. 1987; 80: 458-465

22 Brunner C, Lobentanz EM, Petho-Schramm A, Ernst A, Kang C Dieplinger H, JoMuller HJ, Utermann G. The number of identical kringle IV repeats in apolipoprotein(a) affects its processing and secretion by HepG2 cells. J Biol Chem 1996; 271: 32403-32410

23 Anglés-Cano E, Loyau S, Cardoso-Saldaña G, Courdec R, Gillery P. A novel kringle 4 number-based recombinant apo(a) standar for human apo(a) phenotyping. J Lipid Res 1999; 40 : 354-359

24 Lackner C, Boerwinkle E, Leffert C, Rahming T, Hobbs H. Molecular basis of apolipoprotein (a) isoform size heterogeneity as revealed by pulsed-field gel electrophoresis. J Clin Invest. 1991; 87: 2153-2161

25 Gaw A, Boerwinkle E, Cohen JC, Hobbs HH. Comparative analysis of the apo(a) gene, apo(a) glycoprotein, and plasma concentrations of Lp(a) in three ethnic groups. Evidence for non common "null" allele at the apo(a) locus. J Clin Invest 1994; 93: 2526-2534

26 De Bruin TWA, van Barlingen H, van Linde-Sibenius, Trip M, van Vuurst de Vries AR, Akveld MJ, Erkelens WD. Lipoprotein (a) and apolipoprotein B plasma concentrations in hypothyroid, euthyroid and hyperthiroid subjects. J Clin Endocrinol Metab 1993; 76: 121-126

27 Haenggi W, Reisen W, Birkhaeuser MH. Postmenopausal hormone replacement therapy with tibolone decreases serum lipoprotein(a). Eur J Clin Biochem 1993; 31: 645-650

28 Henriksson P, Angelin B, Berglund D. Hormonal regulation of serum Lp(a) levels. J Clin Invest 1992; 89: 1166-1171

29 Soma MR, Meschia M, Bruschi F, Morriset JD, Paoletti R , Fumagalli R, Cosignani P. Hormonal agents used in lowering lipoprotein (a). Chem. Phys Lipids 1994; 67: 345-350

30 Taskinen MR, Puolakka J, Pyorala T, Luotola H, Bjaorn M, Kaarianen J, Lahdenpera S, Ehnholm C. Hormone replacement therapy lowers plasma Lp(a) concentrations. Comparison of cyclic transdermal and continuos estrogen-progestin regimens. Arterioscler Thromb Vasc Biol 1996; 16: 1215-1221

31 Albers JJ, Taggart HM, Applebaum-Bowden D., Haffner S, Chesnut CH and Hazzard WR. Reduction on lecithin-colesterol acyltransferase, apolipoprotein D and the Lp(a) lipoprotein with the anabolic steroid stanazol. Biochim Biophys Acta 1984; 795: 293

32 Eden F, Wiklund O, Oscarsson J, Rosen T, Bengtsson BA. Growth hormone treatment of growth hormone-deficient adults result in a marked increase in Lp(a) and HDL cholesterol concentrations. Artherioscler Thromb 1993; 13: 296-301

33 Joseph Loscalzo. Lp(a) and the fibrinolytic system. In: Lipoprotein (a). Scanu AM Ed 1990. Academic Press, Inc.p 103-112

34 Noma A, Abe A, Maeda S, Seishima M, Makino K, Yano Y, Shimokawa K. Lp(a): an acute phase reactant?. Chem Phys Lipids 1994; 67/68: 411-417

35 Dahlen GH. Indications of an autoimmune component in Lp(a) associated disorders. Eur J Immunogenet 1994; 21: 301-312

36 Andreassen AK, Berg K, Torsvik H. Changes in Lp(a) lipoprotein and other plasma proteins during acute myocardial infarction. Clin Genet 1994; 46: 410-416

37 Klausen IC, Gerdes UL, Schmidt BE, Dyerberg J, Faergeman O. Differences in apolipoprotein (a) polymorphism in West Greenland Eskimos and Caucasians Danes. Human Genet 1992; 89: 384-388

38 Marcovina S, Albers JJ, Jacobs DR, Perkins LL, Lewis CE, Howard B, Savage P. Lipoprotein (a) concentrations and apolipoprotein (a) phenotypes in caucasians and african americans. The CARDIA study. Arterioscler. Thromb. 1993; 13: 1037-1045

39 Abe A, Noma A, Itakura H. Lipoprotein (a) phenotyping using a computarized micro scale and phenotype frequencies in a healthy Japanese population. Clin Chim Acta 1993; 219: 149-157

40 Kraft HG, Windegger M, Menzel HJ, Uterman G. Significant impact of the +93 C/T polymorphism in the apolipoprotein (a) gene on Lp(a) concentrations in africans but not in caucasians: confounding effect of linkage. Human Mol Genet 1998, 7: 257-264

41 Cobbaert C, Mulder P, Lindemans J, Kesteloot H. Serum LP(a) levels in african aboriginal pygmies and bantus, compared with caucasian and asian population samples. J Clin Epidemiol 1997; 50: 1045-1053

42 Mooser V, Scheer D, Marcovina SM, Wang J, Guerra R, Cohen J, Hobbs HH. The apo(a) gene is the major determinant of variation in plasma Lp(a) levels in african americans. Am J Hum Genet 1997; 61: 402-417

43 Marcovina SM, Albers JJ, Wijsman E, Zhang Z, Chapman NH, Kennedy H. Differences in Lp(a) concentrations and apo(a) polymorphs between black and white american. J Lipid Res 1996; 37: 2569-2585

44 Schreiner PJ, Heiss G, Tyroler HA, Morriset JD, Davis CE, Smith R. Race and gender differences in the association of Lp(a) with carotid artery wall thickness. The artheriosclerosis risk in communities (ARIC) study. Arterioscler Thromb Vasc Biol. 1996; 16: 471-478

45 Guyton B Jr, Dahlen GH, Patsch W, Kautz JA, Gotto AM Jr Relationship of plasma lipoprotein Lp(a) levels to race and to apolipoprotein. Arteriosclerosis 1985;5:265-72

46 Csaszar A, Romics L, Lackner C, Dieplinger H, Karadi I, Utermann G. Plasma concentration of lipoprotein(a) and distribution of its subtypes in the healthy population of Hungary.OrvHetil 1990;131:2071-5

47 Cuchel M, Merati G, Clerici M, Grumelli B, Giudici GA, Vergani C. Determination of Lp(a) and APO(a) in an Italian population. Ric Clin Lab 1991;21:127-34

48 Helmhold M, Bigge J, Muche R, Mainoo J, Thiery J, Seidel D, Armstrong VW. Contribution of the apo[a] phenotype to plasma Lp[a] concentrations shows considerable ethnic variation. J Lipid Res 1991;32:1919-28

49 Cobbaert C, Kesteloot H Serum lipoprotein(a) levels in racially different populations. Am J Epidemiol 1992;136:441-9

50 Sandholzer C, Saha N, Kark JD, Rees A, Jaross W, Dieplinger H, Hoppichler F, Boerwinkle E, Utermann G. Apo(a) isoforms predict risk for coronary heart disease. A study in six populations. Arterioscler Thromb 1992;12:1214-26

51 Kikuchi S, Nakagawa A, Kobayashi K, Li L, Yanagi H, Arinami T, Kozu Y, Miyazaki R, Tsuchiya S, Hamaguchi H. High degree of genetic polymorphism in apolipoprotein(a) associated with plasma lipoprotein(a) levels in Japanese and Chinese populations. Hum Genet 1993;92:537-44

52 Arenas Jimenez JJ, Quilez Ivorra C, Sastre Pascual JF, Arenas Adarve M, Sevadilla Soler L, Roca Villanueva B, Segui Molto R. Levels of lipoprotein(a), other lipids and lipoproteins in adolescents from the health area of Alcoy. An Med Interna 1993;10:9-15

53 Kamboh MI, Svitko CM, Williams ER, Ferrell RE, Pollitzer WS. Hypervariable polymorphism of APO(a) in blacks and whites as reflected by phenotyping. Chem Phys Lipids 1994;67-68:283-92

54 Koukkou E, Watts GF, Mazurkiewicz J, Lowy C. Ethnic differences in lipid and lipoprotein metabolism in pregnant women of African and Caucasian origin. J Clin Pathol 1994;47:1105-7

55 Rainwater DL. Genetic basis for multimodal relationship between apolipoprotein (a) size and lipoprotein (a) concentration in Mexican-Americans. Atherosclerosis 1995;115:165-71

56 Ichinose A, Kuriyama M. Detection of polymorphisms in the 5'-flanking region of the gene for apolipoprotein(a). Biochem Biophys Res Commun 1995;209:372-8

57 Klausen IC, Hansen PS, Povlsen JV, Gerdes LU, Lemming L, Faergeman O.A unique pattern of apo(a) polymorphism in an isolated east Greenlandic Inuit (Eskimo) population. Eur J Epidemiol 1995;11:563-8

58 Low PS, Heng CK, Saha N, Tay JS. Racial variation of cord plasma lipoprotein(a) levels in relation to coronary risk level: a study in three ethnic groups in Singapore.Pediatr Res 1996;40:718-22

59 Gomez Gerique JA, Porres A, Lopez Martinez D, Alvarez Sala LA, Blazquez E, Montoya MT, DeOya M. Levels of lipoprotein(a) and plasma lipids in Spanish children aged from 4 to 18 years.Acta Paediatr 1996;85:38-42

60 Dionyssiou-Asteriou A, Rizos I.Serum lipoprotein(a) levels in a Greek population sample without a history of premature myocardial infarction. J Cardiovasc Risk 1996 Jun;3:277-80

61 Cobbaert C, Mulder P, Lindemans J, Kesteloot H. Serum LP(a) levels in African aboriginal Pygmies and Bantus, compared with Caucasian and Asian population samples. J Clin Epidemiol 1997;50:1045-53

62 Rainwater DL, Kammerer CM, VandeBerg JL, Hixson JE. Characterization of the genetic elements controlling lipoprotein(a) concentrations in Mexican Americans. Evidence for at least three controlling elements linked to LPA, the locus encoding apolipoprotein(a). Atherosclerosis 1997;128:223-33

63 Carstens ME, Burgess LJ, Taljaard JJ.Lp (a) levels and apo (a) phenotypes in urban black South African men.S Afr Med J 1998;88:139-42

64 Noto D, Barbagallo CM, Cavera G, Caldarella R, Marino G, Pace A, Notarbartolo A, Averna MR. Lipoprotein(a) levels and apoprotein(a) phenotypes in a Sicilian population. Ann Ital Med Int 1998;13:205-8

65 Aasvee K, Jauhiainen M, Kurvinen E, Jordania R, Sundvall J, Ehnholm C. Lipoprotein(a), apolipoprotein A-I and B serum levels in young families from Tallinn, Estonia. Relationships with other cardiovascular risk factors and nationality. Scand J Clin Lab Invest 1999 May;59(3):179-89

66 Chien KL, Lee YT, Sung FC, Su TC, Hsu HC, Lin RS. Lipoprotein (a) level in the population in Taiwan: relationship to sociodemographic and atherosclerotic risk factors. Atherosclerosis 1999 Apr;143(2):267-73

67 Gaubatz JW, Heideman C, Gotto AM, Morrisett JD, Dahlen GH. Human plasma lipoprotein(a)-structural propieties. J Biol Chem 1983; 258: 4582-4589

68 Guevara J, Spurlino J, Jan AY, Yang CY, Tulinsky A, Prasad B VV, Gaubatz JW, and Morriset JD. Proposed mechanisms for binding of apo(a) kringle type 9 to apo B-100 in human lipoprotein(a). Biophys J 1993; 64: 686-700

69 Fless GM, Snyder ML,Furbee JWJ, Garcia-Hedo MT, Mora R. Subunit composition of lipoprotein (a) protein. Biochemistry 1994; 33: 13492-13501

70 Sommer A, Georges R, Kostner GM, Paltauf F, Hermetter A. Sulfhydryl-selective fluorescense labeling of lipoprotein (a) reveals evidence for one single disulfide linkage between apoprotein(a) and B-100. Biochemistry 1991; 30 : 11245-11249

71 Huby T, Doucet C, Dieplinger H , Chapman J, Thillet J. Structural domains of apolipoprotein(a) and its interaction with apolipoprotein B-100 in the lipoprotein(a) particle. Biochemistry 1994; 33: 3335-3341

72 Albers JJ, Kennedy H, Marcovina SM. Evidence that Lp(a) contains one molecule of apo(a) and one molecule of apo B: evaluation of aminoacid analysis data. J Lipid Res. 1996: 37: 192-196

73 Kamboch ML, Ferrel RE, Kottke BA. Expressed hypervariable polymorphism of apolipoprotein (a). Am J Hum Gent. 1991; 49: 1063-1074

74 Guevara J, Knapp RD, Honda S, Northup SR, Morriset JD. A structural assessment of the apo(a) protein of human lipoprotein(a). Proteins 1992; 12: 188-199

75 Fless GM, ZumMallen ME, Scanu M. Physicochemical properties of apolipoprotein (a) and Lp(a-) derived from the dissociation of human plasma Lp(a). J Biol Chem 1986; 261: 8712-8718

76 Suzuki K, Kuriyama M, Saito T, Ichinose A. Plasma lipoprotein(a) and expression of the apolipoprotein(a) gene are dependent on the nucleotide polymorphisms in its 5'-flanking region. J Clin Invest, 1997; 97: 1361-1366

77 Tulinsky A, Park CH, Mao B, Llinás M. Lysine/fibrin binding sites of kringles modeled after the structure of kringle 1 of prothrombin. Proteins1988; 3: 85-96

78 Boonmark NW, Lawn RM. The lysine-binding function of Lp(a). Clin Genet 1997; 52:355-360

79 Menhart N, Sehl LC, Kelley RF,Castellino FJ. Construction, expression and purification of recombinant kringle 1 of human plasminogen and analysis of its interactions with w-amino acids. Biochemistry 1991; 30: 1948-1957

80 Rejante MR, Byeon IJL, Llinas M. Ligand specificity of human plasminogen kringle 4. Biochemistry 1991; 30: 11081-11092

81 Guevara J, Knapp RD, Tulinsky A, Morriset JD. Comparison of ligand binding sites of modeled apo(a) kringle like sequences in human lipoprotein (a) Arterioscle. Thromb. 1993; 13: 758-770

82 Sangrar W, Marcovina SM, and Koschinsky ML. Expression characterization of apolipoprotein(a) kringle IV types 1,2 and 10 in mammalian cells. Protein Engineering 1994; 7: 723-731

83 Binder BR. Physiology and Pathophysiology of the Fibrinolytic System. Fibrinolysis 1995; 9: 3-8

84 Anglés-Cano E. Overview on fibrinolysis: plasminogen activation pathways on fibrin and cell surfaces. Chem Phys Lipids 1994; 67/68: 353

85 Hajjar KA, Gavish D, Breslow JL, Nachman RL. Lipoprotein (a) modulation of endothelial cell surface fibrinolysis and its potential role in atherosclerosis. Nature 1989; 339: 303-305

86 Liu JN, Kung W, Harpel PC, Gurewich V. Demostration of covalent binding of lipoprotein (a). [Lp(a)] to fibrin and endothelial cells. Biochemistry 1998; 37: 3949-3954

87 Hervio L, Durlach V, Girard-Globa A, Anglés-Cano E. Multiple binding with identical linkage: a mechanism that explains he effect of lipoprotein (a) on fibrinolysis. Biochemistryy 1995; 34: 13353

88 Hervio, J. Chapman, J Thillet, S. Loyau and E. Anglés-Cano. Does apolipoprotein (a) heterogeneity influence lipoprotein (a) effects on fibrinolysis?. Blood 1993; 82: 392-397

89 Kang C, Durlach V, Soulat T, Fournier C, Anglés-Cano E. Lipoprotein(a) isoforms display differences in affinity for plasminogen-like binding to human mononuclear cells. Arterioscler Thromb Vasc Biol 1997; 17: 2036-2043

90 Miles LA, Fless GM, Scanu AM, Baynham P, Sebald MT, Skocir P, Curtiss LK, Levin EG, Hoover-Plow JL, Plow EF. Interaction of Lp(a) with plasminogen binding sites on cells. Thomb Haemost 1995; 73: 458-465

91 Gonzalez-Goronow M, Edelberg JM and Pizzo SV. Further characterization of the cellular plasminogen binding site: evidence that plasminogen 2 and lipoprotein (a) compete for the same site. Biochemistry 1989; 28: 2374-2377

92 Ezralty A, Simon DI and Loscalzo J. Lipoprotein(a) binds to human platelets and attenuates plasminogen binding and activation. Biochemistry 1993; 32: 4628-4633

93 Miles LA, MT Sebald, GM Fless, AM Scanu, LK Curtiss, EF Plow, JL Hoover-Plow. Interaction of lipoprotein (a) with the extracellular matrix. Fibrinolysis & Proteolysis. 1998; 12: 79-87

94 Li XN, Grenett HE, Benza RL, Demissie S, Brown SL, Tabengwa EM, Gianturco SH, Bradley WA, Fless GM, Booyse FM. Genotype-specific transcriptional regulation of PAI-1 expression by hypertriglyceridemic VLDL and Lp(a) in cultures human endothelial cells. Arterioscler Thromb Vasc Biol 1997; 17: 3215-3223

95 Ross R. The pathogenesis of ahterosclerosis a perspective fo the 1990s. Nature 1993; 362: 801-809

96 Kovanen PT. Atheroma formation: defective control in the intimal round-trip of cholesterol. Eur Heart Journal 1990; 11: 238-246

97 Collen D, Juhan-Vague I. Fibrinolysis and Atherosclerosis. Sem. Thromb. Haemost 1988; 14: 180-183

98 Stary HC, Blankenhorm DH, Chandler AB, Glagov S, Insull W, Richardson M, Rosenfeld ME, Schaffer SA, Schwartz CJ, Wagner WD, Wissler RW. A definition of the intima of human arteries and of its atherosclerosis-prone regions. Circulation 1992; 85:391-405

99 Harpel P, Hermann A, Zhang X, Ostfeld I, Borth W. Lipoprotein(a), Plasmin modulation, and Atherogenesis. Thrombosis and Haemostasis 1995; 74: 382-386

100 Allen S, Khan S, Tam S, Koschinsky M, Taylor P, Yacoub M. Expression of adhesion molecules by Lp(a): a potential novel mechanism for its atherogenicity. FASEB J 1998; 12: 1765-1776

101 Scanu AM. Atherothrombogenicity of lipoprotein(a): the debate. Am J Cardiol 1998; 82: 26Q-33Q

102 Anglés-Cano E, Hervio L, Loyau S. Relevance of lipoprotein (a) in cardiovascular disease: methodological approaches. Fibrinolysis 1993; 7:66

103 Fleury V, Anglés-Cano E. Characterization of the binding of plasminogen to fibrin surfaces: the role of carboxy-terminal lysines. Biochemistry 1991: 30: 7630-7638

104 Fleury V, Loyau S, Lijnen HR, Nieuwenhuizen W, Anglés-Cano E. Molecular assembly of plasminogen and tissue-type plasminogen activator on an evolving fibrin surface. Eur J Biochem 1993; 216: 549-556

105 Rouy D, Laplaud PM, Saboreau M, Anglés-Cano E. Hedgehog lipoprotein(a) is a modulator of activation of plasminogen at the fibrin surface. An in vitro study. Arterioscler Thromb 1992; 12: 146-154

106 Anglés-Cano E. A spectrophotometric solid-phase fibrin-tissue plasminogen activator activity assay (SOFIA-tPA) for high-fibrin-affinity tissue plasminogen activators. Anal Biochem 1986; 43: 129-132

107 Rouy D, Grailhe P, Nigon F, Chapman J, Anglés-Cano E. Lipoprotein(a) impairs generation of plasmin by fibrin-bound tissue-type plasminogen activator. In vitro studies in a plasma milieu. Arterioscler Thromb. 1991; 11:629-638

108 Hervio L, Girard Globa A. Durlach V, Anglés-Cano E. The antifibrinolytic effect of lipoprotein(a) in heterozygous subjects is modulated by the relative concentration of each of the apolipoprotein (a) isoforms and their affinity for fibrin. Eur J Clin Invest. 1996; 26: 411-417

109 Selhub J and D'Angelo A. Hyperhomocysteinemia and thrombosis: acquired conditions. Thromb and Haemost. 1997: 78: 527-531

110 Harpel PC. Homocysteine, atherogenesis and thrombosis. Fibrinolysis and Propteolysis 1997; 11:77-80

111 Scanu AM and Edelstein C. Kringle-dependent structural and functional polymorphism of apolipoprotein (a). Biochim. Biophys. Acta 1995; 1256: 1-12

112 Caplice NM, Panetta C, Peterson TE, Kleppe LS, Mueske CS, Kostner GM, et al. Lipoprotein (a) binds and inactivates tissue factor pathway inhibitos: a novel link between lipoproteins and thrombosis. Blood 2001;98 2980-87

113 Peynet J, Beaudeux JL, Wolmant F, Flourie F, Giraudeaux V, Vicaut E, Launay JM. Apolipoprotein (a) size polymorphism in young adults with ischemic stroke. Atherosclerosis 1999; 142: 233-239

114 Armstrong VW, Cremer P, Eberle E, Manke A, Schulze F, Wieland H, Kreuzer H, Seidel D. The association between Lp(a) concentrations and angiographically assessed coronary atherosclerosis. Atherosclerosis 1986; 62: 249-257

115 Dahlen GH, Guyton JR, Attar M, Farmer JA, Kautz JA Gotto AM. Association of levels of lipoprotein Lp(a), plasma lipids, and other liporpoteins with coronary artery disease documented by angiography. Circulation 1986;74:758-765

116 Rosengren A, Wilhelmsen L, Eriksson E, Risberg B, Wedel H . Lipoprotein (a) and coronary heart disease: a prospective case-control study in a general population sample of middle aged men. Br Med J 1990; 301: 1248

117 Cremer P, Nagel D, Labrot B. LDL cholesterol and other risk factors: results from the prospective Göttingen Risk Incidence and Prevalence Study (GRIPS). Eur. J Clin Invest 1994; 24: 444

118 Wald NJ, Law M, Watt HC, y col. Apolipoproteins and ischaemic heart disease: implications for screening. Lancet 1994: 343: 75

119 Schaefer EJ, Lamon-Fava S, Jenner JL, y col. Lipoprotein (a) levels and risk of coronary heart disease in men. The lipid research clinics coronary primary prevention trial. JAMA 1994; 271: 999-1003

120 Bostom A, Gagnon D, Cupples A, Wilson P. Jenner J, Ordovas J, Schaefer E, Castelli W. A prospective investigation of elevated lipoprotein (a) detected by electrophoresis and cardiovascular disease in women. The Framingham Heart Study. Circulation 1994;90: 1688-1699

121 Dahlen GH, Stenlund H. Lp(a) lipoprotein is a major risk factor for cardiovascular disease: pathogenic mechanisms ans clinical significance. Clin Genet 1997; 52: 272- 280

122 Bostom A, Gagnon D, Cupples A, Wilson P. Jenner J, Ordovas J, Schaefer E, Castelli W. A prospective investigation of elevated lipoprotein (a) detected by electrophoresis and cardiovascular disease in women. The Framingham Heart Study. Circulation 1994;90: 1688-1699

123 Jauhiainen M, Koskinen P, Ehnholm, Frick MH, Manttari M, Manninen V, Huttunen JK. Lipoprotein (a) and coronary heart disease risk. A nested case-control study of the Helsinki Heart Study participants. Atherosclerosis 1991; 89: 59-67

124 Ridker PM, Hennekens CH, Stampfer MJ. A prospective study of lipoprotein (a) and the risk of myocardial infarction. JAMA 1993; 270: 2195-2199

125 Rouy D, Koschinsky ML, Fleury V, Chapman MJ, Anglés-Cano E. Apolipoprotein(a) and plasminogen interactions with fibrin: a study with recombinant apolipoprotein(a) and isolated plasminogen fragments. Biochemistry 1992; 31: 6333-6339

126 Craig W, Neveux M, Palomaki G, Cleveland M, Haddow J. Lipoprotein(a) as a risk factor for ischemic heart disease: metaanalysis of prospective studies. Clin Chem 1998; 44: 2301-2306

127 Alfthan G, Pekkanen J, Jauhiainen M, Pitkaniemi J, Karvonen M, Tuomilehto J y col. Relation of serum homocysteine and lipoprotein (a) concentrations to atherosclerotic disease in a prospective Finnish population based study. Atherosclerosis 1994, 106: 9-19.

128 Assmann G, Schutle H, von Eckardstein A. Hypertriglyceridemia and elevated Lp(a) are risk factors for major coronary events in middle-aged men. Am J Cardiol 1996; 77: 1179-1184.

129 Coleman MP, Key TJA, Wang DY, Hermon C, Fentiman IS, Allen DS y col. A prospective study of obesity, lipids, apolipoproteins and ischaemic heart disease in women. Atherosclerosis 1997; 132:77-84.

130 Jauhiainen M, Koskinen P, Ehnholm C, Frick MH, Mänttäri M, Manninen V y col. Lipoprotein (a) and coronary heart disease risk: a nested case-control study of the Helsinki Heart Study participants. Atherosclerosis 1991; 89: 59-67.

131 Klausen IC, SjØl A, Hansen PS, Gerdes LU, Møller L, Lemming L y col. Apolipoprotein (a) isoforms and coronary heart disease in men. A nested case-control study. Atherosclerosis 1997; 132: 77-84.

132 Sigurdsson G, Baldursdottir A, Sigvaldason H, Agnarsson U, Throgeirsson G, Sigfusson N. Predective value of apolipoproteins in a prospective survey of coronary artery disease in men. Am J Cardiol 1992; 69: 1251-1254.

133 Wild SH, Fortmann SP, Marcovina SM. A prospective case-control study of lipoprotein (a) levels and apo(a) size and risk of coronary heart disease in Stanford Five-City Project participants. Aterioscler Thromb Vasc Biol 1997; 17: 239-245.

134 Koppert PW, Huisjmans CMG, Nieuwenhuizen W. A monoclonal antibody specific for human fibrinogen, fibrinopeptide A-containing fragments and not reacting with free fibrinopeptide A. Blood 1985; 66: 503-507

135 Sorell L, Rojas G, Ramos C, Torres L, Torres MB. A sándwich ELISA based on anti-apo(a) and anti-apoB monoclonal antibodies for lipoprotein(a) measurement. Clin Chim Acta 1995; 236: 59-70.

136 Kronenberg F, Kronenberg MF,Kiechl S, Trenkwalder E, Santer P, Oberhollenzer F et al. Role of lipoprotein (a) and apolipoprotein (a) phenotype in atherogenesis: prospective results from the Bruneck study. Circulation 1999; 100: 1154-60.

137 Danesh J, Collins R, Peto R. Lipoprotein (a) and coronary heart disease. Meta-analysis of prospective studies. Circulation 2000; 102: 1082-85.

138 Jürgens G, Taddei-Peters W, Költringer P, Petek W, Chen Q, Greilberger J, y col. Lipoprotein (a) serum concentration and apolipoprotein (a) phenotype correlate with severity and presence of ischemic cerebrovascular disease. Stroke 1995; 26: 1841-48

139 Rosenberg RD, Aird WC. Vascular-bed-specific hemostasis and hypercoagulable states. N Engl J Med 1999;340:1555

# European Journal of Clinical Investigation

Editor-in-Chief W. H. Hörl
Editorial Secretary T. Szkibik

Associate Editors M. Roden
M. D. Saemann
P. Valent

E. Anglés-Cano
INSERM 13, U460
CHU Bichat-Claude Bernard
46, rue Henri Huchard
F-75877 Cdx Paris 18, France

Date: 7.10.2002

manuscript number: 02-599

authors: De la Pena A et al.

titel: Functional approach to investigate Lp(a) in ischemic heart and cerebral diseases

Dear Colleague,

Thank you for your recent letter, enclosing your revised manuscript, and detailed response to the referee's comments. I am pleased to inform you that your paper is accepted for publication without further changes.

Your manuscript will be forwarded to the publishers in due course and you will next hear from them with page-proofs for correction.

I look forward to publishing your manuscript and hope that you will continue to support the Journal with your work in the future.

With best wishes,

W. H. Hörl, MD, PhD, FRCP
Professor of Medicine
Editor-in-Chief

Editorial Office EJCI
W. H. Hörl, Editor-in-Chief
Division of Nephrology, Department of Medicine III
Wahringer Gürtel 18-20
A-1090 Vienna, Austria

Telephone: +43-1-40400-4390
Telefax: +43-1-40400-4392
E-mail: walter.hoerl@nephro.imed3.akh-wien.ac.at

Published for the European Society for Clinical Investigation by Blackwell Science Ltd

# FUNCTIONAL APPROACH TO INVESTIGATE Lp(a) IN ISCHAEMIC HEART AND CEREBRAL DISEASES

De la Peña-Díaz A [1], Cardoso-Saldaña G [1], Zamora-González J[1], Barinagarrementeria F[2], Loyau S[3], Izaguirre R[1], Anglés-Cano E[3].

1 Instituto Nacional de Cardiología "Ignacio Chávez", 2 Instituto Nacional de Neurología, México, D.F. 3 INSERM U460, Paris, France.

Correspondence and reprints request: E. Anglés-Cano.

INSERM 13, U460

46, rue Henri Huchard

F-75877-Cdx Paris 18, France.

Phone: 33-1 40 25 86 11 Fax: 33-1 40 25 86 10 E-mail: angles@infobiogen.fr

**Running head:** Assay to detect Lp(a) fibrin binding capacity

**Acknowledgments.** We are most grateful to Dr. Luis Sorell (Center for Genetic Engineering and Biotechnology (Havane, Cuba) for providing the peroxidase-labeled monoclonal antibody directed against apo(a), and to Dr G. Baños de Mac Carthy for editorial assistance. This work was supported in part by an INSERM-CONACYT (France-Mexico) research exchange programme.

Word-character count: 2,533

**Abstract**

Background. Lp(a), a major cardiovascular risk factor, contains a specific apolipoprotein, apo(a), which by virtue of structural homology with plasminogen inhibits the formation of plasmin, the fibrinolytic enzyme. A number of clinical reports support the role of Lp(a) as a cardiovascular or cerebral risk factor, and experimental data suggest that it may contribute to atherothrombosis by inhibiting fibrinolysis.

Design. A well characterized model of a fibrin surface and an apo(a) specific monoclonal antibody were used to develop a functional approach to detect pathogenic Lp(a). The assay is based on the competitive binding of Lp(a) and plasminogen for fibrin, and quantifies fibrin-bound Lp(a). High Lp(a) binding to fibrin is correlated with decreased plasmin formation. In a transversal case-control study we studied 248 individuals, 105 had a history of ischemic cardiopathy (IC), 52 had cerebro-vascular disease (CVD) of thrombotic origin, and 91 were controls.

Results. The remarkably high apo(a) fibrin-binding in CVD (0.268±0.15 nmol/L) as compared to IC (0.155±0.12 nmol/L) suggests the existence of peculiar and poorly understood differences in pro- or anti-thrombotic mechanisms in either cerebral and/or coronary arteries.

Conclusions Our results demonstrated that Lp(a) fibrin-binding and small Apo(a) isoforms are associated with athero-thrombotic disease.

**Key words:** fibrinolysis, lipoprotein (a), plasminogen, ischemic cardiopathy, cerebrovascular disease.

**Introduction**

Atherothrombosis is a leading cause of serious morbidity and death due to myocardial infarction and cerebrovascular accidents. Within this context, the Lp(a) has emerged as a major risk factor and a clear association between high plasma Lp(a) and ischemic cardiopathy (IC) or cerebrovascular disease (CVD) has been demonstrated in several case-control and prospective studies [1]. Lp(a) plasma concentration depends on its hepatic synthesis [2, 3] and varies from one individual to another within an approximate range of <10 mg/dL to > 100 mg/dL; it is independent of factors such as diet, cholesterol, obesity, and smoking, and is maintained within small variations during the life span [4]. Lp(a) is an LDL-like particle containing apo(a), a distinctive plasminogen-like glycoprotein. Apo(a) consists of a serine-proteinase region and several kringle domains, derived from those of plasminogen but presenting some distinctive features [5]. Only plasminogen's kringles 5 (one copy) and 4 (multiple copies) are represented in apo(a). The copies of apo(a) kringle 4 are not alike, ten different types have been recognized [6]. Each type is present only once, except for kringle 4 type 2, which appears in multiple copies originating a structural heterogeneity and different size isoforms (molecular weight between 280 and 800 kDa) that are in inverse correlation with Lp(a) plasma concentration [7]. The serine-proteinase region of apo(a) contains the same residues that constitute the potential active site in plasminogen, however a Ser-Ile substitution at the Arg-Val activation cleavage site renders apo(a) insensitive to plaminogen [5,8].

On the basis of these homologies and activation differences, several studies have pointed out that Lp(a) has an antifibrinolytic capacity. The mechanism by which Lp(a) inhibits fibrinolysis remains controversial, *in vitro* studies [9, 10] have demonstrated that Lp(a) competes with plasminogen for similar fibrin binding sites. The formation of a ternary complex between plasminogen, tissue-type plasminogen activator (t-PA) and fibrin is required for efficient plasminogen activation [11], this activation mechanism is reduced when apo(a)/Lp(a) is bound to fibrin [12,13].

It has also been shown that solution phase interactions between apo(a) and plasminogen inhibit the binding of plasminogen to plasmin-modified fibrin surfaces [14].

However, a mechanism that explains the direct role of Lp(a) in promoting intravascular thrombosis has not been identified as yet. Recently [15], it has been described that Lp(a) binds to tissue factor pathway inhibitor (TFPI), with consequent TFPI inhibition. TFPI main role is to modulate factor VIIa/tissue factor catalytic activity and it is now recognized as a physiological anticoagulant. TFPI is present in the vascular smooth muscle cells [15] and the Lp(a)-TFPI interaction suggests a procoagulant mechanism in the atherosclerotic plaque.

These features suggest that Lp(a) has both, antifibrinolytic and prothrombotic capacities.

In spite of these molecular interaction data, and epidemiological evidence, the general approach for the study of Lp(a) as a vascular risk factor has until now, been limited to the determination of Lp(a) plasma concentration. A normal value for plasma concentration has not been agreed upon, some authors consider this to be up to 20 mg/dL [16] and others up to 30 mg/dL [17], pointing out that the use of different

monoclonal or poyclonal antibodies, generates differences in results [18]. In order to assess concordance in results among different methods, a calibrator and a reference ELISA method has been evaluated and demonstrated to be insensitive to apo(a) isoform size heterogeneity [19].

In the present work, we propose a novel approach that allows identification of individuals with a high Lp(a) binding capacity to plasmin degraded fibrin surfaces. This functional method that allows unambiguous estimation of the effect of Lp(a) on fibrinolysis is based on the competitive binding of Lp(a) and plasminogen to fibrin surfaces [10].

We hypothesize that the direct assessment of the ability of Lp(a) to bind to fibrin and decrease thereby plasminogen binding may provide a better and straightforward estimation of its potential atherothrombotic risk.

We performed a transversal case-control study which pointed out Lp(a) fibrin binding association with CVD.

## Methods

### Study group

Patients were selected among subjects attending the out-patient clinics of the National Institutes of Cardiology and Neurology (Tlalpan, D.F., Mexico). A total of 248 individuals were studied, 157 patients and 91 controls. The patients had a history of ischaemic cardiopathy n=105 (66.9 %), including myocardial infarction (67.7%), and stable/unstable angina ( 32.3%) or thrombotic cerebro-vascular disease n=52 (33.1%). The diagnosis of ischemic cardiopathy was made by the clinical and

electrocardiographic studies as well as by coronary angiography. The diagnosis of cerebral vascular disease was based on the clinical signs of transient cerebral ischemia or cerebral infarction, as well as doppler and computerized tomography. The patients selected had none of the following exclusion pathologies criteria: uncontrolled arterial hypertension, hepatic or renal failure, neurological deficit with dementia, cerebrovascular emboli of cardiac origin or after carotid angiography, and recent (less than two months before the blood sample collection) myocardial infarct or surgical interventions. Patients with clinical evidence of ischemic disease were, if necessary, under pharmacological treatment in order to control hyperlipidemia, hypertension, diabetes mellitus or platelet aggregation.

Controls were healthy volunteers with no history of IC or CVD. Written consent was obtained from all participants, the study was approved by the Ethical Committee and the procedures were in accordance with the guidelines established by our institutions.

## Blood samples

Fasting venous blood (up to 10 mL) was drawn from the forearm into sterile polypropylene tubes containing 4 mmol/L EDTA as anticoagulant. The samples were immediately centrifuged at 2000 x g for 15 min at 4°C. Plasma was removed by aspiration, supplemented with 100 KIU/mL aprotinin (Trasylol®, Bayer Pharma, Puteaux, France) and stored in aliquots at -70°C for a maximum of six months.

## Lp(a) fibrin-binding assay

The assay is based on the ability of Lp(a) to compete with plasminogen for fibrin binding either in purified systems [10] or at the plasma/fibrin interface [20]. Conditions for this competition in plasma have been established [20] and advantageously used to develop

a functional assay to detect pathogenic Lp(a). Fibrin surfaces used as a template for the competitive interaction between plasminogen and Lp(a) present in plasma, were prepared and characterized as previously described [21,22]. Buffer A (0.05 mmol/L sodium phosphate, pH 7.4, 0.08 mmol/L NaCl, 0.01% (w/v) Thymerosal, 0.01 % (w/v) Tween 20) supplemented with BSA where indicated, was used in all experiments. To perform the assay, a volume of 50 µL of plasma (diluted1:16 in buffer A supplemented with 40 mg/mL BSA) was incubated in duplicate with fibrin surfaces for 12 h at 4°C, to allow plasminogen/Lp(a) equilibrium competition for fibrin binding. Unbound proteins were removed by washing three times with buffer A supplemented with 2 mg/mL of bovine serum albumin and a volume of 50 µL of a peroxidase-conjugated monoclonal mouse antibody, directed against apo(a) [23], was added. This antibody does not cross-react with plasminogen. After 2 h at 37°C the plate was washed and a solution of 1 mg/mL 2,2'-azino-bis(3-ethylbenzthiazoline)-6-sulphonic acid (ABTS, Boehringer Mannheim) was used for colour development. The change in absorbance ($\Delta A_{405/min}$) was measured with a microtitration plate counter (MR 5000, Dynatech). The amount of Lp(a) bound is expressed in nmol/L of apo(a) by referring to a calibration curve. The standard curve was obtained from a reference standard composed of equimolar amounts of five recombinant apo(a) isoforms of different lenghts (10 to 34 kringles) [24].

### Lp(a) plasma concentration

Lp(a) plasma concentration was measured employing an immunonephelometric method (reagents and equipment from Beckman Co, Palo Alto CA, USA).

Apo(a) phenotyping

Apo(a) isoforms were identified as described [24] using an immunoblot procedure and a standard composed of recombinant apo(a)s containing 10, 14, 18, 26 and 34 kringles. Each apo(a) band image of the immunoblots was digitalized (Molecular Analyst TM/PC) and analyzed by densitometric scanning (Bio-Rad GS-670). The densitometric data from each band was expressed as a percentage relative to the sum of isoform (one or two) considered as 100%. The molar concentration of each apo(a) isoform was then calculated by relating their molecular mass and relative proportion in plasma with the concentration of Lp(a). For statistical purposes (see below and Tables 2 and 3) isoforms present at the highest concentration were considered as phenotype 1 whereas phenotype 2 represented the low concentration isoform in heterozygous subjects.

Statistical Analysis

For all variables, the mean and SD or the median and range inter-quartile were calculated. Quartiles cut-off values for Lp(a) concentration, apo(a) fibrin-binding capacity, and apo(a) phenotypes were calculated from the entire study population (IC, CVD and controls) and the proportion of patients with IC, CVD and control interquartil was analyzed by $\chi^2$ test. Differences between groups were determined by $\chi^2$ for discrete variables. For continuous variables comparisons between groups were evaluated with parametric or non-parametric analysis of variance according to the variable distribution. Least significant difference test (LSD) for parametric variables or U test of Mann-Whitney for non-parametric variables were used to determine significant differences between groups. Covariance analyses were also used to assess the

potentially confusing effect of age. Lp(a) concentrations and Lp(a) binding distribution were compared by the Kolmogorov-Smirnov test.

The association of Lp(a) plasma concentration, apo(a) binding and apo(a) isoforms with IC and CVD in the fourth quartile versus the first quartile was evaluated by contingency tables, odd ratios and 95% confidence intervals were calculated.

All statistical analyses were performed with the SPSS-10.1 Package (SPSS, USA).

Results

The group of patients with clinical evidence of ischaemic disease did not significantly differ from the group of control subjects, regarding gender and body mass index (Table 1). The age was significantly higher in both ischaemic disease groups compared to the control group ($p<0.001$). Even if the Lp(a) plasma concentration is not age dependent, the results for Lp(a) concentrations and apo(a) binding were adjusted by age (ANCOVA analyse). By ANCOVA the results showed that apo(a) binding was significantly higher in the CVD patients as compared to IC patients and the control group. No differences were found in the Lp(a) concentrations among the studied groups of patients. All haemostatic variables tested (PT, PTT, TT) were normal in all subjects, (data not shown).

Table 2 illustrates the percentage of patients and controls in each quartile of Lp (a) concentration, apo(a) fibrin binding and apo(a) phenotype. Lp(a) concentration and apo(a) fibrin binding were skewed to lower values in controls, IC Lp(a) concentration showed an increment in the fourth quartile and an inverse relation was observed with large apo(a) phenotype.

CVD subjects differ substantially from controls and IC, increasing values for Lp(a) concentration and apo(a) fibrin binding were observed among the higher quartiles.

Table 3 shows the possible association between ischaemic cardiopathy or cerebrovascular disease and either Lp(a) concentration, apo(a) binding, or apo(a) phenotypes. The ischaemic cardiopathy was not associated with Lp(a) levels, apo(a) fibrin binding or apo(a) phenotypes in this particular group of Mexican patients. The cerebrovascular disease was significantly associated with high Lp(a) plasma concentration and high apo(a) fibrin binding. The presence of IC or CVD was associated, though not statistical significant to small size apo (a) isoforms.

Figure 1 shows the more important contribution, though not statistical significant, of low molecular weight phenotypes, less than 22 kringles, to Lp(a) plasma concentration in IC and CVD than in controls, (76.86%, 74.32% and 56.3%, respectively). This analysis allowed us to notice that K17-K19 phenotypes are more frequent in IC and CVD than in controls.

Furthermore, analyzing (in the same manner) only the subjects with high apo(a) fibrin-binding, the contribution of low molecular weight phenotypes, less than 19 kringles, to plasmatic Lp(a) concentration became more evident in IC (80.15 %), CVD (61.97%) and in controls, (56.65%), providing further evidence of the greater antifibrinolytic potential associated with small size isoforms and high Lp(a) concentration.

## Discussion

Lp(a) affinity for fibrin shows an inverse relationship with the size of the apo(a) isoform within a dissociation constant (Kd) range from 50 to 500 nmol/L [10]. The importance of this structural and functional relationship is the fact that the Lp(a) antifibrinolytic potential depends, firstly, on the affinity of each of the two apo(a) isoforms found in plasma and, secondly, on Lp(a) concentration [10,25,26]. Therefore, similar Lp(a)

concentrations may display differences in atherothrombotic risk, depending on apo(a) size and affinity for fibrin. In agreement with these findings, it has been recently reported that small apo(a) isoforms are strong indicators of advanced stenotic atherosclerosis [27]. However, the lack of a method to evaluate the functional heterogeneity of Lp(a) is indeed a main obstacle for the correct assessment of coronary artery disease and CVD risk associated with Lp(a). This fact and the diversity of assay methods to measure Lp(a) plasma concentration may explain the discrepancy of some prospective studies that do not find a correlation between Lp(a) plasma level and coronary artery disease or CVD [28,29,30,31, 32].

Despite these drawbacks, a recent meta-analysis of 27 prospective coronary heart disease studies [1] concluded that individual concentrations of plasma Lp(a) in the highest third of baseline measurements versus those in the lowest third, result in a combined risk ratio of 1.6 (95% CI 1.4 to 1.8, $p<0.00001$). The recognition that Lp(a) pathogenicity may not be alike in all individuals encouraged us to develop a new strategy for the evaluation of Lp(a). The original approach of this method to the pathogenesis of Lp(a)-related vascular disease, resides in its capacity to unveil the functional behaviour of apo(a) with regard to fibrinolysis impairment. This procedure is based on the antifibrinolytic mechanism of Lp(a) and takes into account both the affinity of apo(a) isoforms for fibrin and the plasma concentration of Lp(a).

A pathophysiological patient profile due to Lp(a) fibrin-binding is thus generated. Under the conditions of the assay, the competitive binding of Lp(a) and plasminogen can only be affected by lysine-analogues [10]. Our experimental system mimics the possible behavior of plasminogen and Lp(a) when challenged by fibrin formation and

fibrinolysis activation. Its relevance for in vivo biology and disease has been previously evaluated in the pathophysiological model represented by the nephrotic syndrome [20,33]. The variations in plasminogen and Lp(a) plasma concentrations observed in this pathology result in significant differences in plasmin formation. We provided direct evidence that the more Lp(a) binds to fibrin, the less plasminogen is bound and plasmin is generated. Plasminogen binding to fibrin was shown to be decreased as a result of Lp(a) plasma concentration and isoform size. This mechanism results in low plasmin formation and a potential antifibrinolytic effect.

In the present work our findings provide experimental evidence for: 1) the potential antifibrinolytic effect associated, though not statistical significant, with small size isoforms of apo(a) in IC and CVD, and 2) the strong association between Lp(a) fibrin-binding and our CVD group. The high apo(a) fibrin binding observed in CVD patients as compared to controls is indicative of an efficient competition between plasminogen and Lp(a) for fibrin binding sites. This finding suggests that under conditions of fibrin challenging in the vasculature or in tissues, Lp(a) may inhibit plasmin formation, an important condition for the pathophysiology of atherothrombosis. Because of the small CVD case number it is not reasonable to draw final conclusions from our study regarding the unexpectedly high final Odd ratios results as there is only one case in the first quartile. However, there is a significantly increased relation between Lp(a) fibrin-binding and CVD. Our results agree with the hypothesis that elevated plasma Lp(a) is the primary factor associated with the presence of ischemic cerebrovascular disease as reported by Jürgens et al. [34].

Furthermore, our data suggest the existence of peculliar and poorly understood differences in antithrombotic mechanisms in cerebral and coronary arteries in these patients. Further studies will be necessary to determine whether these differences are of ethnical and/or environmental origin. In fact, a well documented model that provides evidence for the differences in the endothelial-cell-derived anticoagulant and procoagulant activities from one vascular bed to another, has been recently proposed [35]. The model supports the possibility of the existence of regionally distinct haemostatic balances. As a matter of fact, thrombomodulin is more important in maintaining the haemostatic balance in the lungs and heart than in the liver, whereas tissue-type plasminogen activator (t-PA) and urokinase-type plasminogen activator (u-PA) are important antithrombotic mechanisms in all three vascular beds. In the brain the factors mantaining haemostatic balance have not been identified as yet and the significant association between CVD and the antifibrinolytic effect of Lp(a) in this vascular bed could be an important clue.

Figure legends

Figure 1. Distribution and frequency of apo(a) phenotypes by kringle number in plasma Lp(a) from patients with ischaemic cardiopathy (IC), cerebrovascular disease (CVD) and controls.

Table 1

Demographic data, Lp(a) plasma concentration and apo(a) fibrin binding

| Variable | Control | CVD | IC |
|---|---|---|---|
| Gender[+] | | | |
| Male (%) | 78.6 | 76.1 | 82.5 |
| Female (%) | 21.4 | 23.9 | 17.5 |
| Age (years)[§] | 40.2±13.1 | 53.4±10.5* | 62.5±10.6* |
| Body Mass Index ($Kg/m^2$) [§] | 26.3±5.6 | 27.0±3.5 | 27.2±3.4 |
| Lp(a) (mg/dL)[†] | 16.3±18 | 20.3±22.8 | 20.5±25.29 |
| Apo(a)binding (nmol/L)[†] | 0.158±0.09* * | 0.268±0.15 | 0.155±0.12** |

[+] The results are mean ± SD. CVD: cerebrovascular disease, IC: ischaemic cardiopathy,

[§]ANOVA, *<0.001 vs control. [†]ANCOVA **p<0.0001 Vs CVD

Table 2.

Distribution of patients with ischaemic cardiopathy (IC), cerebrovascular disease (CVD), and control subjects according to Lp(a) concentration, Apo(a) binding and Apo(a) phenotypes .

| Quartile | 1 | 2 | 3 | 4 | P* |
|---|---|---|---|---|---|
| Lp(a) concentration | <6.1 | 6.1-13.8 | 13.9-22.45 | >22.45 | |
| IC (%) | 33.3 | 19 | 20.2 | 27.4 | 0.074 |
| CVD (%) | 12.8 | 23.1 | 25.6 | 38.5 | <0.001 |
| CONTROL (%) | 20.5 | 33.3 | 30.8 | 15.4 | 0.009 |
| Apo (a)binding (nM) | <0.098 | 0.098-0.162 | 0.163-0.231 | >0.231 | |
| IC (%) | 34.3 | 24.8 | 20 | 21 | 0.089 |
| CVD (%) | 1.9 | 9.6 | 32.7 | 55.8 | <0.001 |
| CONTROL (%) | 27.5 | 34.1 | 26.4 | 12.1 | 0.003 |
| Apo(a) fenotype 1 (No. of K4) | <20 | 20-23 | 23.1-26 | >26 | |
| IC (%) | 20.8 | 35.1 | 28.6 | 15.6 | 0.006 |
| CVD (%) | 28.9 | 18.4 | 21.1 | 31.6 | 0.102 |
| CONTROL(%) | 23.1 | 18.5 | 32.3 | 26.2 | 0.187 |
| Apo(a) fenotype 2 (No. of K4) | <16.5 | 16.6-19 | 19.1-21 | >21 | |
| IC (%) | 28.3 | 28.2 | 17.4 | 26.1 | 0.217 |
| CVD (%) | 24.1 | 17.2 | 31 | 27.6 | 0.118 |
| CONTROL (%) | 20.6 | 20.6 | 29.4 | 29.4 | 0.332 |

IC= ischaemic cardiopathy, CVD=cerebrovascular disease * $\chi^2$ test.

Table 3
Association between ischaemic cardiopathy or cerebrovascular disease.and and Lp(a) parameters

| | Highest Quartile | Lowest Quartile | OR (95 % CI) |
|---|---|---|---|
| | Lp(a) (mg/dL) | | |
| IC (%) | 27.4 | 33.3 | 1.10 (0.43-2.78) |
| CONTROL (%) | 15.4 | 20.5 | |
| CVD (%) | 38.5 | 12.8 | 4 (1.13-14.09) |
| CONTROL (%) | 15.4 | 20.5 | |
| | Apo(a) Binding (nM) | | |
| IC (%) | 21.0 | 34.3 | 1.39 (0.57-3.37) |
| CONTROL (%) | 12.1 | 27.5 | |
| CVD (%) | 55.8 | 1.9 | 65.9 (7.95-546.8) |
| CONTROL (%) | 12.1 | 27.5 | |
| | Apo(a) Phenotype 1 | | |
| IC (%) | 15.6 | 20.8 | 0.66 (0.24-1.84) |
| CONTROL (%) | 26.2 | 23.1 | |
| CVD (%) | 31.6 | 28.9 | 0.96 (0.33-2.84) |
| CONTROL (%) | 26.2 | 23.1 | |
| | Apo(a) Phenotype 2 | | |
| IC (%) | 26.1 | 28.3 | 0.64 (0.19-2.24) |
| CONTROL (%) | 29.4 | 20.6 | |
| CVD (%) | 27.6 | 24.1 | 0.80 (0.20-3.25) |
| CONTROL (%) | 29.4 | 20.6 | |

haemic cardiopathy (n=105), CVD=cerebrovascular disease (n=52), OR= Odds ratio,

CI=confidence intervals, Apo(a) phenotypes by kringle number

References

1 Danesh J, Collins R, Peto R. Lipoprotein(a) and coronary heart disease. Meta-analysis of prospective studies. Circulation 2000; 102:1082-85.

2 Kraft HG, Menzel HJ, Hoopichler F, Vogel W, Uterman G. Changes of genetic apolipoprotein phenotypes caused by liver transplantation. J Clin Invest 1989; 83: 137-42.

3 Kostner GM, Wo X, Frank S, Kostner K, Zimmermann R, Steyrer E. Metabolism of Lp(a): assembly and excretion. Clin Genet 1997; 52: 347-54.

4 Berg K. Lp(a) lipoprotein: an over-view. Chem Phys Lipids 1992;67/68:9-18.

5 McLean JW, Tomlinson JE, Kuang WJ,Eaton DL, Chen EY, Fless GM , Scanu A, et al. cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. Nature 1987;300:132-37.

6 Guevara J Jr, Knapp RD, Honda S, Northup SR, Morrisett JD. A structural assessment of the apo(a) protein of human lipoprotein(a). Proteins 1992;12:188-99.

7 Utermann G, Menzel HJ, Kraft HG, Duba HC, Kemmler HG, Seitz C. Lp(a) glycoprotein phenotypes: Inheritance and relation to Lp(a)-lipoprotein concentrations in plasma. J Clin Invest 1987;80:458-65 .

8 Hervio L, Brunner C, Sorell L, Kang C, Muller H, Anglés-Cano E.. Effect of plasminogen activators on human recombinant apolipoprotein(a) having the plasminogen activation cleavage site. Biochim Biophys Acta 1999;1434:124-34.

9 Rouy D, Koschinsky ML, Fleury V, Chapman MJ, Anglés-Cano E. Biochemistry 1992 ;31 :6333-39.

10 Hervio L, Durlach V, Girard-Globa A, Anglés-Cano E. Multiple binding with identical linkage: a mechanism that explains the effect of lipoprotein(a) on fibrinolysis. Biochemistry 1995;34:13353-8.

11 Hoylaerts M, Rijken DC, Lijnen HR, Collen D. Kinetics of the activation of plasminogen by human tissue plasminogen activator. Role of fibrin. J Biol Chem 1982; 257: 2912-19.

12 Anglés-Cano E, de la Peña-Díaz A, Loyau S. Inhibition of fibrinolysis by lipoprotein (a). Ann N Y Acad Sci 2001;936:261-75.

13 De la Peña-Díaz A, Izaguirre Avila R, Anglés-Cano E. Lipoprotein Lp(a) and atherothrombotic disease. Arch Med Res 2000;31:353-59.

14 Sangrar W, Gabel BR, Boffa MB, Walker JB, Hancock MA, Marcovina SM, et al. The solution phase interaction between apolipoprotein (a) and plasminogen inhibits the binding of plasminogen to a plasmin-modified fibrinogen surface. Biochemistry 1997 ; 36 :10353-63.

15 Caplice NM, Panetta C, Peterson TE, Kleppe LS, Mueske CS, Kostner GM, et al. Lipoprotein (a) binds and inactivates tissue factor pathway inhibitos: a novel link between lipoproteins and thrombosis. Blood 2001;98 2980-87.

16 Dahlen GH, Stenlund H. Lp(a) lipoprotein is a major risk factor for cardiovascular disease: pathogenic mechanisms ans clinical significance. Clin Genet 1997; 52: 272- 280.

17 Rosengren A, Wilhelmsen L, Eriksson E, Risberg B, Wedel H . Lipoprotein (a) and coronary heart disease: a prospective case-control study in a general population sample of middle aged men. BMJ 1990; 301: 1248-51.

18 Dahlen GH. Lp(a) Lipoprotein in cardiovascular disease. Atherosclerosis 1994; 108: 111-126.

19 Marcovina SM, Albers JJ, Scanu AM, Kennedy H, Giaculli F, Berg K, et al. Use of a reference material proposed by the International Federation of Clinical Chemistry and Laboratory Medicine to evaluate analytical methods for the determination of plasma lipoprotein (a). Clin Chem 2000;46: 1956-67.

20 Soulat T, Loyau S, Baudouin V, Maisonneuve L, Hurtaud-Roux MF, Schlegel N et al. Effect of individual plasma lipoprotein(a) variations in vivo on its competition with plasminogen for fibrin and cell binding: An in vitro study using plasma from children with idiopathic nephrotic syndrome. Arterioscler Thromb Vasc Biol 2000;20:575-84.

21 Fleury V, Anglés-Cano E. Characterization of the binding of plasminogen to fibrin surfaces: role of carboxy-terminal lysines. Biochemistry 1991; 30:7630-8.

22 Anglés-Cano E. A spectrophotometric solid-phase fibrin tissue plasminogen activator activity assay (SOFIA-tPA) for high-fibrin affinity tissue plasminogen activators. Anal Biochem 1986;153:201-10

23 Sorell L, Rojas G, Rodriguez M, Ramos C, Torres L, Torres MB. A sandwich ELISA based on anti-apo(a) and anti-apo B monoclonal antibodies for lipoprotein(a) measurement. Clin Chim Acta 1995, 236: 59-70.

24 Anglés-Cano E, Loyau S, Cardoso-Saldaña G, Courdec R, Gillery R. A novel kringle-4 number based recombinant apo(a) standard for human apo(a) phenotyping. J Lipid Res 1999;40:354-59.

25 Hervio L, Chapman MJ, Thillet J, Loyau S, Anglés-Cano E. Does apolipoprotein(a) heterogeneity influence lipoprotein (a) effects on fibrynolisis? Blood 1993;82:392-7.

26 Hervio L, Girard-Globa A, Durlach V, Anglés-Cano E. The antifibrinolytic effect of lipoprotein (a) in heterozygous subjects is modulated by the relative concentration of each of the apolipoprotein (a) isoforms and their affinity for fibrin. Eur J Clin Invest 1996; 26: 411-7.

27 Kronenberg F, Kronenberg MF, Kiechl S, Trenkwalder E, Santer P, Oberhollenzer F, et al. Role of lipoprotein(a) and apolipoprotein (a) phenotype in atherogenesis: prospective results from the Bruneck study. Circulation 1999;100:1154-60.

28 Hachinski V, Graffagnino C, Beaudry M, Bernier G, Buck C, Donner A, et al. Lipids and stroke: a paradox resolved. Arch Neurol 1996;53:303-8.

29 Glader CA, Stegmayr B, Boman J, Stenlund H, Weinehall L, Hallmans G,et al. Chlamydia pneumoniae antobodies and high lipoprotein(a) levels do not predict ischemic cerebral infarctions. Results from a nested case-control study in Northern Sweden. Stroke 1999; 30: 2013-8.

30 Alfthan G, Pekkanen J, Jauhaiainen M, Pitkaniemi J, Karvonen M, Tuomilehto J, et al. Relation of serum homocysteine and lipoprotein(a) concentrations to atherosclerotic disease in a propspective Finnish population based study. Atherosclerosis 1994;106: 9-19.

31 Ridker PM, Stampfer MJ, Hennekens CH. Plasma concentration of lipoprotein(a) and the risk of future stroke. JAMA 1995;273:1269-73.

32 Wityk RJ, Kittner SJ, Jenner JL, Hebel JR, Epstein A, Wozniak MA, et al. Lipoprotein(a) and the risk of ischemic stroke in young women. Atherosclerosis 2000; 150: 389-96.

33 Soulat T, Loyau S, Baudouin V, Durlach V, Gillery P, Roselyne G, et al. Evidence that modifications of Lp(a) in vivo inhibit plasmin formation on fibrin. Thromb Haemostas 1999; 82: 121-27.

34 Jürgens G, Taddei-Peters W, Költringer P, Petek W, Chen Q, Greilberger J, et al. Lipoprotein (a) serum concentration and apolipoprotein (a) phenotype correlate with severity and presence of ischemic cerebrovascular disease. Stroke 1995; 26: 1841-48.

35 Rosenberg RD, Aird WC. Vascular-bed-specific hemostasis and hypercoagulable states. N Engl J Med 1999;340:1555.

# LIPOPROTEIN(a) POLYMORPHISM IN MEXICAN AMERINDIAN POPULATIONS

Cardoso-Saldaña G[1], De la Peña-Díaz A[1], Zamora-González[1] J, Posadas-Romero C[1], Izaguirre-Avila R[1], Malvido-Miranda E[2], Morales-Anduaga ME[2], Gomez R ,Anglés-Cano E[3].

[1]Instituto Nacional de Cardiología Ignacio Chávez, 14080 México, D.F.

[2]Instituto Nacional de Antropología, 14080 México, D.F.

[3]Institut National de la Santé et de la Recherche Médicale, U. 460, 75870 París, Francia.

Number of pages: 18

Abbreviated title: Lp(a) in Mexican-Amerindian

KEY WORDS: Lp(a), fibrinolysis, kringle domains, Mazahuas, Mayas, Mayos, Mestizos

Correspondence: Dr. E. Anglés-Cano

INSERM U460

UFR de Médecine Xavier Bichat

16, rue Henri Huchard-BP 416

F-75870-Cdx, PARIS 18, France.

Phone: 33-1 44 85 61 50 Fax: 33-1 44 85 61 56.

Email:angles@infobiogen.fr

*Abstract.* Lp(a) is a complex particle of unknown function mainly present in primates. It consists of a low-density lipoprotein and apo(a), a polymorphic glycoprotein homologous to plasminogen, the precursor of plasmin the fibrinolytic enzyme. Apo(a) is, however, an inactive zymogen that inhibits fibrinolysis through binding to fibrin. By virtue of its composite structure, Lp(a) is currently considered as a link between atherosclerosis and thrombosis. Marked inter-ethnic differences in Lp(a) concentration probably related to the genetic polymorphism of apo(a) has been reported. In this work we have evaluated the Lp(a) pattern in three Amerindian groups (Mayos n=68, Mazahuas n=62 and Mayas n=36) and 41 Mestizo subjects (admixture of Amerindian and Spaniards). Lp(a) plasma concentrations, apo(a) phenotypes and fibrin binding were analyzed. The Mestizo group presented the less skewed distribution and the highest median Lp(a) concentration (13.25 mg/dL) relative to Mazahuas (8.2 mg/dL), Mayas (8.25 mg/dL) and Mayos (6.5 mg/dL). There was not significant difference between groups in the prevalence of Lp(a) concentrations greater that the conventional cut-off for cardiovascular risk (> 30 mg/dL). Phenotype distribution was different in Mayas and Mazahuas as compared to the Mestizo group. The higher Lp(a) fibrin binding was found in the Maya population. There was an inverse relationship between the size of apo(a) polymorphs and both Lp(a) levels and Lp(a) binding to fibrin in all groups. In conclusion, the Lp(a) levels and distribution in Amerindians is highly skewed toward lower levels and differs significantly from the Mestizo ethnic group.

The lipoprotein Lp(a) is a low density lipoprotein (LDL)-like particle mainly found in primate species and currently considered as a major risk factor for cerebrum and cardiovascular atherosclerotic disease (Djurovic and Berg, 1997). Lp(a) composition is indeed similar to that of the atherogenic LDL particle in terms of lipid core composition (cholesterol, triglycerides, phospholipids) and the apolipoprotein Apo B-100. The distinctive feature of Lp(a) is the glycoprotein apo(a), coded by a gene that arose from a duplicate copy of the plasminogen gene during primate evolution (Lawn et al., 1995). Apo(a) is therefore homologous to plasminogen, the precursor of plasmin the fibrinolytic enzyme (McLean et al., 1987). Plasminogen contains five different types of a structure termed kringle consisting of 80 to 90 amino acid residues arranged in a disulfide-bridge triple loop structure. Kringles 1 and 4 of plasminogen allow binding to fibrin, thus ensuring its *in situ* transformation into plasmin by plasminogen activators and thereby the lysis of a fibrin clot. Apo(a) contains 10 different types of plasminogen-like kringle 4 (K-IV) and a copy of each, kringle 5 and the serine-protease region. Although apo(a) shares with plasminogen its fibrin-binding function, it cannot be transformed into a plasmin-like serine-protease. Lp(a) may, therefore, compete with plasminogen for binding to fibrin and inhibit fibrinolysis, linking thereby the pathophysiological process of atherosclerosis and thrombosis (de la Peña-Diaz et al., 2000).

More than 30 apo(a) isoforms have been identified (Marcovina et al., 1993) that differ by the number of copies of K-IV type 2 which, in any given individual, is determined by the apo(a) gene (Boerwinkle, 1992). This polymorphism could be involved in determining Lp(a) levels as epidemiological evidence indicates an inverse relationship between Lp(a) concentration and the size of apo(a) (Gaubatz et al., 1990). The apo(a) gene is indeed a

major determinant of Lp(a) concentrations in plasma, it accounting for greater than 90% of its variations (Boerwinkle et al., 1992). A similar inverse relationship has also been found between apo(a) size and the ability of Lp(a) to compete with plasminogen for binding to fibrin (Hervio et al. 1996).

Differences in Lp(a) concentration and apo(a) phenotype distribution have been described in Caucasian, African and Asian groups (Sandolzer et al., 1991: Wong et al., 1999; Sorensen et al., 1994). Data in Amerindian populations have not been reported until now. The existence of different ethnic groups in Mexico and the prevalence of an important Mestizo population prompted us to investigate the distribution of Lp(a) concentration, apo(a) kringle IV phenotypes and the binding of Lp(a) to fibrin in these populations. The Mestizo can be distinguished from Amerindians by higher Lp(a) concentration from a lees skewed distribution.

Subjects. A total of 240 non-related healthy subjects, women and men from four different ethnic groups, were studied. All subjects were selected at random from healthy individuals living in four states from Mexico. Anthropometric data are shown in Table 1. The historical, cultural and linguistic characteristics were different for each group as determined by the Instituto Nacional de Antropología e Historia de México. Plasma samples were obtained from Mayos (north-west of the country at Capomito, Estado de Sinaloa), Mazahuas (Santa Rosa and Pueblo Nuevo, Estado de Mexico) and Mestizos (Mexico City). The Maya samples were obtained from Tahdzibichen, near Merida, Yucatan. The present study was approved by the ethical committee of our institutions. Informed consent was obtained from all individuals. After an overnight fast, blood (10 ml) was drawn from the antecubital vein from each apparently healthy subject. Blood was collected into sodium

EDTA tubes (1mg/ml) and kept on ice until separation of plasma within the next 2 hours by centrifugation at 2000 g for 20 minutes at 4°C. Samples of plasma were supplemented with a proteinase inhibitor (Aprotinin 100 IU/ml, Trasylol® Bayer) and stored at -70°C until analysis (less than 6 months from sampling).

## METHODS

Plasma Lp(a) concentrations. Lp(a) in plasma was measured by kinetic immunonephelometry (Gillery et al., 1993) using a commercial antibody in an automated Array protein system (Beckman Co, Palo Alto, CA, USA). The intra-assay and inter-assay coefficients of variation were less than 8%.

Apo(a) Kringle IV isoforms determination.

Sample preparation. Plasmas were adjusted to 15 mg/dl of Lp(a) or directly diluted 1:2 in sample buffer made of 0.34 M N-ethylmorpholine in 1.5 M Tris pH 8.8, 0.83 M 2-mercaptoethanol and 17.8% bromophenol blue in 10% glycerol. A mix of five recombinant apo(a) isoforms containing 10, 14, 18, 26 and 34 kringles were used as standard (Anglés-Cano et al., 1999). Samples and the standard were supplemented with 0.1% SDS and boiled for 5 minutes in a water bath.

Electrophoresis on a 3.75% and 6% discontinuous polyacrylamide gel and immunoblotting were performed as previously described (Anglés-Cano et al., 1999). A volume of 12 µl of plasma or recombinant apo(a) was applied to the gel and electrophoresis was run at room temperature using 35V for the first half an hour, followed by 70 V for another 6 hrs.

Apo(a) size determination. Blots were scanned in a GS-670 Bio-Rad densitometer using the Molecular Analyst™/PC software. The procedure sensitivity was 50 ng. The apo(a) isoform

size number was determined by reference to a standard curve made by plotting the relative migration of five recombinant apo(a) isoforms against the log of the number of kringle units. The inter and intra-assay coefficients of variation were less than 2%.

Lp(a) fibrin-binding assay. The experiments were performed with fibrin surfaces prepared and characterized as previously described (Fleury and Anglés-Cano, 1991). The procedure to assess binding of plasma Lp(a) to fibrin in competition with plasminogen was essentially as described (Soulat et al., 2000) with minor modifications. The amount of Lp(a) bound was expressed in nanomoles of apo(a) by referring to a calibration curve built with a reference standard composed of equimolar amounts of 5 recombinant apo(a) isoforms of different length (10 to 34 kringles) (Anglés-Cano et al., 1999)

Statistical methods. The overall shape of the distribution of plasma Lp(a) and apo(a) kringle IV phenotypes were compared between populations using the Kolmogorov-Smirnov test. Because of the highly skewed distribution of Lp(a) levels in the four ethnic groups, non-parametric Kruskal-Wallis tests were used to compare the median Lp(a) concentrations between groups. To estimate the influence of the apo(a) size polymorphism on Lp(a) plasma levels, Spearman rank correlation coefficients (Rs) were calculated. The prevalence of higher Lp(a) levels (> 30mg/dL) were compared with the $\chi^2$ test. Multiple group means were compared by analysis of variance (ANOVA). The Statistical Package for Social Science (SPSS) V. 10 was used.

## RESULTS

General characteristics of the four ethnic groups are shown in Table 1. There were no associations between Lp(a) and either age, weigh, height, waist, BMI or gender; no adjustment was therefore necessary for the analysis.

Plasma Lp(a) concentrations. The distribution of plasma Lp(a) levels in each of the four ethnic groups is shown in figure 1. In all four groups the distribution is skewed toward low concentrations. The plasma levels of Lp(a) were less skewed in the Mestizo group than in the other groups. The median Lp(a) concentration was significantly higher in Mestizos than in the three indigenous groups (Table 2). The prevalence of Lp(a) greater than 30 mg/dL, the conventional cut-off for atherosclerotic risk, was slightly higher in Mazahuas than in the other groups (Table 2).

Apo(a) phenotypes. **Twenty six different apo(a) phenotypes were identified between all ethnic groups.** Apo(a) phenotype distribution in each of the four ethnic groups is shown in Figure 2. Phenotype distribution was significantly different in Mayas and in Mazahuas as compared to the Mestizo group. DifferenceS in distribution were also observed between Mazahuas and Mayos. There was an inverse relationship between Lp(a) levels and apo(a) kringle number in Mestizos, Mazahuas and Mayos (Table 3); surprisingly, the Maya population studied showed a direct relationship between apo(a) kringle IV number and Lp(a) concentration.

Lp(a) binding to fibrin. Mean and median for Lp(a) binding to fibrin surfaces was significantly higher in Mayas when compared to each of the other ethnic groups (Table 2). Lp(a) median binding in Mazahuas was significantly lower than binding in Mestizos. An inverse relationship was found between Lp(a) binding and apo(a) kringle number in all populations (Table 3).

## DISCUSSION

Several reports have suggested that plasma Lp(a) concentration is determined by the apo(a) gene locus (Kraft et al., 1992, Boerwinkle, 1992). However, variations among populations have been observed. As a matter of fact, in Caucasians the apo(a) gene size explains 40-60% of plasma Lp(a) concentration, whereas in other ethnic groups it varies from as low as 28% in Sudanese to as high as 70% in Chinese (Scholz et al., 1999). Indeed, the number of kringle IV type 2 that define the apo(a) polymorphism is inversely related not only to the plasma concentration of Lp(a) ( Utermann et al., 1987) but also to its ability to bind to fibrin (Hervio et al., 1996). Therefore, genetic susceptibility to the effects of Lp(a) in a given population may be better assessed by estimation of the relationship between these parameters. The availability of a reliable fibrin-binding assay and a well defined standard composed of recombinant apo(a) isoforms of known kringle number, allowed us to perform the first population study integrating Lp(a) concentration determination, apo(a) kringle IV number analysis and the functional fibrin-binding assay. The Lp(a) concentration frequency distribution in the Mexican indigenous group was skewed to levels (<10 mg/dl) lower than those observed in Mestizo, Caucasian, Chinese, Black African, African-American and Mexican-American populations (Haffner et al., 1992; Gaw et al., 1994; Kraft et al., 1996). Another major finding in this study is the significant difference in Lp(a) plasma concentration between Mestizo and indigenous populations. The Mestizo population currently living in the Mexican Republic arose after the Spanish colonization started in 1502. Note that the mean concentration of Lp(a) found in the Mexican Mestizo group (16.6±18 mg/dl) is similar to that found in Spanish populations (18.2±14.8 mg/dl) (GomezGerique et al., 1996; Muros et al., 1996). However, the prevalence of Lp(a) greater

than the conventional cut-off value usually used to define cardiovascular risk (>30 mg/dl) is higher in apparently healthy European/Caucasian populations (15-20 %) than in the Mexican groups (Table 2). This difference is probably related to a less skewed distribution of Lp(a) concentrations in the Spanish population relative to the distribution observed in our study and reflects in a certain way cross genetic influences resulting from the admixture of Amerindians with Spaniards. The distribution of apo(a) phenotypes was bimodal in Mestizos and multimodal in the indigenous Amerindian groups. In agreement with this finding is the observation by Haffner et al. (1992) that Lp(a) concentrations in Mexican-Americans with greater Native Amerindian genetic admixture, are close to the values found in our Amerindian populations.

An interesting finding was the direct correlation (Table 3) between apo(a) phenotype and Lp(a) concentration in the Maya population we have studied. This finding suggests that the number of repetitive sequences in the apo(a) gene coding for kringle IV type 2, and therefore the size of apo(a), is not a major determinant of Lp(a) concentration in this population. Mayas also showed the greatest fibrin-binding but no correlation was found with apo(a) size. Because these observations may be of great value to our understanding of the influence of apo(a) size on both Lp(a) concentration and binding to fibrin, we are currently performing another study in a Maya population from Guatemala.

In conclusion, Lp(a) distribution in Mexican Amerindians is highly skewed toward lower levels relative to the Mestizo population. Apo(a) size distribution in Amerindians also differs significantly from the apo(a) size distribution in Mestizos. Our data provide further support to a genetic hypothesis for the difference in Lp(a) parameters among Amerindians and Mestizos.

## ACKNOWLEDGEMENTS

We are most grateful to all Amerindian populations that made possible the realization of these studies. We thank the schoolteachers of Santa Rosa and Pueblo Nuevo (Estado de Mexico), the University of Yucatan and the Government of Sinaloa for their collaboration and help with sampling. This work was supported by a special grant from the INSERM, the Réseau Nord-Sud program for the development of biomedical research in south latitude countries.

LITERATURE CITED

Anglés-Cano E, Loyau S, Cardoso-Saldaña G, Couderc R, and Gillery PA. 1999. A novel kringle-4 number based recombinant apo(a) standard for human apo(a) phenotyping. J Lipid Res 40: 354-359.

Boerwinkle E. 1992. Genetics of Plasma Lipoprotein(a) Concentrations. Curr Opin Lipidol 3:128-136.

Boerwinkle E, Leffert CC, Lin J, Lackner C, Chiesa G, and Hobbs HH. 1992. Apolipoprotein (a) gene accounts for greater than 90% of the Variation in plasma lipoprotein (a) concentrations. J Clin Invest 90:52-60.

De la Peña-Diaz A, Izaguirre Avila R, and Anglés-Cano E. 2000. Lipoprotein Lp(a) and atherothrombotic disease. Arch Med Res 31: 353-359.

Djurovic S, and Berg K. 1997. Epidemiology of Lp(a) lipoprotein: its role in atherosclerotic/thrombotic disease. Clin Genet 52:281-292.

Fleury V, and Anglés-Cano E. 1991. Characterization of the binding of plasminogen to fibrin surfaces: role of carboxy-terminal lysines. Biochemistry 30:7630-7638.

Gaubatz JW, Ghanem KI, Guevara J, Nava ML, Patsch W, and Morrisett, J. D. 1990. Polymorphic forms of human apolipoprotein (a) : inheritance and relationship of their molecular weights to plasma levels of lipoprotein (a). J.Lipid.Res. 31:603-613.

Gaw A, Boetwinkle E, Cohen JC, and Hobbs HH. 1994. Comparative analysis of the apo(a) gene, Apo(a) glycoprotein, and plasma concentrations of Lp(a) in three ethnic groups. J Clin Invest 93:2526-2534.

Gillery P, Arthuis JP, Cuperlier C, Circaud R. 1993 Rate nephelometric assay of serum lipoprotein(a). Clin Chem 39:503-508

GomezGerique JA, Porres A, LopezMartinez D, AlvarezSala LA, Blazguez E, Montoya MT, and DeOya M. 1996. Levels of lipoprotein(a) And plasma lipids in Spanish children aged from 4 to 18 years. Acta Pediatr 85:38-42.

Haffner SM, Gruber KK, Morales PA, Hazuda HP, Valdez RA, Mitchell BD, and Stern MP. 1992. Lipoprotein (a) concentrations in Mexican-Americans and non-hispanic whites: the San Antonio heart study.Am J Epidemiol 136:1060-1068.

Hervio L, Girard-Globa A, Durlach V and Anglés-Cano E. 1996. The antifibrinolytic effect of lipoprotein(a) in heterozygous subjects is modulated by the relative concentration of each of the apolipoprotein(a) isoforms and their affinity for fibrin. Eur J Clin Invest 26: 411-417.

Kraft HG, Kochl S, Mezel HJ, Sandholzer C, Uterman G. 1992. The apolipoprotein gene: A transcribed hypervariable locus controlling plasma lipoprotein(a) concentration. Hum.Genet 90:220-230.

Kraft HG, Lingenhel A, Pang RWC, Delport R, Trommsdorff M, Vermaak H, Janus ED, and Utermann G. 1996. Frequency distributions of apolipoprotein(a) kringle IV repeat alleles and their effects on lipoprotein(a) levels in caucasian, asian, and african populations: the distribution of null alleles is non-random. Eur J Hum Gen 4:74-87.

Lawn RM, Boonmark NW, Schwartz K, Lindahl GE, Wade DP, Byrne CD, Fong KJ, Meer K, Patthy L. 1995. The recurring evolution of lipoprotein(a). J Biol Chem 270:24004-24009.

Marcovina SM, Zhang ZH, Gaur VP, and Albers JJ. 1993.Identification of 34 apolipoprotein (a) isoforms: differential expression of apolipoprotein (a) alleles between american blacks and whites. Biochem Biophys Res Commun 191:1192-1196.

McLean JW, Tomlinson JE, Kuang WJ, Eaton DL, Chen EY, Fless GM, Scanu AM, and Lawn RM. 1987. cDNA sequence of human apolipoprotein (a) is homologous to plasminogen. Nature 300:132-137.

Muros, M. and Rodriguez-Ferrer, C. 1996 Apolipoprotein E polymorphism Influence on lipids, apolipoproteins and Lp(a) in a Spanish population Underexpressing apo E4. Atherosclerosis. 121:13-21.

Sandholzer C, Hallman DM, Saha N, Sigurdsson G, Lackner C, Csaszar A, Boerwinkle E, and Utermann G. 1991 Effects of the apolipoprotein(a) size polymorphism on the lipoprotein(a) concentration in 7 ethnic groups. Hum Genet 86(6):607-14.

Schol, M, Kraft HG, Lingenhel A, Delport R, Vorster EH, Bickeboller H, and Utermann, G. 1999. Genetic control of lipoprotein(A) concentrations is different in Africans and Caucasians. Eur J Hum Gen 7:169-178.

Sorensen KE, Celermajer DS, Georgakopoulos D, Hatcher G, Betteridge DJ, and Deanfield JE. 1994. Impairment of endothelium-dependent dilation is an early event in children with familial hypercholesterolemia and is related to the lipoprotein(a) level. J Clin Invest 93:50-55.

Soulat T, Loyau S, Baudouin V, Maisonneuve L, Hurtaud-Roux MF, Schlegel N, Loirat C, and Angles-Cano E. 2000. Effect of individual plasma lipoprotein(a) variations in vivo on its competition with plasminogen for fibrin and cell binding: An in vitro study using plasma from children with idiopathic nephrotic syndrome. Arterioscler Thromb Vasc Biol. 20:575-84.

Utermann G, Menzel HJ, Kraft HG, Duba HC, Kemmler HG, and Seitz C. 1987. Lp(a) glycoprotein phenotypes: inheritance and relation to Lp(a)-lipoprotein concentrations in plasma. J Clin Invest 80:458-465.

Wong MS, Chew WLS, and Aw TC. 1999. Serum lipoprotein(A) profiles in a Singaporean population. Pathology 31:225-229.

**Figure Legends**

Fig. 1. Distribution of lipoprotein Lp(a) concentrations in the populations studied. Difference between Mestizos and Amerindians is significant ($p<0.001$)

Fig. 2. Distribution of apolipoprotein(a) isoforms by kringle number. The X axis indicates the total number of plasminogen-like kringles including the single copy of kringle V. Kolmogorow)Smirnov Z test: Mayas $p=0.05$, Masahuas $p<0.001$, vs Mestizos.

Table 1. Anthropometric characteristics of populations studied.

| | Mayos | Mayas | Mazahuas | Mestizos |
|---|---|---|---|---|
| n = | 68 | 36 | 60 | 76 |
| Age (years) | 55.8 ± 14.5 | 39.3 ± 21.3 | 19.35 ± 10.9 | 34.0 ± 9.9 |
| Weight, Kg | 65.6 ± 12.7 | NA | 43.2 ± 12.7 | 71.0 ± 13.4 |
| Height, cm | 167 ± 12.2 | NA | 147.3 ± 9.7 | 162.0 ± 26.0 |
| Waist, cm | 95.5 ± 9.1 | NA | NA | 86.8 ± 14.5 |
| BMI, $kg/m^2$ | 23.6 ± 4.5 | NA | 19.0 ± 4.0 | 26.1 ± 3.8 |

n = number of subjects studied.

Values are Mean ± SD

BMI, body mass index. NA, not available.

Table 2. Lp(a) plasma concentrations and fibrin-binding by ethnic group.

| | | Lp(a) Concentration | | | Fibrin-Binding | Fibrin-binding |
|---|---|---|---|---|---|---|
| | n | Mean ± SD | Median | >30 mg/dl[¶] | Mean ± SD | Median |
| Mayos | 68 | 10.5 ± 1.3 | 6.55 | 7.4 | 0.153 ± 0.12 | 0.10 |
| Mayas | 36 | 10.6 ± 8.5 | 8.25 | 5.6 | 0.257 ± 0.14** | 0.28** |
| Mazahuas | 60 | 11.6 ± 12 | 8.20 | 11.7 | 0.146 ± 0.14 | 0.09 |
| Mestizos | 76 | 16.6 ± 18* | 13.25** | 7.5 | 0.155 ± 0.09 | 0.14 |

n = number of subjects studied.

Lp(a) concentration is expressed in mg/dl. Lp(a) binding is expressed in nanomol bound.

[¶]Proportion of subjects with Lp(a) concentration >30mg/dl, the conventional cut-off value for cardiovascular risk. Values in %.

Mean *= p< 0,05 vs. Mestizos. ** p<0,01 vs. Mayas

ANOVA LSD post hoc Median: p <0,001

Table 3. Correlation between apo (a) size and Lp(a) fibrin-binding and concentration.

| Ethnic Group | Apo (a) phenotype/Lp (a) level | Apo(a) phenotype/fibrin-binding |
|---|---|---|
| Mayos | -0.361* | -0.07 |
| Mayas | 0.541* | -0.261 |
| Mazahuas | -0.007 | -0.248 |
| Mestizo | -0.422** | -0.003 |

• $p < 0.05$
**$p < 0.005$

ELSEVIER

Archives of Medical Research 31 (2000) 353–359

Archives of Medical Research

REVIEW ARTICLE

# Lipoprotein Lp(a) and Atherothrombotic Disease

Aurora de la Peña-Díaz,* Raúl Izaguirre-Avila,* and Eduardo Anglés-Cano**

*Departamento de Hematología, Instituto Nacional de Cardiología Ignacio Chávez, México, D.F., Mexico
**Institut National de la Santé et de la Recherche Médicale, U 143, Hôpital de Bicêtre, Paris, France

Received for publication October 15, 1999; accepted March 17, 2000 (99/191).

High plasma concentrations of lipoprotein (a) [Lp(a)] are now considered a major risk factor for atherosclerosis and cardiovascular disease. This effect of Lp(a) may be related to its composite structure, a plasminogen-like inactive serine-proteinase, apoprotein (a) [apo(a)], which is disulfide-linked to the apoprotein B100 of an atherogenic low-density lipoprotein (LDL) particle. Apo(a) contains, in addition to the protease region and a copy of kringle 5 of plasminogen, a variable number of copies of plasminogen-like kringle 4, giving rise to a series of isoforms. This structural homology endows Lp(a) with the capacity to bind to fibrin and to membrane proteins of endothelial cells and monocytes, and thereby inhibits binding of plasminogen and plasmin formation. This mechanism favors fibrin and cholesterol deposition at sites of vascular injury and impairs activation of transforming growth factor-beta (TGF-β) that may result in migration and proliferation of smooth muscle cells into the vascular intima. It is currently accepted that this effect of Lp(a) is linked to its concentration in plasma, and an inverse relationship between apo(a) isoform size and Lp(a) concentrations that is under genetic control has been documented. Recently, it has been shown that inhibition of plasminogen binding to fibrin by apo(a) from homozygous subjects is also inversely associated with isoform size. These findings suggest that the structural polymorphism of apo(a) is not only inversely related to the plasma concentration of Lp(a), but also to a functional heterogeneity of apo(a) isoforms. Based on these pathophysiological findings, it can be proposed that the predictive value of Lp(a) as a risk factor for vascular occlusive disease in heterozygous subjects would depend on the relative concentration of the isoform with the highest affinity for fibrin. 

*Key Words:* Lipoprotein(a), Atherosclerosis, Thrombosis, Fibrinolysis.

## Introduction

In 1963, Kâre Berg identified the presence of lipoprotein(a) [Lp(a)] in plasma (1); nevertheless, it was not until 1987 when Eaton et al. (2) partially identified the apoprotein(a) [apo(a)] glycoprotein sequence. The protein was later cloned (3) and showed a strong structural similarity between one of the Lp(a) components, apo(a) glycoprotein, and the plasmin precursor, plasminogen. This finding stimulated the interest of different research groups who found a common point between atherosclerosis and thrombosis (4–11). Different epidemiological studies were performed from this perspective and identified a positive correlation between high Lp(a) plasma concentration and an increase in cerebrovascular (12) and cardiovascular diseases, as well as coronary restenosis, postangioplasty reocclusion, and premature development of atherosclerosis related to high low-density lipoprotein (LDL) concentrations and/or low high-density lipoprotein (HDL) concentrations (13,14). Most prospective studies have confirmed these results (15–21). Even when a normal value for plasma concentration has not been agreed upon, some investigators considered this to be 20 mg/dL (20) and others suggested 30 mg/dL (15), pointing out that the use of different antibodies, monoclonal or polyclonal, generates differences in results (5).

Nevertheless, other studies do not find a relationship between Lp(a) and coronary arterial disease (22,23). This discrepancy may be a reflection of the large structural hetero-

Address reprint requests to: Eduardo Anglés-Cano, INSERM U 143, Hôpital de Bicêtre, 94276 Le Kremlin Bicêtre, Paris, France. Tel.: (+33-1) 4959 5601; FAX: (+33-1) 4959-5611; E-mail: angles@kb.inserm.fr

0188-4409/00 See front matter 

neity of the apo(a) molecule, which is conducive to a nctional heterogeneity as a plasminogen competitive inbitor of fibrin because it lowers the formation of plasmin ',24).

Not all organisms synthesize apo(a). Its presence has een identified in humans, in some Asian, European, and merican primates (25), and in hedgehogs (26).

## Function

he function of apo(a) in the organism is unknown. It proides cholesterol from the liver to organs that synthesize teroidal hormones (28) and to tissues for cell repair (29). urthermore, the function of apo(a) found in testicles and rain is unknown and is independent from Lp(a); that is, it unctions without being part of the Lp(a) molecule (30).

## Concentration

Lp(a) plasma concentration, which depends on its hepatic ynthesis (31,32), varies from one individual to another within an approximate range of <10 mg/dL to >100 mg/ dL; it is independent on other factors such as diet, cholesterol, obesity, and smoking, and is maintained within small variations throughout the lifespan (33,34). In general, an individual inherits, in a codominant autosomic fashion, two apo(a) isoforms that may be identified by sodium dodecylsulfate polyacrylamide gel electrophoresis (SDS-PAGE) and, subsequently, by immunotransference, making use of monoclonal or polyclonal antibodies against apo(a). By means of this method, almost 37 isoforms have been identified, which, according to the Utermann classification are: F (faster); B (i.e., apoB-100); S1 (slow), and S2, S3, or S4, depending on their migration velocity, in comparison with that of apoB-100 (35). The method does not allow for distinction of differences between isoforms of close molecular weight. Nevertheless, it has recently become possible to visualize the isoforms with the help of a reference, accounting for apo(a) recombinant isoforms with different molecular weights, correlating ($r$ = 0.97) with the technique of pulsed-field electrophoresis (36). With the use of this reference it has been possible to obtain a linear relationship between the log r-apo(a) kringle number and the relative migration using SDS-PAGE, successfully identifying apo(a) isoforms over a wide range of molecular sizes. Pulsed-field electrophoresis has been employed to identify different genotypes codified for apo(a) (37). With the aforementioned technique, 19 different alleles have been described in a U.S. study of a population of whites (38).

Apo(a) isoform size accounts for an inverse correlation with Lp(a) plasma concentration (39), probably because, as apo(a) size increases, less protein is secreted from the cell, as occurs in the case of the human hepatocarcinoma cell line HepG2 (40).

Some hormones can modify Lp(a) plasma concentration, such as estrogens (41–44); anabolic steroids might reduce it (45) and growth hormone can increase it (46), but it cannot be modified by lipid-lowering medication or by diet (33,47). The plasma concentration of Lp(a) increases in diseases such as diabetes mellitus, nephrotic syndrome, rheumatoid arthritis, and in a transitory fashion after myocardial infarction or surgical intervention (5,33,34,48–50).

There are variations both in its average concentration and in the abundance of isoforms in genetically different populations (51–54). Lp(a) plasma concentration in blacks is at least three times greater than in whites (55–58).

## Chemical Structure

Lp(a) composition is similar to that of LDLs. Both of these lipoproteins contain cholesterol, triglycerides, and phospholipids that may be dissolved and transported by plasma, due to the presence of a protein, apoB-100, which surrounds the lipid group and cholesterol (Figure 1).

The difference between them is that Lp(a) contains another glycoprotein, apo(a), which is bound to apoB-100 by a disulfide bridge between Cys in the 69 position for KIV-9 of apo(a) and Cys 3734 for apoB-100; the union is stabilized by hydrogen bonds and van der Waals interactions in other areas of both proteins (59–61). ApoB-100 (62) has the same structure and conformation in Lp(a) and the LDL molecule. The ratio apo(a):apoB-100 is 1:1 (63), and in considering their physicochemical variables there may be entities in a 2:1 ratio (64).

As usually occurs with some plasma proteins, Lp(a) may have different sizes with weights, ranging between 800 and 1300 kDa, and thus different densities. These differences are a reflection, although to a lesser extent, of lipid core composition, and especially of apo(a) structural polymorphism (65).

There are technical difficulties involved in obtaining apo(a) in its native form, and predictions of its secondary structure suggest an absence of an α-helix (66). Nevertheless, after reducing sulfhydryl binding, and by means of the circular dichroism technique, 8% α-helix, 21% β-sheet, and 71% random arrangements have been observed (67). Apo(a) belongs to the serine protease family, together with plasminogen, prothrombin, tissue plasminogen activator, urokinase-type plasminogen activator, and factor XII. These proteins derive from an ancestral gene common to all of them. Apo(a) is very similar to plasminogen. Genes codifying both proteins are very close, in chromosome 6, band q26-27 (35). The genes are a 50-kb distance apart in a head-to-head position (68,69) in the terminal region 5′. In the case of apo(a), the gene presents a polymorphism, which can be expressed by modifying the efficiency of the transcription. This, in turn, originates differences in Lp(a) plasma concentration, not only between individuals but between different ethnic groups (68). The apo(a) gene may have different sizes, each

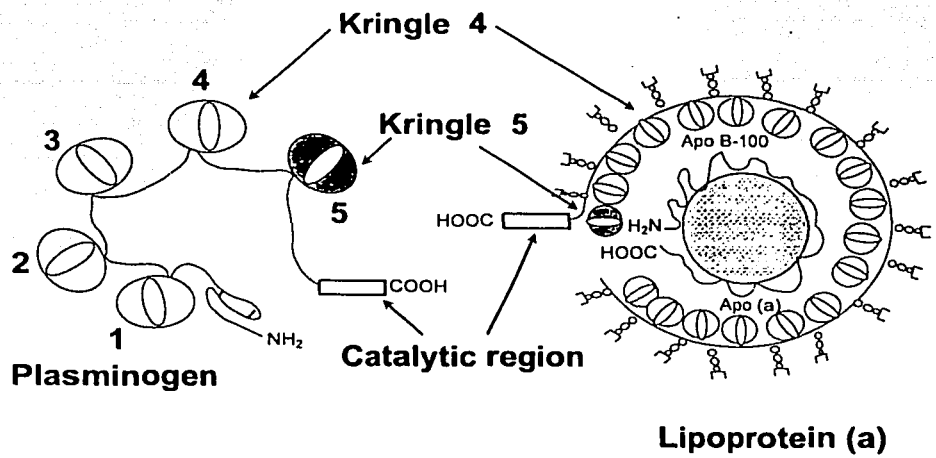



**Figure 1.** Chemical structure of lipoprotein(a) and plasminogen.

corresponding to the number of times in which a 5.5-kb sequence is present, with its number varying between 12 and 51 (3). The gene-promoting region of apo(a) possesses, in a distal position, a 1-kb fragment with a sequence showing sites with a different interaction potential. For interleukin-6 (IL-6), it shows seven sites; this conformation could explain the increase in Lp(a) plasma concentration during acute inflammation states and the three sites with specific elements (HNF-1, CEBP, and LF-A1) for hepatic transcription (69).

Apo(a) and plasminogen contain a protease region with a 94% similarity, but in the case of apo(a) it lacks the ability to become active and perform its enzymatic function due to the presence of arginine instead of serine at the activation site (3).

Plasminogen and apo(a) also include a different number of modules, called kringles. Through kringles they bind and recognize other macromolecules and/or specific sites in the cell membrane (73,74).

Kringles connect with each other by interkringle regions that are segments of 26–36 serine-, proline-, and/or threonine-rich amino acids (3); each interkringle region contains six potential O-glycosylation sites.

The kringle recognizance function includes the participation of a structure generated in the inner loop surrounding a hydrophobic region formed by different aromatic amino acids that are stabilized by means of hydrogen bonds and separate a cationic from an anionic group (73). This region is known as the lysine-binding site (LBS). Its structural characteristics generate a relatively rigid geometry that allows selective access and binding of 6.8-Å aliphatic or aromatic ligands of ω-amino-carboxylic acid type, such as ω-amino-hexanoic acid or similar compounds (74). Plasminogen comprises five very similar kringle types, but with small differences at the lysine-binding site, which modify the degree of affinity for different ligands. Plasminogen K I has a high-affinity LBS; the cationic pole has Arg-35 and Arg-71, and the anionic pole has Asp-55 and Asp-57 (74,75). The LBS in plasminogen K IV has an intermediate affinity; its V-shaped hydrophobic region generates a topography in which the aromatic rings of Phe-64, Trp-62, and Trp-72 separate the anionic group, which is formed by Asp-55 and Asp-57, and from the cationic group by Lys-35 and Arg-71 (76).

### Apolipoprotein (a) Polymorphism

Apo(a) shares kringle V and a variable number of kringle IVs with plasminogen (35). Not all kringles IV of apo(a) are alike; they are classified into 10 different subtypes (77). In the apo(a) molecule, each is present only once, except for kringle IV-2, which appears on multiple copies, originating structural heterogeneity and different size isoforms that account for molecular weights of between 280 and 800 kDa.

Kringle IV-10 is most similar to the kringle IV of plasminogen, with the high-affinity, lysine-binding site being formed by Asp-55 and Asp-57 in the anionic pole, and by Arg-71 and Arg-35 in the cationic pole. Between the two poles there is a hydrophobic microenvironment that is formed by three aromatic amino acids: Trp-62, Phe-64, and Trp-72. KIV-10 of apo(a) differs from K-IV of plasminogen due to the presence of Arg instead of Lys in position 35. This kringle has a very important role in the Lp(a) union with lysine (78), preventing plasminogen access to the fibrin clot, thus blocking the action of the tissue plasminogen activator (79–82). This generates fibrinolytic insufficiency that, in turn, promotes atherosclerosis and thrombosis.

Another apo(a) polymorphism source is glycosylation;

apo(a) KIV has a potential site for N-glycosylation. If kringle glycosylation is considered, 30% of each mole apo(a) corresponds to carbohydrates as follows: man-; galactose; galactosamine; glucosamine, and sialic acid atios of close to 3:7:5:4:7, respectively (83).

### a) Plasminogen Competitive Inhibitor

e fibrinolytic system destroys fibrin deposits in blood sels, either those remaining from hemostatic activity or e formed and accumulated during the development of atherosclerotic plaque. Because there is a balance be- en plasminogen activators and different types of inhibi- of these activators, its response varies either toward a ic condition or a fibrinolytic activity.

As a result of fibrinolytic activation, plasmin is gener- l, a proteolytic enzyme that may have effects on differ- plasma substrates. In the present case, however, this s not occur, because fibrin itself locates and signals the inolytic activation sites. Within the fibrin mesh, plasmin- n is bound precisely at the lysine-binding site and per- s and properly directs the binding of its tissue activator .. tissue plasminogen activator). Therefore, plasmin is erated in the inner section of the fibrin deposit, breaking and exposing other affinity sites such as terminal carboxy- ine residues, to amplify its response. Once fibrin is disin- rated, the mechanism stops, and the vascular endothe- m does not release additional activator; the remaining culating plasmin and tissue activator find their corre- nding inhibitors, α2-antiplasmin and type 1 tissue plas- nogen activator inhibitor (PAI-1).

The similarity between plasminogen and apolipoprotein(a) ows different apo(a) isoforms to compete with plasminogen fibrin affinity sites. The affinity of each isoform depends on size and its plasma concentration (7,84,85); in addition, the smin formed at the surface of fibrin may vary with modifi- ions of the concentration of Lp(a) *in vivo* (94).

Plasminogen and the different Lp(a) isoforms (86) also mpete for lysine residues on the surface receptors of en- thelial cells (81,87), U937 monocytes (87,88), platelets 9), mononuclear cells (86), and on matrices for *in vitro* odels simulating the extracellular membrane (90).

Another mechanism that alters the fibrinolytic system bal- ce is either the decrease or increase in synthesis of the tis- e plasminogen activator (t-PA) or of the tissue plasminogen tivator inhibitor (PAI-1), respectively (33,91), which is ob- rved in cultures of endothelial cells exposed to Lp(a).

## p(a) and Atherogenesis

p(a) favors atherogenesis through different mechanisms: acrophages phagocytize Lp(a) (92), migrate, and settle in e subendothelium, becoming transformed into foam cells, enerating deformities that decrease the lumen of the blood vessels. It has been observed that, in cultures of endothelial cells from the coronary artery, Lp(a) stimulates the expression of vascular adhesion molecule-1 (VCAM-1) and selectin E, a process that triggers attraction to macrophages (93). Another proposed mechanism is related to a decrease in plasmin generation that accounts for: (a) prolonged permanence of fibrin deposits (74), with the consequent increase of cholesterol deposits and formation of atherosclerotic plaque, and (b) decrease of activation, by partial hydrolysis with plasmin, of TGF-β, which prevents the growth of vascular smooth muscle cells.

### *Lp(a) Inhibits Fibrinolysis Depending on Apo(a) Isoforms*

A high plasma concentration of Lp(a) does not always interfere with normal fibrinolysis. Apo(a) isoforms show different antifibrinolytic activity (84,85), hence the importance of taking into account the antifibrinolytic activity of the isoforms in the prediction of cardiovascular diseases (95).

Many different strategies have been applied in *in vitro* studies. While making use of a solid-phase fibrin model (96–100), it has been possible to identify, with high specificity and sensitivity, fibrinolysis inhibition by apo(a) isoforms. These studies have permitted identification of the mechanisms and different variables involved in plasminogen competitive inhibition, due either to the different Lp(a) native isoforms (84) or through apo(a) recombinant forms (24).

These studies demonstrate the following: (1) Lp(a) affinity for fibrin shows an inverse relationship with the size of the apo(a) isoform within a $K_d$ range of 50–500 nM (84); (2) both plasminogen and Lp(a) compete for the same binding sites, which corresponds to a saturable competitive inhibition mechanism, and (3) Lp(a) antifibrinolytic potential depends on the affinity and concentration of each of the two apo(a) isoforms found in plasma (101).

## Lp(a) and Homocysteinemia

Hyperhomocysteinemia is related to an increase in the incidence of thrombotic and atherosclerotic diseases (102). Thrombosis is favored because of alterations in different antithrombosis-regulation mechanisms; in addition, it increases tissue factor activity, lowers the expression and activity of thrombomodulin necessary for protein C activation, and lowers the anticoagulant activity of antithrombin III as well as the binding capacity of the tissue plasminogen activator to its receptor on cell surfaces (anexin II) (103). In addition, an increased plasma homocysteine concentration manifests itself as an increase in Lp(a) binding to fibrin (104,105).

## Conclusions

Most epidemiological studies point out the relationship between lipoprotein (a) plasma concentration and the risk of suffering cardiovascular and cerebrovascular diseases, espe-

cially when other risk factors are present, such as high levels of low-density lipoproteins and smoking. It is also important to point out that there are differences in the concentration and in the presence/nonpresence of some isoforms within genetically different populations. Therefore, identification of fibrinolysis inhibition behavior of different Lp(a) isoforms necessitates applying a strategy to the study that will allow for the identification of individuals and ethnic groups suffering from fibrinolytic deficiency due to the presence of high concentrations of Lp(a). This line of investigation represents a challenge to the various disciplines in studying the role of Lp(a) in the pathogenesis and progression of atherosclerosis.

## Acknowledgments

This work was supported in part by the Institut National de la Santé et de la Recherche Médicale (INSERM), Project 4N001B (Réseau Nord-Sud). We are grateful for the expert help of Dr. Guadalupe Baños de MacCarthy in the preparation of this manuscript.

## References

1. Berg K. A new serum type system in man–the Lp system. Acta Pathol Microbiol Scand 1963;59:369.
2. Eaton DL, Fless GM, Kohr WJ, McLean JW, Xu QT, Miller CG, Lawn RM, Scanu AM. Partial amino acid sequence of apolipoprotein (a) shows that it is homologous to plasminogen. Proc Natl Acad Sci USA 1987;84:3224.
3. McLean JW, Tomlinson JE, Kuang WJ, Eaton OL, Chen EY, Fless GM, Scanu AM, Lawn RM. cDNA sequence on human apolipoprotein(a) is homologous to plasminogen. Nature 1987;330:132.
4. Berg K. Lp(a) Lipoprotein: an overview. Chem Phys Lipids 1992;67/68:9.
5. Dahlen GH. Lpta) Lipoprotein in cardiovascular disease. Atherosclerosis 1994;108:111.
6. Brown MS, Goldstein JL. Plasma lipoproteins: teaching old dogmas new tricks. Nature 1987;83:113.
7. Anglés-Cano E, Hervio L, Rouy D. Effects of lipoprotein(a) on the binding of plasminogen to fibrin and its activation by fibrin-bound tissue-type plasminogen activator. Chem Phys Lipids 1994;67/68;369.
8. Brown MS, Goldstein JL. Plasma lipoproteins: teaching old dogmas new tricks. Nature 1987;330:113.
9. MBewu AD, Durrington PN. Lipoprotein(a): structure, properties and possible involvement in thrombogenesis and atherogenesis. Atherosclerosis 1990;85:1.
10. Miles LA, Fless GM, Levin EG, Scanu AM, Plow EF. A potential basis for the thrombotic risks associated with lipoprotein (a). Nature 1989;339:301.
11. Nachman RL. Thrombosis and atherogenesis: molecular connections. Blood 1992;79:1897.
12. Peynet J, Beaudeux JL, Wolmant F, Flourie F, Giraudeaux V, Vicaut E, Launay JM. Apolipoprotein (a) size polymorphism in young adults with ischemic stroke. Atherosclerosis 1999;142:233.
13. Armstrong VW, Cremer P, Eberle E, Manke A, Schulze F, Wieland H, Kreuzer H, Seidel D. The association between Lp(a) concentrations and angiographically assessed coronary atherosclerosis. Atherosclerosis 1986;62:249.
14. Dahlen GH, Guyton JR, Attar M, Farmer JA, Kautz JA, Gotto AM. Association of levels of lipoprotein Lp(a), plasma lipids, and other lipoproteins with coronary artery disease documented by angiography. Circulation 1986;74:758.
15. Rosengren A, Wilhelmsen L, Eriksson E, Risberg B, Wedel H. Lipoprotein (a) and coronary heart disease: a prospective case-control study in a general population sample of middle aged men. BMJ 1990; 301:1248.
16. Cremer P, Nagel D, Labrot B. LDL cholesterol and other risk factors: results from the prospective Gottingen Risk Incidence and Prevalence Study (GRIPS). Eur J Clin Invest 1994;24:444.
17. Wald NJ, Law M, Watt HC, Wu T, Bailey A, Johnson AM, Craig WY, Ledue TB, Haddow E. Apolipoproteins and ischaemic heart disease: implications for screening. Lancet 1994;343:75.
18. Schaefer EJ, Lamon-Fava S, Jenner JL, MacNamara JR, Ordovas JM, Davis CE, Abolafia JM, Lippel K, Levy JR. Lipoprotein (a) levels and risk of coronary heart disease in men. The Lipid Research Clinics Coronary Primary Prevention Trial. JAMA 1994;271:999.
19. Bostom AG, Cupples LA, Jenner JL, Ordovas JM, Seman LJ, Wilson PW, Schaefer EJ, Castelli WP. Elevated plasma lipoprotein(a) and coronary heart disease in men aged 55 years and younger. A prospective study. JAMA 1996;276:544.
20. Dahlen GH, Stenlund H. Lp(a) lipoprotein is a major risk factor for cardiovascular disease: pathogenic mechanisms and clinical significance. Clin Genet 1997;52:272.
21. Bostom A, Gagnon D, Cupples A, Wilson P, Jenner J, Ordovas J, Schaefer E, Castelli W. A prospective investigation of elevated lipoprotein (a) detected by electrophoresis and cardiovascular disease in women. The Framingham Heart Study. Circulation 1994;90:1688.
22. Jauhiainen M, Koskinen P, Ehnholm E, Frick MH, Manttari M, Manninen V, Huttunen JK. Lipoprotein (a) and coronary heart disease risk. A nested case-control study of the Helsinki Heart Study participants. Atherosclerosis 1991;89:59.
23. Ridker PM, Hennekens CH, Stampfer MJ. A prospective study of lipoprotein (a) and the risk of myocardial infarction. JAMA 1993; 270:2195.
24. Rouy D, Koschinsky ML, Fleury V, Chapman MJ, Anglés-Cano E. Apolipoprotein(a) and plasminogen interactions with fibrin: a study with recombinant apolipoprotein(a) and isolated plasminogen fragments. Biochemistry 1992;31:6333.
25. Guo HC, Michel JB, Blouquit Y, Chapman MJ. Lipoprotein (a) and apolipoprotein (a) in a New World monkey, the common marmoset (*Callithrix jacchus*). Arterioscler Thromb 1991;11:1030.
26. Laplaud M, Beaubatie L, Rall SC, Luc G, Saboureau M. Lipoprotein (a) is the major apoB-containing lipoprotein in the plasma of a hibernator, the hedgehog (*Erinaceus europaeus*). J Lipid Res 1988;29: 1157.
27. Rath M, Pauling L. Immunological evidence for the accumulation of lipoprotein (a) in the atherosclerotic lesion of the hyperascorbemic guinea pig. Proc Natl Acad Sci USA 1990;87:9388.
28. Kostner GM. The physiological role of Lp(a). In: Scanu AM, editor. Lipoprotein (a). San Diego, CA: Academic Press:1990. p. 183.
29. Cardoso GC, Posadas C, Orvañanos OO, Peniche C, Zamora J, Aguilar R, Olguín JA, Raynaud S, Morriset JD, Guevara J Jr. Long distance runners and body-builders exhibit elevated plasma levels of lipoprotein (a). Chem Phys Lipids 1994;67/68:207.
30. Lawn RM, Tomlinson JE, McLean JW, Eaton DL. Molecular biology of lipoprotein(a). In: Scanu AM, editor. Lipoprotein(a). San Diego, CA: Academic Press;1990. p. 25.
31. Kraft HG, Menzel HJ, Hoopichler F, Vogel W, Uterman G. Changes of genetic apolipoprotein phenotypes caused by liver transplantation. J Clin Invest 1989;83:137.
32. Kostner GM, Wo X, Frank S, Kostner K, Zimmermann R, Steyrer E. Metabolism of Lp(a): assembly and excretion. Clin Genet 1997; 52:347.
33. Anglés-Cano E, Hervio L, Loyau S. Lipoproteine, hypofibrinolyse et atherothrombose, quo vadis? STV 1995;7:315.
34. Aznar J, Estelles A. Lipoproteína (a) y trombosis. Rev Iberoam Tromb Hemostasia 1993;2:123.
35. Utermann G. The mysteries of lipoprotein (a). Science 1989;246:904.
36. Anglés-Cano E, Loyau S, Cardoso-Saldaña G, Courdec R, Gillery P.

A novel kringle 4 number-based recombinant apo(a) standard for human apo(a) phenotyping. J Lipid Res 1999;40:354.
Lackner C, Boerwinkle E, Leffert C, Rahming T, Hobbs H. Molecular basis of apolipoprotein (a) isoform size heterogeneity as revealed by pulsed-field gel electrophoresis. J Clin Invest 1991;87:2153.
Gaw A, Boerwinkle E, Cohen JC, Hobbs HH. Comparative analysis of the apo(a) gene, apo(a) glycoprotein, and plasma concentrations of Lp(a) in three ethnic groups. Evidence for non common lnulli allele at the apo(a) locus. J Clin Invest 1994;93:2526.
Utermann G, Menzel HJ, Kraft G, Duba HC, Kemmler HG, Seitz C. Lp(a) glycoprotein phenotypes: inheritance and relation to Lp(a)-lipoprotein concentrations in plasma. J Clin Invest 1987;80:458.
Brunner C, Lobentanz EM, Petho-Schramm A, Ernst A, Kang C, Dieplinger H, Jomuller HJ, Utermann G. The number of identical kringle IV repeats in apolipoprotein(a) affects its processing and secretion by HepG2 cells. J Biol Chem 1996;271:32403.
Haenggi W, Reisen W, Birkhaeuser MH. Postmenopausal hormone replacement therapy with tibolone decreases serum lipoprotein(a). Eur J Clin Biochem 1993;31:645.
Henriksson P, Angelin B, Berglund D. Hormonal regulation of serum Lp(a) levels. J Clin Invest 1992;89:1166.
Soma MR, Meschia M, Bruschi F, Morriset JD, Paoletti R, Fumagalli R, Cosignani P. Hormonal agents used in lowering lipoprotein (a). Chem Phys Lipids 1994;67:345.
Taskinen MR, Puolakka J, Pyorala T, Luotola H, Bjaorn M, Kaarianen J, Lahdenpera S, Ehnholm C. Hormone replacement therapy lowers plasma Lp(a) concentrations. Comparison of cyclic transdermal and continuous estrogen–progestin regimens. Arterioscler Thromb Vasc Biol 1996;16:1215.
Albers JJ, Taggart HM, Applebaum-Bowden D, Haffner S, Chesnut CH, Hazzard WR3d. Reduction on lecithin-cholesterol actyltransferase, apolipoprotein D and the Lp(a) lipoprotein with the anabolic steroid stanazol. Biochim Biophys Acta 1984;795:293.
Eden F, Wiklund O, Oscarsson J, Rosen T, Bengtsson BA. Growth hormone treatment of growth hormone-deficient adults result in a marked increase in Lp(a) and HDL cholesterol concentrations. Arterioscler Thromb 1993;13:296.
Loscalzo J. Lp(a) and the fibrinolytic system. In: Scanu AM, editor. Lipoprotein(a). San Diego, CA: Academic Press;1990. p. 103.
Noma A, Abe A, Maeda S, Seishima M, Makino K, Yano Y, Shimokawa K. Lp(a): an acute phase reactant? Chem Phys Lipids 1994;67/68:411.
Dahlen GH. Indications of an autoimmune component in Lp(a) associated disorders. Eur J Immunogenet 1994;21:301.
Andreassen AK, Berg K, Torsvik H. Changes in Lp(a) lipoprotein and other plasma proteins during acute myocardial infarction. Clin Genet 1994;46:410.
Klausen IC, Gerdes UL, Schmidt BE, Dyerberg J, Faergeman O. Differences in apolipoprotein (a) polymorphism in West Greenland Eskimos and Caucasian Danes. Hum Genet 1992;89:384.
Marcovina S, Albers JJ, Jacobs DR, Perkins LL, Lewis CE, Howard B, Savage P. Lipoprotein (a) concentrations and apolipoprotein (a) phenotypes in Caucasians and African Americans. The CARDIA study. Arterioscler Thromb 1993;13:1037.
Abe A, Noma A, Itakura H. Lipoprotein (a) phenotyping using a computerized micro scale and phenotype frequencies in a healthy Japanese population. Clin Chim Acta 1993;219:149.
Kraft HG, Windegger M, Menzel HJ, Uterman G. Significant impact of the −93 C/T polymorphism in the apolipoprotein (a) gene on Lp(a) concentrations in Africans but not in Caucasians: confounding effect of linkage. Hum Mol Genet 1998;7:257.
Cobbaert C, Mulder P, Lindemans J, Kesteloot H. Serum LP(a) levels in African aboriginal pygmies and Bantus, compared with Caucasian and Asian population samples. J Clin Epidemiol 1997;50:1045.
Mooser V, Scheer D, Marcovina SM, Wang J, Guerra R, Cohen J, Hobbs HH. The apo(a) gene is the major determinant of variation in plasma Lp(a) levels in African Americans. Am J Hum Genet 1997;61:402.
Marcovina SM, Albers JJ, Wijsman E, Zhang Z, Chapman NH, Kennedy H. Differences in Lp(a) concentrations and apo(a) polymorphs between black and white American. J Lipid Res 1996;37:2569.
Schreiner PJ, Heiss G, Tyroler HA, Morriset JD, Davis CE, Smith R. Race and gender differences in the association of Lp(a) with carotid artery wall thickness. The arteriosclerosis risk in communities (ARIC) study. Arterioscler Thromb Vasc Biol 1996;16:471.
Guevara J, Spurlino J, Jan AY, Yang CY, Tulinsky A, Prasad BVV, Gaubatz JW, Morriset JD. Proposed mechanisms for binding of apo(a) kringle type 9 to apo B-100 in human lipoprotein(a). Biophys J 1993;64:686.
Fless GM, Snyder ML, Furbee JWJ, García-Hedo MT, Mora R. Subunit composition of lipoprotein (a) protein. Biochemistry 1994;33: 13492.
Sommer A, Georges R, Kostner GM, Paltauf F, Hermetter A. Sulfhydryl-selective fluorescence labeling of lipoprotein (a) reveals evidence for one single disulfide linkage between apoprotein(a) and B-100. Biochemistry 1991;30:11245.
Huby T, Doucet C, Dieplinger H, Chapman J, Thillet J. Structural domains of apolipoprotein(a) and its interaction with apolipoprotein B-100 in the lipoprotein(a) particle. Biochemistry 1994;33:3335.
Albers JJ, Kennedy H, Marcovina SM. Evidence that Lp(a) contains one molecule of apo(a) and one molecule of apo B: evaluation of aminoacid analysis data. J Lipid Res 1996;37:192.
Fless GM, Snyder ML, Furbee JWJ, García-Hedo MT, Mora R. Subunit composition of lipoprotein(a) protein. Biochemistry 1994;33: 13492.
Kamboch ML, Ferrel RE, Kottke BA. Expressed hypervariable polymorphism of apolipoprotein (a). Am J Hum Genet 1991;49:1063.
Guevara J, Knapp RD, Honda S, Northup SR, Morriset JD. A structural assessment of the apo(a) protein of human lipoprotein(a). Proteins 1992;12:188.
Fless GM, ZumMallen ME, Scanu AM. Physicochemical properties of apolipoprotein (a) and Lp(a-) derived from the dissociation of human plasma Lp(a). J Biol Chem 1986;261:8712.
Suzuki K, Kuriyama M, Saito T, Ichinose A. Plasma lipoprotein(a) and expression of the apolipoprotein(a) gene are dependent on the nucleotide polymorphisms in its 5'-flanking region. J Clin Invest 1997;97:1361.
Ichinose A, Kuriyama M. Detection of polymorphisms in the 5'-flanking region of the gene for apo(a). Biochem Biophys Res Commun 1995;209:372.
Vali Z, Patthy L. Location of the intermediate and high affinity ω-aminocarboxylic acid binding sites in human plasminogen. J Biol Chem 1982;257:2104.
Hajjar KA, Nachman RL. The role of lipoprotein(a) in atherogenesis and thrombosis. Annu Rev Med 1996;47:423.
McLean JW, Tomlinson JE, Kuang WJ, Eaton DL, Chen EY, Fless GM, Scanu AM, Lawn RM. cDNA sequence of human apolipoprotein (a) is homologous to plasminogen. Nature 1987;330:132.
Tulinsky A, Park CH, Mao B, Llinas M. Lysine/fibrin binding sites of kringles modeled after the structure of kringle 1 of prothrombin. Proteins 1988;3:85.
Boonmark NW, Lawn RM. The lysine-binding function of Lp(a). Clin Genet 1997;52:355.
Menhart N, Sehl LC, Kelley RF, Castellino FJ. Construction, expression and purification of recombinant kringle 1 of human plasminogen and analysis of its interactions with ω-amino acids. Biochemistry 1991;30:1948.
Rejante MR, Byeon IJL, Llinas M. Ligand specificity of human plasminogen kringle 4. Biochemistry 1991;30:11081.
Guevara J, Knapp RD, Tulinsky A, Morriset JD. Comparison of ligand binding sites of modeled apo(a) kringle like sequences in human lipoprotein (a). Arterioscler Thromb 1993;13:758.
Sangrar W, Marcovina SM, Koschinsky ML. Expression and characterization of apolipoprotein(a) kringle IV types 1, 2 and 10 in mammalian cells. Prot Eng 1994;7:723.

79. Binder BR. Physiology and pathophysiology of the fibrinolytic system. Fibrinolysis 1995;9:3.
80. Anglés-Cano E. Overview on fibrinolysis: plasminogen activation pathways on fibrin and cell surfaces. Chem Phys Lipids 1994;67/68:353.
81. Hajjar KA, Gavish D, Breslow JL, Nachman RL. Lipoprotein (a) modulation of endothelial cell surface fibrinolysis and its potential role in atherosclerosis. Nature 1989;339:303.
82. Liu JN, Kung W, Harpel PC, Gurewich V. Demonstration of covalent binding of lipoprotein (a), [Lp(a)] to fibrin and endothelial cells. Biochemistry 1998;37:3949.
83. Fless GM, ZumMallen ME, Scanu S. Physicochemical properties of apolipoprotein (a) and Lp(a-) derived from the dissociation of human plasma Lp(a). J Biol Chem 1986;261:8712.
84. Hervio L, Durlach V, Girard-Globa A, Anglés-Cano E. Multiple binding with identical linkage: a mechanism that explains the effect of lipoprotein (a) on fibrinolysis. Biochemistry 1995;34:13353.
85. Hervio L, Chapman J, Thillet J, Loyau S, Anglés-Cano E. Does apolipoprotein (a) heterogeneity influence lipoprotein (a) effects on fibrinolysis? Blood 1993;82:392.
86. Kang C, Durlach V, Soulat T, Fournier C, Anglés-Cano E. Lipoprotein(a) isoforms display differences in affinity for plasminogen-like binding to human mononuclear cells. Arterioscler Thromb Vasc Biol 1997;17:2036.
87. Miles LA, Fless GM, Scanu AM, Baynham P, Sebald MT, Skocir P, Curtiss LK, Levin EG, Hoover-Plow JL, Plow EF. Interaction of Lp(a) with plasminogen binding sites on cells. Thromb Haemost 1995;73:458.
88. González-Gronow M, Edelberg JM, Pizzo SV. Further characterization of the cellular plasminogen binding site: evidence that plasminogen 2 and lipoprotein (a) compete for the same site. Biochemistry 1989;28:2374.
89. Ezralty A, Simon DI, Loscalzo J. Lipoprotein(a) binds to human platelets and attenuates plasminogen binding and activation. Biochemistry 1993;32:4628.
90. Miles LA, Sebald ST, Fless GM, Scanu AM, Curtiss LK, Plow EF, Hoover-Plow JL. Interaction of lipoprotein (a) with the extracellular matrix. Fibrinol Proteol 1998;12:79.
91. Li XN, Grenett HE, Benza RL, Demissie S, Brown SL, Tabengwa EM, Gianturco SH, Bradley WA, Fless GM, Booyse FM. Genotype-specific transcriptional regulation of PAI-1 expression by hypertriglyceridemic VLDL and Lp(a) in cultured human endothelial cells. Arterioscler Thromb Vasc Biol 1997;17:3215.
92. Harpel P, Hermann A, Zhang X, Ostfeld I, Borth W. Lipoprotein(a), plasmin modulation, and atherogenesis. Thromb Haemost 1995; 74:382.
93. Allen S, Khan S, Tam S, Koschinsky M, Taylor P, Yacoub M. Expression of adhesion molecules by Lp(a): a potential novel mechanism for its atherogenicity. FASEB J 1998;12:1765.
94. Soulat T, Loyau S, Baudouin V, Durlach V, Gillery P, Garnotel R, Loirat C, Anglés-Cano E. Evidence that modifications of Lp(a) *in vivo* inhibit plasmin formation on fibrin, a study with individual plasmas presenting natural variations of Lp(a). Thromb Haemost 1999;82:121.
95. Anglés-Cano E, Hervio L, Loyau S. Relevance of lipoprotein (a) in cardiovascular disease: methodological approaches. Fibrinolysis 1993;7:66.
96. Fleury V, Anglés-Cano E. Characterization of the binding of plasminogen to fibrin surfaces: the role of carboxy-terminal lysines. Biochemistry 1991;30:7630.
97. Fleury V, Loyau S, Lijnen HR, Nieuwenhuizen W, Anglés-Cano E. Molecular assembly of plasminogen and tissue-type plasminogen activator on an evolving fibrin surface. Eur J Biochem 1993;216:549.
98. Rouy D, Luplaud PM, Saboreau M, Anglés-Cano E. Hedgehog lipoprotein(a) is a modulator of activation of plasminogen at the fibrin surface. An *in vitro* study. Arterioscler Thromb 1992;12:146.
99. Anglés-Cano E. A spectrophotometric solid-phase fibrin-tissue plasminogen activator activity assay (SOFIA-tPA) for high-fibrin-affinity tissue plasminogen activators. Anal Biochem 1986;43:129.
100. Rouy D, Grailhe P, Nigon F, Chapman J, Anglés-Cano E. Lipoprotein(a) impairs generation of plasmin by fibrin-bound tissue-type plasminogen activator. *In vitro* studies in a plasma milieu. Arterioscler Thromb 1991;11:629.
101. Hervio L, Girard Globa A, Durlach V, Anglés-Cano E. The antifibrinolytic effect of lipoprotein(a) in heterozygous subjects is modulated by the relative concentration of each of the apolipoprotein (a) isoforms and their affinity for fibrin. Eur J Clin Invest 1996; 26:411.
102. Selhub J, D'Angelo A. Hyperhomocysteinemia and thrombosis: acquired conditions. Thromb Haemost 1997;78:527.
103. Harpel PC. Homocysteine, atherogenesis and thrombosis. Fibrinol Proteol 1997;11:77.
104. Scanu AM, Edelstein C. Kringle-dependent structural and functional polymorphism of apolipoprotein (a). Biochim Biophys Acta 1995; 1256:1.
105. Gavish D, Breslow JL. Lipoprotein(a) reduction by N-acetylcysteine. Lancet 1991;337:203.

# *Lipoproteína (a), ¿es un factor de riesgo en la enfermedad aterotrombótica coronaria?*

Aurora de la Peña,* Raúl Izaguirre,* Eduardo Anglés-Cano**

**Palabras clave:** Lipoproteína (a). Aterotrombosis. Enfermedad arterial coronaria. Fibrinólisis.
**Key words:** Lipoprotein (a). Atherothrombosis. Coronary artery disease. Fibrinolysis.
(Arch Cardiol Mex 2001; 71:188-192).

A pesar de que numerosos estudios *in vitro* e *in vivo* confirman la participación de la Lp(a) en los procesos fisiopatológicos de la aterosclerosis y de que los estudios retrospectivos consistentemente confirman la asociación entre Lp(a) y la enfermedad isquémica coronaria y cerebral, algunos estudios prospectivos han reportado resultados inconsistentes.

Varios motivos contribuyen en esta controversia, entre ellos destacan la falta de un estándar universal de referencia, el método analítico de estudio, las condiciones en el manejo de la muestra y sobre todo las características estructurales distintivas de la partícula de la lipoproteína (a) o Lp(a). La Lp(a) está formada por una lipoproteína de baja densidad o LDL[1] que contiene un núcleo de colesterol, triglicéridos y fosfolípidos, rodeado de una proteína apoB-100 a la cual se une la glicoproteína apo(a). Como es frecuente en las lipoproteínas plasmáticas, la Lp(a) puede tener diferentes tamaños; estas diferencias se deben, en menor medida, a la composición del núcleo de lípidos y, principalmente, al polimorfismo estructural de apo(a).[2] La apo(a) pertenece a la familia de serino proteasas, al igual que el plasminógeno, la protrombina, el activador tisular del plasminógeno, el activador del plasminógeno tipo urocinasa y el factor XII. Estas proteínas derivan de un gene ancestral común a todas ellas.

La apo(a) es muy parecida al plasminógeno *(Fig. 1)*. Los genes que codifican para ambas proteínas se encuentran muy cercanos, pero el gene de la apo(a) tiene la peculiaridad de tener diferentes tamaños que dependen del número de veces que se repite una secuencia de 5.5 kb. El tamaño de las isoformas de la apo(a) tienen una relación inversa con la concentración plasmática de la Lp(a)[3] y a medida que se incrementa el tamaño de apo(a), se incrementa la dificultad para secretarse de las células hepáticas, lo que da lugar a diferencias en la concentración plasmática de Lp(a), no sólo entre individuos, sino entre diferentes grupos étnicos. Algunas hormonas pueden modificar su concentración plasmática: la hormona tiroidea,[4] los estrógenos,[5-8] y los esteroides anabólicos la reducen[9] en tanto la hormona de crecimiento la incrementa,[10] pero no puede modificarse por medicamentos hipolipemiantes o por la dieta.[11]

La apo(a) y el plasminógeno, el precursor de la plasmina, tienen una región proteasa muy similar. La presencia de arginina en lugar de serina en el sitio de activación impide que la apo(a) pueda ser activada y por ende que esta región exprese una actividad enzimática.

* Instituto Nacional de Cardiología "Ignacio Chávez". (INCICH. Juan Badiano No. 1, 14080, México, D.F).
** INSERM U460, Remodelage CardioVasculaire, UFR de Médicine Xavier-Bichat, 16 rue Henri Huchard-BP 416, F-75870-Cdx, Paris, France.

Correspondencia:
Dr. Eduardo Anglés-Cano. INSERM U460, Remodelage CardioVasculaire, UFR de Médicine Xavier-Bichat, 16 rue Henri Huchard-BP 416, F-75870-Cdx, Paris, France. Tel 33 1 44 85 61 50 Fax 33 1 44 85 61 56 E-mail: angles@infobiogen.fr

Recepción: 16 de abril de 2001.
Aceptado: 3 de mayo de 2001.

Esta similitud estructural y diferencia enzimática despertó el interés de numerosos grupos de investigación que encontraron en la Lp(a) el punto común entre la aterosclerosis y la trombosis.[12-18] Bajo esta perspectiva, se realizaron numerosos estudios epidemiológicos, que describen una asociación positiva entre la elevada concentración plasmática de Lp(a) y un incremento en las enfermedades cerebrovasculares,[19] cardiovasculares, la reestenosis de puentes coronarios, la reoclusión de angioplastías y el desarrollo prematuro de aterosclerosis asociada a altas concentraciones de LDL y/o a bajas concentraciones de HDL.[20,21] La mayoría de estudios prospectivos confirman estos resultados.[22-28] Aun cuando no existe un acuerdo en el límite de corte para considerar una concentración plasmática normal, algunos autores consideran que es de 20 mg/dL, otros hasta 30 mg/dL y que el empleo de anticuerpos diferentes, monoclonales o policlonales, generan divergencias en los resultados.

Sin embargo, otros estudios no encuentran asociación entre la Lp(a) y la enfermedad arterial coronaria.[29,30] Esta discrepancia en los resultados podría ser el reflejo de la gran heterogeneidad estructural de la molécula de apo(a), que se traduce en una heterogeneidad funcional como inhibidor competitivo del plasminógeno por la fibrina, disminuyendo así la formación de plasmina.[15,31]

Craig y col.[32] hacen un meta-análisis de diferentes estudios prospectivos en población caucásica, evalúan a la Lp(a) como factor de riesgo en la enfermedad arterial coronaria, ponen especial atención en los criterios de selección de los trabajos a fin de que los resultados fueran comparables. Concluyen que en los individuos que desarrollaron enfermedad arterial coronaria la concentración de Lp(a) es mayor que en el grupo control (excepto en los pacientes masculinos de un estudio)[33] y sugieren que la variabilidad puede ser efecto del manejo pre-analítico de la muestra, especialmente de la temperatura en que ésta haya sido almacenada.

Recientemente[34] un meta-análisis de 27 estudios prospectivos de Lp(a) y enfermedad isquémica coronaria, concluye que independientemente del lugar geográfico del estudio, edad promedio de los individuos, tiempo de duración del estudio, métodos de ensayo y grado de ajuste de las variables de confusión, el riesgo relativo es de 1.7 (intervalo de confianza 95% de 1.4-1.9, 2P < 0.00001) para 18 estudios en población general que incluyeron un total de 4,044 casos, y un riesgo relativo de 1.3 (intervalo de confianza 95 de 1.1-1.6, 2P < 0.001) para 9 estudios que incluyeron un total de 1,392 individuos con antecedentes de enfermedad coronaria. Este meta-análisis incluyó algunos de los trabajos analizados por

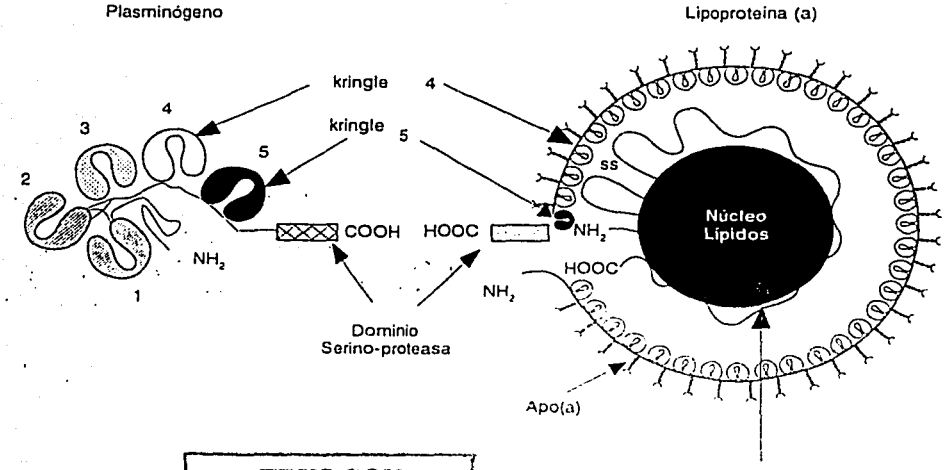



Fig. 1.





27

Craig y col. pero con un criterio diferente de evaluación; comparó la concentración plasmática de Lp(a) en el tercil superior contra el tercil más bajo de distribución.

En ambos meta-análisis resalta sin lugar a dudas, la asociación que existe entre la Lp(a) y la enfermedad isquémica coronaria, frecuentemente con isoformas de bajo peso molecular de apo(a);[35] sin embargo hasta la fecha no es posible afirmar si este hecho es un factor directo de riesgo aterotrombótico o si el riesgo se favorece a través de permitir una mayor concentración plasmática de Lp(a).

El hecho de que la Lp(a) inhibe la fibrinólisis, ofrece un mecanismo fisiopatológico congruente con ambas situaciones;[36] explica porqué una alta concentración de Lp(a) se refleja en diferentes grados de riesgo aterotrombótico que dependen de las isoformas de apo(a)[37] y pone de manifiesto la importancia cualitativa y cuantitativa de las interacciones entre la apo(a), el plasminógeno y la fibrina.

Empleando un modelo de fibrina en fase sólida,[38-42] ha sido posible identificar de manera sensible y específica la inhibición de la fibrinólisis por las isoformas de apo(a). Los estudios concluyen que el potencial anti-fibrinolítico de Lp(a) depende de la concentración y afinidad de cada una de las dos isoformas de apo(a)[43] y muestran una relación inversa con el tamaño de la isoforma en un rango de 50 a 500 nM. Este dato se comprueba al observar que las isoformas de apo(a) de bajo peso molecular son indicadores importantes en la aterosclerosis obstructiva.[35]

El estudio de la Lp(a) en la enfermedad aterotrombótica sigue representando un reto multidisciplinario, en donde una estrategia objetiva de estudio subraye la importancia de considerar la actividad antifibrinolítica de las isoformas en la predicción de la enfermedad cardiovascular.[44] Deberán corregirse también, para determinar la concentración plasmática de Lp(a), la falta de acuerdo que existe para el manejo pre-analítico de la muestra, el empleo de un estándar y técnica de referencia y el empleo de anticuerpos monoclonales o policlonales.

Es también importante llevar a cabo estudios en poblaciones sanas, con la finalidad de identificar a las poblaciones en las que la Lp(a) represente un factor de morbilidad y mortalidad importante y conocer su impacto en la salud pública. Resalta la ausencia de estudios en la población latinoamericana a pesar del gran interés que el tema ha cobrado en los últimos años.

## Referencias


1. Gaubatz JW, Heideman C, Gotto AM, Morrisett JD, Dahlen GH: *Human plasma lipoprotein(a)-structural properties.* J Biol Chem 1983; 258: 4582-4589.
2. Kamboch ML, Ferrel RE, Kottke BA: *Expressed hypervariable polymorphism of apolipoprotein(a).* Am J Hum Gent 1991; 49: 1063-1074.
3. Utermann G, Menzel HJ, Kraft G, Duba HC, Kemmler HG, Seitz C: *Lp(a) glycoprotein phenotypes: inheritance and relation to Lp(a)-lipoprotein concentrations in plasma.* J Clin Invest 1987; 80: 458-465.
4. De Bruin TWA, van Barlingen H, van Linde-Sibenius, Trip M, van Vuurst de Vries AR, Akveld MJ, Erkelens WD: *Lipoprotein (a) and apolipoprotein B plasma concentrationis in hypotyroid, euthiroid and hyperthiroid subjects.* J Clin Endocrinol Metab 1993; 76: 121-126.
5. Haenggi W, Reisen W, Birkhaeuser MH: *Postmenopausal hormone replacement therapy with tibolone decreases serum lipoprotein(a).* Eur J Clin Biochem 1993; 31: 645-650.
6. Henriksson P, Angelin B, Berglund D: *Hormonal regulation of serum Lp(a) levels.* J Clin Invest 1992; 89: 1166-1171.
7. Soma MR, Meschia M, Bruschi F, Morriset JD, Paoletti R, Fumagalli R, Cosignani P: *Hormonal agents used in lowering lipoprotein(a).* Chem Physics Lipids 1994; 67: 345-350.
8. Taskinen MR, Puolakka J, Pyorala T, Luotola H, Bjaorn M, Kaarianen J, et al: *Hormone replacement therapy lowers plasma Lp(a) concentrations. Comparison of cyclic transdermal and continuos estrogen-progestin regimens.* Arterioscler Thromb Vasc Biol 1996; 16: 1215-1221.
9. Albers JJ, Taggart HM, Applebaum-Bowden D, Haffner S, Chesnut CH, Hazzard WR: *Reduction on lecithin-cholesterol acyltransferase, apolipoprotein D and the Lp(a) lipoprotein with the anabolic steroid stanazol.* Biochim Biophys Acta 1984; 795: 293-296.
10. Eden F, Wiklund O, Oscarsson J, Rosen T, Bengtsson BA: *Growth hormone treatment of growth hormone-deficient adults result in a marked increase in Lp(a) and HDL cholesterol concentrations.* Artherioscler Thromb 1993; 13: 296-301.
11. Anglés-Cano E, Hervio L, Loyau S: *Lipoprotéine, hypofibrinolyse et athérothrombose, quo vadis?* Sang Thrombose Vaisseaux 1995; 7: 315-322.

12. Berg K: *Lp(a) Lipoprotein: an overview.* Chem Phys Lipids 1992; 67/68: 9-18.
13. Dahlén GH: *Lp(a) Lipoprotein in cardiovascular disease.* Atherosclerosis 1994; 108: 111-126.
14. Brown MS, Goldstein JL: *Plasma lipoproteins: teaching old dogmas new tricks.* Nature 1987; 330: 113-114.
15. Anglés-Cano E, Hervio L, Rouy D: *Effects of lipoprotein(a) on the binding of plasminogen to fibrin and its activation by fibrin-bound tissue-type Plasminogen activator.* Chem Phys Lipids 1994; 67/68: 369-380.
16. MBewu AD, Durrington PN: *Lipoprotein(a): structure, properties and possible involvement in thrombogenesis and atherogenesis.* Atherosclerosis 1990; 85: 1-14.
17. Miles LA, Fless GM, Levin EG, Scanu AM, Plow EF: *A potential basis for the thrombotic risks associated with lipoprotein (a).* Nature 1989; 339; 301-303.
18. Nachman RL. *Thrombosis and atherogenesis: molecular conections.* Blood 1992; 79: 1897-1906.
19. Peynet J, Beauldeux JL, Wolmant F, Flourie F, Giraudeaux V, Vicaut E, Launay JM: *Apolipoprotein (a) size polymorphism in young adults with ischemic stroke.* Atherosclerosis 1999; 142: 233-239.
20. Armstrong VW, Cremer P, Eberle E, Manke A, Schulze F, Wieland H, et al: *The association between LP(a) concentrations and angiographically assessed coronary atherosclerosis.* Atherosclerosis 1986; 62: 249-257.
21. Dahlén GH, Guyton JR, Attar M, Farmer JA, Kautz JA, Gotto AM: *Association of levels of lipoprotein Lp(a), plasma lipids, and other lipoproteins with coronary artery disease documented by angiography.* Circulation 1986; 74: 758-765.
22. Rosengren A, Wilhelmsen L, Eriksson E, Risberg B, Wedel H: *Lipoprotein (a) and coronary heart disease: a prospective case-control study in a general population sample of middle aged men.* Br Med J 1990; 301: 1248-1251.
23. Cremer P, Nagel D, Labrot B: *LDL cholesterol and other risk factor: results from the prospective Göttingen Risk Incidence and Prevalence Study (GRIPS).* Eur J Clin Invest 1994; 24: 444-453.
24. Wald NJ, Law M, Watt HC, Wu T, Bailey A, Johson AM, et al: *Apolipoproteins and ischaemic heart disease: implications for screening.* Lancet 1994; 343: 75-79.
25. Schaefer EJ, Lamon-Fava S, Jenner JL, Mc Namara JR, Ordovas JM, Davis CE, et al: *Lipoprotein (a) levels and risk of coronary heart disease in men. The lipid research clinics coronary primary prevention trial.* JAMA 1994; 271: 999-1003.
26. Bostom A, Gagnon D, Cupples A, Wilson P, Jenner J, Ordovas J, et al: *Prospective investigation of elevated lipoprotein (a) detected by electrophoresis and cardiovascular disease in women.* The Framingham Heart Study. Circulation 1994; 90: 1688-1699.
27. Dahlén GH, Stenlund H: *Lp(a) lipoprotein is a major risk factor for cardiovascular disease: pathogenic mechanisms and clinical significance.* Clin Genet 1997; 52: 272-280.
28. von Eckardstein A, Schulte H, Cullen P, Assmann G: *Lipoprotein (a) further increases the risk of coronary events in men with high global cardiovascular risk.* Am Coll Cardiol 2001; 37: 434-439.
29. Jauhiainen M, Koskinen P, Ehnholm, Frick MH, Manttari M, Manninen V, Huttunen JK: *Lipoprotein (a) and coronary heart disease risk. A nested case-control study of the Helsinki Heart Study participants.* Atherosclerosis 1991; 89: 59-67.
30. Ridker PM, Hennekens CH, Stampfer MJ: *A prospective study of lipoprotein (a) and the risk of myocardial infarction.* JAMA 1993; 270: 2195-2199.
31. Rouy D, Koschinsky ML, Fleury V, Chapman MJ, Anglés-Cano E: *Apolipoprotein(a) and plasminogen interactions with fibrin: a study with recombinant apolipoprotein(a) and isolated plasminogen fragments.* Biochemistry 1992; 31: 6333-6339.
32. Craig W, Neveux M, Palomaki G, Cleveland M, Haddow J: *Lipoprotein(a) as a risk factor for ischemic heart disease: metaanalysis of prospective studies.* Clin Chem 1998; 44: 2301-2306.
33. Alfthan G, Pekkanen J, Jauhiainen M, Pitkaniemi J, Karvonen M, Tuomilehto J, et al: *Relation of serum homocysteine and lipoprotein(a) concentrations to atherosclerotic disease in a prospective Finish population based study* . Atherosclerosis 1994; 106: 9-19.
34. Danesh J, Collins Rory, Peto Richard: *Lipoprotein(a) and coronary heart disease meta-analysis of prospective studies.* Circulation 2000; 102: 1082-1085.
35. Kronenberg F, Kronenberg MF, Kiechl S, Trenwalder E, Santer P, Oberhollenzer F, et al: *Role of lipoprotein(a) and apolipoprotein(a) phenotype in atherogenesis: prospective results from the Bruneck study.* Circulation 1999; 100: 1154-1160.
36. Anglés-Cano E: *High antifibrinolytic activity of lipoprotein(a) containing small apolipoprotein(a) isoforms.* Circulation 2000; 102: E184.
37. Soulat T, Loyau S, Baudouis V, Maisonneuve L, Hurtaud-Roux MF, Schlegel N, et al: *Evidence that modifications of Lp(a) in vivo inhibits plasmin formation on fibrin.* Thromb Haemost 1999; 82: 121-127.
38. Fleury V, Anglés-Cano E: *Characterization of the binding of plasminogen to fibrin surfaces: the role of carboxy-terminal lysines.* Biochemistry 1991: 30: 7630-7638.
39. Fleury V, Loyau S, Lijnen HR, Nieuwenhuizen W, Anglés-Cano E: *Molecular assembly of plasminogen and tissue-type plasminogen activator on an evolving fibrin surface.* Eur J Biochem 1993; 216: 549-556.





29

40. Rouy D, Laplaud PM, Saboreau M, Anglés-Cano E: *Hedgehog lipoprotein(a) is a modulator of activation of plasminogen at the fibrin surface. An in vitro study.* Arterioscler Thromb 1992; 12: 146-154.
41. Anglés-Cano E: *A spectrophotometric solid-phase fibrin-tissue plasminogen activator activity assay (SOFIA-tPA) for high-fibrin-affinity tissue plasminogen activators.* Annal Biochem 1986; 43: 129-132.
42. Rouy D, Grailhe P, Nigon F, Chapman J, Anglés-Cano E: *Lipoprotein(a) impairs generation of plasmin by fibrin-bound tissue-type plasminogen activator. In vitro studies in a plasma milieu.* Arterioscler Thromb 1991; 11: 629-638.
43. Hervio L, Durlach V, Girard-Globa A, Anglés-Cano E: *Multiple binding with identical linkage: a mechanism that explains the effect of lipoprotein(a) on fibrinolysis.* Biochemistry 1995; 34: 13353-13358.
44. Anglés-Cano E, Hervio L, Loyau S: *Relevance of lipoprotein(a) in cardiovascular disease: methodological approaches.* Fibrinolysis 1993; 7: 66-68.

ANNALS OF
THE NEW YORK ACADEMY
OF SCIENCES

VOLUME 936

# FIBRINOGEN

## XVIIth INTERNATIONAL FIBRINOGEN WORKSHOP

*Editors*
WILLEM NIEUWENHUIZEN
MICHAEL W. MOSESSON
MONIEK P.M. DE MAAT

PUBLISHED BY THE NEW YORK ACADEMY OF SCIENCES
ANYAA9 936 1–645 (2001)

ninogen is a significant interme-
rinolysis *in vitro*. J. Biol. Chem.

1. 1997. Production and charac-
(S741C-Fluorescein): a novel
ation of active protease. J. Biol.

veights, mass distribution, chain
ation products released from a
(8): 5201–5212.
lysis of the tissue plasminogen
ited and TAFIa-treated soluble
hem. 276(5): 3138–3148.
alytic domain structure of vam-
) for proteolysis without activa-

0. Identification of the mecha-
f TNK-tissue plasminogen acti-
. Chem. 275: 10112–10120.

# Inhibition of Fibrinolysis by Lipoprotein(a)

EDUARDO ANGLÉS-CANO,[a] AURORA DE LA PEÑA DÍAZ,[b] AND STÉPHANE LOYAU[a]

[a]*Institut National de la Santé et de la Recherche Médicale, U460, Faculté de Médecine Xavier-Bichat, France*

[b]*Instituto Nacional de Cardiología "Ignacio Chávez", Departamento de Hematología, México*

ABSTRACT: **A high plasma concentration of lipoprotein Lp(a) is now considered to be a major and independent risk factor for cerebro- and cardiovascular atherothrombosis. The mechanism by which Lp(a) may favour this pathological state may be related to its particular structure, a plasminogen-like glycoprotein, apo(a), that is disulfide linked to the apo B100 of an atherogenic LDL-like particle. Apo(a) exists in several isoforms defined by a variable number of copies of plasminogen-like kringle 4 and single copies of kringle 5 and the catalytic region. At least one of the plasminogen-like kringle 4 copies present in apo(a) (kringle IV type 10) contains a lysine binding site (LBS) that is similar to that of plasminogen. This structure allows binding of these proteins to fibrin and cell membranes. Plasminogen thus bound is cleaved at $Arg_{561}$-$Val_{562}$ by plasminogen activators and transformed into plasmin. This mechanism ensures fibrinolysis and pericellular proteolysis. In apo(a) a Ser-Ile substitution at the Arg-Val plasminogen activation cleavage site prevents its transformation into a plasmin-like enzyme. Because of this structural/functional homology and enzymatic difference, Lp(a) may compete with plasminogen for binding to lysine residues and impair, thereby, fibrinolysis and pericellular proteolysis. High concentrations of Lp(a) in plasma may, therefore, represent a potential source of antifibrinolytic activity. Indeed, we have recently shown that during the course of the nephrotic syndrome the amount of plasminogen bound and plasmin formed at the surface of fibrin are directly related to *in vivo* variations in the circulating concentration of Lp(a) (*Arterioscler. Thromb. Vasc. Biol.*, 2000, 20: 575–584; *Thromb. Hæmost.*, 1999, 82: 121–127). This antifibrinolytic effect is primarily defined by the size of the apo(a) polymorphs, which show heterogeneity in their fibrin-binding activity—only small size isoforms display high affinity binding to fibrin (*Biochemistry*, 1995, 34: 13353–13358). Thus, in heterozygous subjects the amount of Lp(a) or plasminogen bound to fibrin is a function of the affinity of each of the apo(a) isoforms and of their concentration relative to each other and to plasminogen. The real risk factor is, therefore, the Lp(a) subpopulation with high affinity for fibrin. According to this concept, some Lp(a) phenotypes may not be related to atherothrombosis and, therefore, high Lp(a) in some individuals might not represent a risk factor for cardiovascular disease. In agreement with these data, it has been recently reported that Lp(a) particles containing low molecular mass apo(a) emerged as one of the leading risk conditions in advanced stenotic atherosclerosis (*Circulation*, 1999, 100: 1154–1160). The predictive value of high Lp(a) as a risk factor, therefore,**

Address for correspondence: E. Anglés-Cano, M.D., Sc.D., INSERM U460, Remodelage CardioVasculaire, 16 rue Henri Huchard - BP 416, F-75870-Cdx, Paris, France. Voice: 33-1 44 85 61 50; fax: 33-1 44 85 61 56.
angles@kb.infobiogen.fr

depends on the relative concentration of Lp(a) particles containing small apo(a) isoforms with the highest affinity for fibrin. Within this context, the development of agents able to selectively neutralise the antifibrinolytic activity of Lp(a), offers new perspectives in the prevention and treatment of the cardiovascular risk associated with high concentrations of thrombogenic Lp(a).

KEYWORDS: Fibrin surface; Fibrinogen; Plasminogen; Plasmin; Kringle domains; Lysine binding site; Tissue-plasminogen activator; Urokinase; Atherosclerosis; Thrombosis; Atherothrombosis; Monocytes; Macrophages; Fibrinolysis; Plasminogen activation; Plasminogen activator inhibitor.

## INTRODUCTION

The presence of the lipoprotein Lp(a) in plasma was first identified by Berg in 1963 as an LDL-like particle.[1] Lp(a) composition is indeed similar to that of LDL in terms of cholesterol, triglycerides, phospholipids, and apo B100. It was not until 1987 when Eaton *et al.*[2] and Mc Lean *et al.*[3] identified apo(a), the unique and distinctive component of Lp(a), as a member of the plasminogen gene family,[4] both by amino acid sequence analysis and cDNA clonning. The apo(a) glycoprotein indeed showed a strong structural homology with plasminogen, the plasmin precursor. Apo(a) is disulfide linked to the apo B100 of the LDL-like particle. These findings stimulated the interest of different research groups that found in Lp(a) a common point between atherosclerosis and thrombosis.[5,6] Different epidemiological studies were performed from this perspective and identified high Lp(a) plasma concentration as a major and independent cardiovascular risk factor.[7] Although some studies did not find a relationship between Lp(a) and coronary heart disease,[8,9] most prospective studies (reviewed in Ref. 10) confirmed the initial results and extended its application to coronary restenosis, post-angioplastic occlusion, as well as cerebrovascular accidents, and premature development of atherosclerosis. The mechanism by which Lp(a) favors the atherogenic and/or thrombogenic processes is not clearly understood. However, Lp(a) has been found associated with fibrin deposits in atherosclerotic plaques.[11] Moreover, recent data indicate that the large structural heterogeneity of the apo(a) molecule is conducive to a functional heterogeneity as a plasminogen competitive inhibitor for fibrin binding that lowers the formation of plasmin and thereby decreases fibrinolysis.[12,13] In order to understand the molecular basis of this mechanism we analyze the structural aspects of plasminogen and apo(a) interactions with fibrin and consider recent evidence indicating that Lp(a) behaves as an inhibitor of fibrinolysis.

## STRUCTURAL BASIS FOR THE ANTIFIBRINOLYTIC ACTIVITY OF LP(A)

### *Plasminogen and Apolipoprotein(a): General Structure*

Apo(a) and plasminogen are constituted by structural domains called kringles and a serine-proteinase region (see FIGURE 1). The kringle structure was first described in prothrombin and is found in several copies in proteins that evolved from a common ancestral gene: plasminogen, apo(a) and hepatocyte growth factors.[4] Kringles



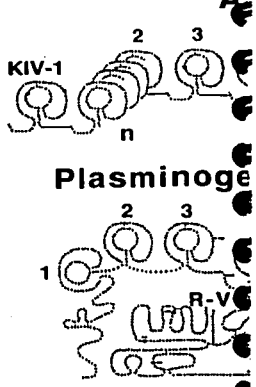



**FIGURE 1.** Structural hom
(adapted from Petersen *et al.*, *J.*
of five kringle domains and a serir
of plasminogen kringle 5 (KV)
minogen kringle 4 (KIV). These
ten different types (KIV-1 to KI
ence of several apo(a) isoforms.
cates the Arg561-Val562 plasmin
by Ser-Ile, S-I (see FIG. 2).

are sequences of 80 to 90 ami
stabilized by three disulfide
nated 1 to 5, differ from each o
that show high affinity for l
between lysine residues in fibr
subsites of plasminogen allo

Apo(a) contains a variable
ogy with kringle 4 of plasmi
lowed by a single copy of plas
94% homology with the corr
connected by regions of 26–3
with six potential O-glycosil
are alike; they are classified
KIV-10 (see FIG. 1). Each of t
appears in a variable number,
isoforms, that account for
including 30% weight due to
residue that ensures the coval

taining small apo(a) ontext, the develop- rinolytic activity of ment of the cardio- bogenic Lp(a).

Plasmin; Kringle or; Urokinase; Ath- tes; Macrophages; or inhibitor.

st identified by Berg in similar to that of LDL in B100. It was not until (a), the unique and dis- n gene family,[4] both by (a) glycoprotein indeed the plasmin precursor. particle. These findings nd in Lp(a) a common epidemiological studies p(a) plasma concentra- Although some studies rt disease,[8,9] most pro- results and extended its usion, as well as cere- osclerosis. The mecha- ogenic processes is not ted with fibrin deposits that the large structural ional heterogeneity as a owers the formation of nderstand the molecular plasminogen and apo(a) ting that Lp(a) behaves

## Y OF LP(A)

### l Structure

nains called kringles and cture was first described at evolved from a com- rowth factors.[4] Kringles



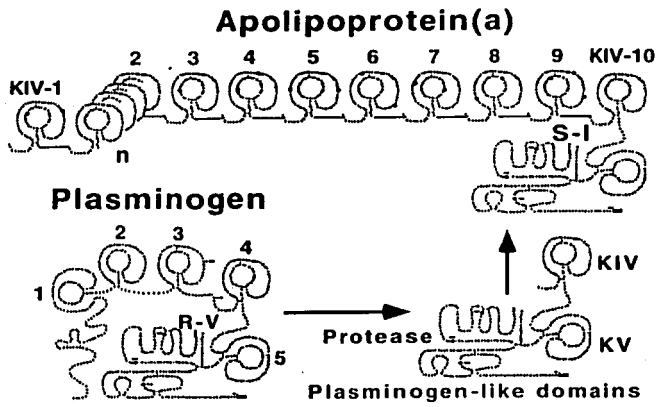



**FIGURE 1.** Structural homologies between apolipoprotein(a) and plasminogen (adapted from Petersen *et al., J. Biol. Chem.*, 1990, 265: 6104–6111). Plasminogen consist of five kringle domains and a serine-protease region. Apo(a) is constituted by single copies of plasminogen kringle 5 (KV) and of the protease region and by multiple copies of plasminogen kringle 4 (KIV). These KIV copies are not identical and have been classified[18] in ten different types (KIV-1 to KIV-10). A variable number of KIV-2 determines the existence of several apo(a) isoforms. All other KIV types are present in single copies. R-V indicates the Arg561-Val562 plasminogen cleavage site, which in apo(a) has been substituted by Ser-Ile, S-I (see FIG. 2).

are sequences of 80 to 90 amino acid residues organized in a triple-loop structure, stabilized by three disulfide bridge.[14] The kringle domains of plasminogen, designated 1 to 5, differ from each other. Kringles 1 and 4 contains lysine binding subsites that show high affinity for lysine residues in fibrin.[15,16] The specific interactions between lysine residues in fibrin or cell membrane proteins and the lysine binding subsites of plasminogen allow plasminogen binding and activation.[17]

Apo(a) contains a variable number of kringle domains that share 61–75% homology with kringle 4 of plasminogen.[2,3] The kringle 4-like repeats of apo(a) are followed by a single copy of plasminogen kringle 5 and a protease domain that shares 94% homology with the corresponding domain of plasminogen. Kringles are interconnected by regions of 26–36 serine-, proline-, and/or threonine-rich amino acids with six potential O-glycosilation sites. Not all plasminogen-like kringle 4 of apo(a) are alike; they are classified in 10 different types,[18] hereafter referred as KIV-1 to KIV-10 (see FIG. 1). Each of these kringles is present only once, except KIV-2, which appears in a variable number, originating structural heterogeneity and different size isoforms, that account for molecular weights between about 280 and 800 kDa including 30% weight due to glycosilation.[19] KIV-9 possesses an additional cysteine residue that ensures the covalent binding between apo(a) and Cys3734 of apo B-100

and, thereby, the formation of the Lp(a) particle.[20] By sequence comparison and molecular modeling[21] it has been shown that KIV-10 contains a lysine binding site (LBS) similar to that of plasminogen kringle 4, and that slightly modified LBS are present in KIV-5 to KIV-8. These kringle copies may confer to apo(a) binding capabilities similar to those of plasminogen.

### *Structure of the Lysine Binding Site*

The structure of the lysine binding site, an elongated hydrophobic depression lined with aromatic residues that separate by 6.8 Å a cationic group from an anionic group positioned at opposite ends of the trough, has been well defined.[22] Its structural characteristics generate a relatively rigid geometry that allows selective access and binding of C-terminal lysine residues in fibrin, as well as aliphatic or aromatic ligands of ω-amino carboxylic acid type. Based on information derived from biochemical,[23] mutagenesis,[24] crystallographic,[25,26] and spectroscopic[27] studies, an appropriate set of amino acids that are key components for lysine binding has been identified (see TABLE 1). Thus, the LBS of plasminogen K1 consists of three aromatic residues, Tyr64/Trp62/Tyr72, lining the hydrophobic trough of the LBS and the double charged anionic, Asp55/Asp57, and cationic, Arg35/Arg71 centres located at opposite ends of the hydrophobic trough. In apo(a) only KIV-10 contains an LBS with similar characteristics that is identical to that of plasminogen kringle 4 except for a conservative Arg→Lys35 substitution also present in plasminogen kringle 1.[28]

**TABLE 1. Key components of the lysine-binding site**

| Kringle | Anionic pole | | Hydrophobic aromatic residues | | | Cationic pole | |
|---|---|---|---|---|---|---|---|
| Pg K1 | Asp 55 | Asp 57 | Tyr 64 | Trp 62 | Tyr 72 | Arg 35 | Arg 71 |
| Pg K4 | Asp 55 | Asp 57 | Phe 64 | Trp 62 | Trp 72 | Lys 35 | Arg 71 |
| Pg K5 | Asp55 | Asp 57 | Tyr 64 | Trp 62 | Trp 72 | Ile 35 | Leu 71 |
| KV | Asp 55 | Asp 57 | Tyr 64 | Trp 62 | Trp 72 | Ser 35 | Leu 71 |
| KIV-10 | Asp 55 | Asp 57 | Phe 64 | Trp 62 | Trp 72 | Arg 35 | Arg 71 |
| KIV-9 | Asp 55 | Gly 57 | Tyr 64 | Trp 62 | Trp 72 | Arg 35 | Arg 71 |
| KIV-8 | Asp 55 | Glu 57 | Tyr 64 | Trp 62 | Trp 72 | Arg 35 | Arg 71 |
| KIV-7 | Asp 55 | Glu 57 | Tyr 64 | Trp 62 | Trp 72 | Arg 35 | Arg 71 |
| KIV-6 | Asp 55 | Glu 57 | Tyr 64 | Trp 62 | Trp 72 | Arg 35 | Arg 71 |
| KIV-5 | Asp 55 | Glu 57 | Tyr 64 | Trp 62 | Trp 72 | Arg 35 | Arg 71 |
| KIV-4 | Asp 55 | Val 57 | Tyr 64 | Trp 62 | Trp 72 | Arg 35 | Arg 71 |
| KIV-3 | Asp 55 | Val 57 | Tyr 64 | Tyr 62 | Trp 72 | Arg 35 | Arg 71 |
| KIV-2 | Asp 55 | Val 57 | Tyr 64 | Tyr 62 | Trp 72 | Arg 35 | Arg 71 |
| KIV-1 | Asp 55 | Val 57 | Tyr 64 | Tyr 62 | Trp 72 | Arg 35 | Arg 71 |

NOTE: Pg. Plasminogen kringles are designated K1, K4, and K5. KV and KIV-1 to KIV-10 correspond to apo(a) kringles.



Both plasminogen K4 and apo(a
1 by two important substitutio
are, therefore, specific structur
apo(a) KIV-10. The critical ro
umented; its hydrophobic indo
and serves as an obvious mark

### *The Serine–Protein*

Serine-proteinases are synth
activated by proteolytic pr
enzymes.[29] These include bind
enzyme ($S_1$–$S_n$ and $S_1'$–$S_n'$)
cleavage site on the substrate[3
$Arg_{561}$-$Val_{562}$, peptide scissil
active plasmin.[31] This cleavage
must restrict the conformation
disulfide bond is of importan
sequence corresponding to thi
of plasminogen that is strictl
bond in apo(a) consist of Ser-
recognition by activators (F
plasminogen prevent its cleava

We have recently conside
residues at, respectively, P–P
of specific fibrinolytic tests r
age site by activators and gen
sis, r-apo(a) mutant plasmid
HepG2 cells; culture superna
and the corresponding r-Lp
were treated with u-PA or

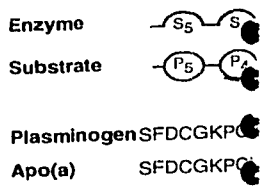



**FIGURE 2.** Scheme of t
region in apo(a). Amino acid s
585, according to Petersen *et*
responding sequence in apo
activators cleave plasminogen
the *arrow*). In apo(a), anino
plasminogen, however the cle

quence comparison and
ins a lysine binding site
ightly modified LBS are
r to apo(a) binding capa-

e

hydrophobic depression
c group from an anionic
well defined.[22] Its struc-
t allows selective access
as aliphatic or aromatic
ation derived from bio-
troscopic[27] studies, an
lysine binding has been
consists of three aromat-
ugh of the LBS and the
Arg71 centres located at
IV-10 contains an LBS
inogen kringle 4 except
lasminogen kringle 1.[28]

| es | Cationic pole | |
|---|---|---|
| 2 | Arg 35 | Arg 71 |
| 2 | Lys 35 | Arg 71 |
| 2 | Ile 35 | Leu 71 |
| 2 | Ser 35 | Leu 71 |
| 2 | Arg 35 | Arg 71 |
| 2 | Arg 35 | Arg 71 |
| 2 | Arg 35 | Arg 71 |
| 2 | Arg 35 | Arg 71 |
| 2 | Arg 35 | Arg 71 |
| 2 | Arg 35 | Arg 71 |
| 2 | Arg 35 | Arg 71 |
| 2 | Arg 35 | Arg 71 |
| 2 | Arg 35 | Arg 71 |
| 2 | Arg 35 | Arg 71 |

KV and KIV-1 to KIV-10



Both plasminogen K4 and apo(a) KIV-10 however, differ, from plasminogen kringle 1 by two important substitutions, Phe64 and Trp72, instead of Tyr64 and Tyr72, that are, therefore, specific structure requirements for the LBS of plasminogen K4 and apo(a) KIV-10. The critical role of this residue in lysine binding has been well documented; its hydrophobic indole is unusually exposed in the LBS of K4 and KIV-10 and serves as an obvious marker of the binding site.[25]

### *The Serine–Proteinase Domain in Plasminogen and Apo(a)*

Serine-proteinases are synthesized and secreted as single-chain zymogens that are activated by proteolytic processing through specific interactions with active enzymes.[29] These include binding forces between subsites of the active site of the enzyme ($S_1$–$S_n$ and $S_1'$–$S_n'$) and amino acid residues ($P_1$–$P_n$ and $P_1'$–$P_n'$) of the cleavage site on the substrate[30] (see FIGURE 2). For instance, cleavage of the $P_1$–$P_1'$, $Arg_{561}$-$Val_{562}$, peptide scissile bond in plasminogen by its activators yields two-chain active plasmin.[31] This cleavage site is located in a small disulfide-bounded loop that must restrict the conformation around this bond. It has been shown, indeed, that this disulfide bond is of importance for the specificity of plasminogen activation.[32] The sequence corresponding to this loop is comprised in the $P_2$–$P_{17}$ / $P_2'$–$P_{23}'$ sequence of plasminogen that is strictly preserved in apo(a); in contrast, the $P_1$–$P_1'$ peptide bond in apo(a) consist of Ser-Ile instead of Arg-Val,[2] a substitution that may impair recognition by activators (FIG. 2). Indeed, the introduction of these substitutions in plasminogen prevent its cleavage by activators.[33]

We have recently considered the possibility that introduction of both Arg and Val residues at, respectively, P–$P_1'$ by site directed mutagenesis together with the use of specific fibrinolytic tests may ensure potential recognition of this activation cleavage site by activators and generation of plasmin-like activity.[34] To test this hypothesis, r-apo(a) mutant plasmids encoding this sequence were expressed in 293 cells and HepG2 cells; culture supernatants containing wildtype r-apo(a), Arg-Val r-apo(a), and the corresponding r-Lp(a)-like particles were thus obtained. These products were treated with u-PA or t-PA in the presence of cofactors known to stimulate

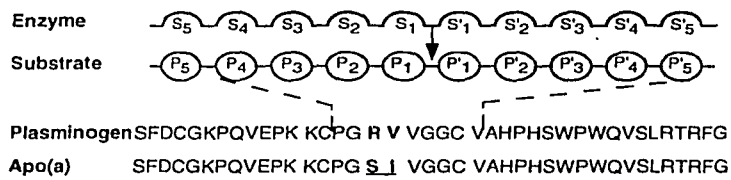



**FIGURE 2.** Scheme of the plasminogen activation cleavage site and the corresponding region in apo(a). Amino acid sequences of the cleavage site in plasminogen (residues 544–585, according to Petersen *et al. J. Biol. Chem.* 1990, 265, 6104–6111) compared to the corresponding sequence in apo(a) (residues 4291–4332, according to Ref. 3). Plasminogen activators cleave plasminogen at the $P_1$–$P_1'$ (Arg-Val) scissile peptide bond (indicated by the *arrow*). In apo(a), amino acid residues $P_2$–$P_{17}$ and $P_2'$–$P_{23}'$ are identical to those of plasminogen, however the cleavage site is substituted by Ser-Ile.

plasminogen activation by t-PA (fibrin and CNBr-fragments of fibrinogen) or u-PA (6-Ahx, a lysine analogue). However, neither cleavage of apo(a) nor plasmin-like activity could be demonstrated on treatment of Arg-Val r-apo(a)/r-Lp(a) mutants with plasminogen activators, either in the presence or in the absence of cofactors known to induce changes in plasminogen conformation or to stimulate plasminogen activation.

These results strongly suggest that to promote efficient cleavage of plasminogen, t-PA recognises complex structural elements on the surface of native plasminogen that are distant from those at P4-P2′.[35] The exact nature of these secondary interactions remains unknown. Because r-apo(a) contains multiple copies of kringle 4 and lacks copies of kringles 1 to 3 of plasminogen, it may not possess the potential for such enzyme–substrate interactions, that are independent of the binding cleft around P1; r-apo(a) Arg-Val may fail, thereby, to induce a better fit of the cycle peptide loop containing the Arg-Val bond with the activators. The presence of multiple kringle repeats in apo(a) does not substitute the specific conformation present in plasminogen that is required for the selective and specific recognition by activators. It is, therefore, apparent that besides the absence of the Arg-Val site, apo(a) has undergone further evolutionary changes which prevent its activation towards a plasmin-like protease. Because of this important enzymatic difference, the ability of selected kringle-4 copies of apo(a) to compete with plasminogen for binding to fibrin and cell surfaces results in decreased fibrinolysis and impaired pericellular proteolysis.[36,37]

## INHIBITION OF PLASMINOGEN BINDING TO FIBRIN, THE MAJOR MECHANISM OF ACTION OF LP(A)

Physiological fibrinolysis involves heterogeneously catalyzed reactions that proceed at the fibrin/plasma interface, where fibrin provides a surface to which tissue plasminogen activator (t-PA) and plasminogen bind specifically.[15,17] Molecular assembly of these proteins results in a ternary complex that efficiently generates plasmin on the surface of fibrin and thereby triggers the dissolution of a clot.[38]

Initial limited degradation of the surface of fibrin by plasmin unveils carboxy-terminal lysine residues and increases the local concentration of plasminogen, a process that amplifies and accelerates the degradation of fibrin.[39] This mechanism is mediated by specific interactions of lysine residues in fibrin with the kringle domains of plasminogen.[40] In a plasma milieu, the progression of this process is markedly influenced by $\alpha_2$-antiplasmin, the specific plasmin inhibitor, and by active carboxy-peptidase B, an exopeptidase that cleaves Arg and Lys residues at C-terminal position.[41] The number of carboxy-terminal lysine residues is thus limited and, thereby, the amount of bound plasminogen.[17] On the other hand, the blockade of such residues by isolated plasminogen kringle 4 has been shown to interfere competitively with clot lysis by a mechanism involving binding to lysine–fibrin residues.[42] The presence in apo(a) of kringle modules structurally related to those of plasminogen endows Lp(a) with the ability to compete with plasminogen for binding to fibrin. The effect of Lp(a) on plasminogen binding to fibrin and cell surfaces has been studied by several groups.[37,43–45] A number of experimental *in vitro* studies resulted in convincing evidence that Lp(a) binds to the fibrin surface and cell membranes and, thereby, competes with plasminogen so as to inhibit its activation.[46,47] We have shown that, indeed, both free recombinant apo(a) (see FIGURE 3) and apo(a) in Lp(a) particles (see

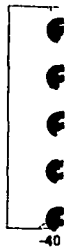



**FIGURE 3.** Determinati
minogen to fibrin (as indicate
formed in the absence and in
(18 kringles). For each concen
bound, and $K_d$, the dissociation
tion. The graph depicts the re
The inhibition constant ($K_i$ = 4
line with the abcissa.

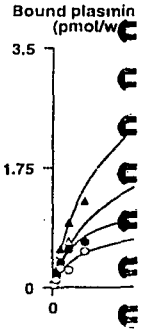



**FIGURE 4.** Inhibition of
Lp(a) (as indicated in Ref. 12)
containing a trace amount o
fibrin in the presence of 0 (▲),
the Langmuir equation allow
bound) and $K_d$ (the dissociatio
By plotting the reciprocal of t
constant $K_i$ = 32 nmol/L was
the abcissa (not shown).

f fibrinogen) or u-PA
)(a) nor plasmin-like
o(a)/r-Lp(a) mutants
absence of cofactors
imulate plasminogen

'age of plasminogen,
native plasminogen
:e secondary interac-
pies of kringle 4 and
sess the potential for
binding cleft around
ıe cycle peptide loop
: of multiple kringle
present in plasmino-
by activators. It is,
e, apo(a) has under-
ı towards a plasmin-
he ability of selected
ling to fibrin and cell
ılar proteolysis.[36,37]

) FIBRIN,
LP(A)

d reactions that pro-
face to which tissue
ally.[15,17] Molecular
efficiently generates
tion of a clot.[38]
ıin unveils carboxy-
plasminogen, a pro-
[39] This mechanism
in with the kringle
ın of this process is
ıibitor, and by active
sidues at C-terminal
ıs limited and, there-
ılockade of such res-
erfere competitively
brin residues.[42] The
ıose of plasminogen
ınding to fibrin. The
has been studied by
resulted in convinc-
branes and, thereby,
We have shown that,
ı Lp(a) particles (see

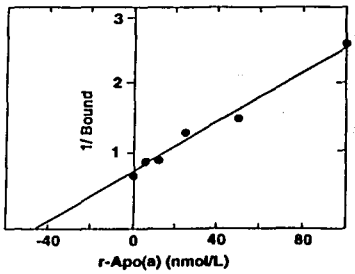



**FIGURE 3.** Determination of the inhibition constant of apo(a) for the binding of plasminogen to fibrin (as indicated in Ref. 48). Plasminogen binding experiments were performed in the absence and in the presence of various concentrations of recombinant apo(a) (18 kringles). For each concentration of apo(a) the binding parameters ($B_{max}$, the maximum bound, and $K_d$, the dissociation constant) were determined according to the Langmuir equation. The graph depicts the reciprocal of $B_{max}$ as a function of the concentration of apo(a). The inhibition constant ($K_i$ = 44 nmol/L) was calculated from the *intercept* of the straight line with the abcissa.

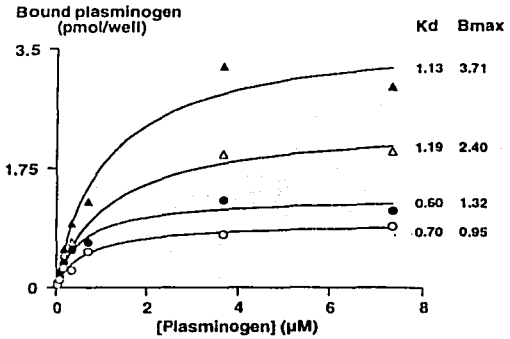



**FIGURE 4.** Inhibition of the binding of plasminogen to fibrin by purified 18 kringles Lp(a) (as indicated in Ref. 12). Increasing concentrations of plasminogen (0 to 8 μmol/L) containing a trace amount of radiolabeled plasminogen (5 nmol/L) were incubated with fibrin in the presence of 0 (▲), 50 (△), 100 (●) and 200 (○) nmol/L Lp(a). Data fitted to the Langmuir equation allowed calculation of $B_{max}$ (maximum amount of plasminogen bound) and $K_d$ (the dissociation constant of the interaction) for each Lp(a) concentration. By plotting the reciprocal of the $B_{max}$ versus the concentration of added Lp(a) an inhibition constant $K_i$ = 32 nmol/L was determined at the *intercept* of the linear regression curve with the abcissa (not shown).

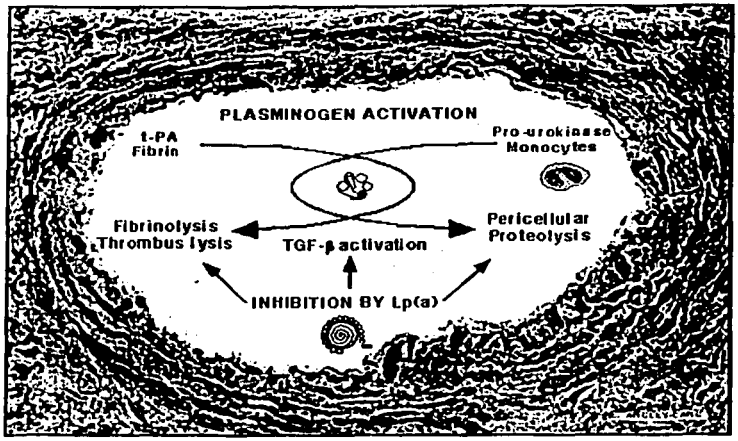



**FIGURE 5.** Competitive inhibition of the binding and activation of plasminogen by Lp(a) on fibrin and cell surfaces. This competitive mechanism results in inhibition of fibrinolysis, pericellular proteolysis and TGF-β activation. The different components of the plasminogen activation system and Lp(a) are represented in the intravascular space of a vessel section.

FIGURE 4) efficiently inhibit ($K_i$ = 44 nmol/L and 30 nmol/L, respectively) the binding of plasminogen to fibrin[12,48] (see FIGURE 5). Such unique behaviour was attributed to the fibrin-binding properties conferred by the kringle 4 repeats of apo(a).[48] Plasminogen and the different Lp(a) isoforms also compete for lysine residues on the surface receptors of endothelial cells,[37] platelets,[49] and mononuclear cells.[45] Thus, Lp(a) interferes with the evolution of fibrinolysis at the surface of fibrin, endothelial cells, monocytes, and platelets through binding of apo(a), an eternal zymogen that decreases the local concentration of plasminogen and cannot be transformed into an active enzyme. Most of the effects of Lp(a)—persistence of fibrin deposits, accumulation of cholesterol and proliferation of smooth muscle cells in the intima, are related to a decrease in plasmin activity (FIG. 5). Hypofibrinolysis and cholesterol accumulation are a direct consequence of the presence of Lp(a) at the surface of fibrin and cell membranes: apo(a) inhibits plasmin formation and the LDL components favors cholesterol accumulation.

## OTHER ANTIFIBRINOLYTIC AND ANTIPROTEOLYTIC EFFECTS OF LP(A)

Lp(a) may stimulate the expression of PAI-1 and inhibit the synthesis of t-PA by endothelial cells in culture.[50,51] Thus, inhibition of t-PA by PAI-1 and low t-PA antigen levels may enhance Lp(a)-dependent hypofibrinolysis by decreasing the amount of

t-PA available for the acti
inhibits the activation of
apo(a), the generation of pl
Insufficient activation of
muscle cells into the intim

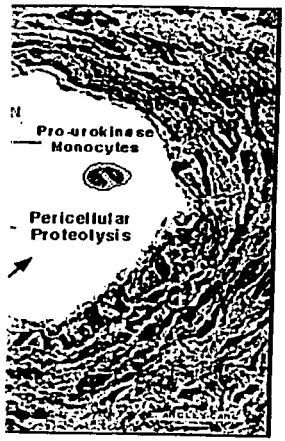
## ANTIFIBRINOLYTI

To date there is no d
binding and activation *in*
imental studies. Occlusiv
age arterial segments w
plasma levels.[54] Biemond
on endogenous and t-PA
bosis; endogenous thromb
in the presence of apo(a
have been shown to exhil
duced by injection of hu
sive report to this respec
apo(a) by disruption of
transgenic mice.[57]

In humans Lp(a) plas
varies from one individu
10 mg/dl to more than 1
cholesterol, obesity, and
the life span. However, d
variations in Lp(a) plasm
obtained in this human
plasminogen binding an
measured the effect of in
nogen activation at the
inverse relationship betw
(see FIGURE 6). A direc
decrease in plasmin form
centration has thus been
may decrease fibrinolysis

## LP(A) INHIBITS FI

In general, an individ
isoforms; only a reduced
The size of the Apo(a) is
concentration.[63] In gener
and, therefore, the size
of Lp(a). A difference in




ictivation of plasminogen by Lp(a)
esults in inhibition of fibrinolysis,
t components of the plasminogen
ular space of a vessel section.

iol/L, respectively) the binding
ue behaviour was attributed to
4 repeats of apo(a).[48] Plasmi-
r lysine residues on the surface
ionuclear cells.[45] Thus, Lp(a)
ace of fibrin, endothelial cells,
eternal zymogen that decreas-
be transformed into an active
ibrin deposits, accumulation of
in the intima, are related to a
and cholesterol accumulation
surface of fibrin and cell mem-
components favors cholesterol

IC AND
S OF LP(A)

hibit the synthesis of t-PA by
by PAI-1 and low t-PA antigen
by decreasing the amount of

t-PA available for the activation of plasminogen. It has also been shown that Lp(a) inhibits the activation of TGF-β[52] and that, in transgenic mice expressing human apo(a), the generation of plasmin and thereby the activation of TFG-β are decreased.[53] Insufficient activation of TGF-β may result in migration and proliferation of smooth muscle cells into the intima, an important mechanism in atheroma plaque formation.

## ANTIFIBRINOLYTIC ACTIVITY OF APOLIPOPROTEIN(A) *IN VIVO*

To date there is no definitive evidence that Lp(a) interferes with plasminogen binding and activation *in vivo*. Some evidence has been obtained from a few experimental studies. Occlusive arterial thrombosis with incorporation of Lp(a) into damage arterial segments was observed in cynomolgous monkeys with high Lp(a) plasma levels.[54] Biemond *et al.*[55] studied the effect of a recombinant form of apo(a) on endogenous and t-PA mediated lysis in an *in vivo* model of experimental thrombosis; endogenous thrombolysis but not t-PA induced lysis was significantly reduced in the presence of apo(a). In contrast, transgenic mice that express human apo(a) have been shown to exhibit reduced t-PA induced lysis of pulmonary emboli produced by injection of human platelet-rich plasma clots.[56] However, the most conclusive report to this respect concerns the elimination of the pathogenic activity of apo(a) by disruption of the KIV-10 lysine binding site using genetic engineering in transgenic mice.[57]

In humans Lp(a) plasma concentration, which depends on its hepatic synthesis,[58] varies from one individual to another within an approximate range of less than 10 mg/dl to more than 100 mg/dl; it is independent from other factors, such as diet, cholesterol, obesity, and smoking and is maintained within small variations during the life span. However, during the course of the nephrotic syndrome, large individual variations in Lp(a) plasma concentration have been observed.[59] Data have been obtained in this human model system,[60,61] indicating that Lp(a) interferes with plasminogen binding and activation under conditions fashioned *in vivo*. We have measured the effect of individual variations in the concentration of Lp(a) on plasminogen activation at the surface of fibrin and have determined the existence of an inverse relationship between plasmin formation and quantity of Lp(a) that is bound (see FIGURE 6). A direct relationship between binding of Lp(a) to fibrin and the decrease in plasmin formation due to modifications *in vivo* of the Lp(a) plasma concentration has thus been established.[60,61] These studies strongly suggest that apo(a) may decrease fibrinolysis *in vivo*.

## LP(A) INHIBITS FIBRINOLYSIS DEPENDING ON APO(A) ISOFORMS

In general, an individual inherits, in a codominant autosomic fashion, two apo(a) isoforms; only a reduced number of individuals are homozygous for the apo(a) trait.[62] The size of the Apo(a) isoforms accounts for an inverse correlation with Lp(a) plasma concentration.[63] In general, the smaller the hypervariable region of the apo(a) allele and, therefore, the size of the apo(a) isoform, the higher is the plasma concentration of Lp(a). A difference in the distribution of apo(a) isoforms between patients with

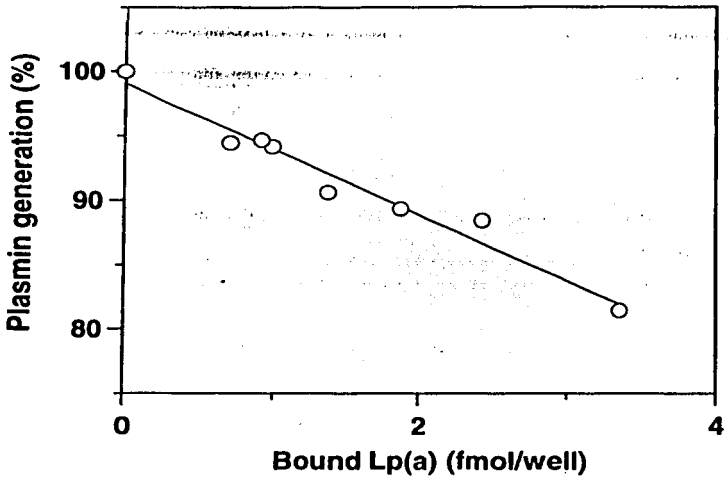



**FIGURE 6.** Inhibition of plasmin generation by Lp(a). Varying concentrations of Lp(a) (18 kringles) were incubated with 200 nmol/L of Glu-plasminogen (final concentration) on fibrin bound-t-PA. Plasminogen activation was followed at 37°C by measuring the amidolytic activity of plasmin with a synthetic substrate (CBS 00-65, Stago). At the end of the activation, the plate was washed and bound Lp(a) was detected with a radiolabeled antibody directed against apo(a); bound radioactivity was transformed in fmol of Lp(a). Plasminogen activation expressed as a percentage relative to the maximum of plasmin generated in the absence of Lp(a), is plotted against the amount of Lp(a) bound. Data represent the mean of three determinations. A linear correlation ($r = 0.985$) was found between the inhibition of plasmin generation and the increase in the amount of Lp(a) bound.

atherosclerosis and a control population has been recently reported.[64–66] Low molecular mass isoforms were found more frequently in subjects with high Lp(a) concentration and a history of myocardial infarction or intermitent claudication. Thus, short apo(a) alleles may favor atherogenesis by increasing the concentration of Lp(a). However, the difference in allele distribution between patients at risk and controls is not always observed, and the inverse relationship between apo(a) size and Lp(a) concentration is not linear, thus suggesting the existing of a functional diversity among apo(a) isoforms. Indeed, some plasmas with a high Lp(a) concentration may fail to induce a decrease in fibrinolysis.[44] The question remains as to whether short apo(a) isoforms are *per se* an atherothrombotic risk factor, especially when associated with high Lp(a) plasma concentration. Recent experimental and clinical evidence provide arguments that favor this hypothesis.



Since the atherogenic po
conferred by the kringle 4
Lp(a) phenotypes may have
ity for fibrin. We demonst
jects containing a single, di
effect and that this heterog
low molecular mass showed
ing suggests that the varia
bind to fibrin.[67] In heterozy
lation,[62] differences in affi
demonstrated.[13] Under thes
function of the affinity of
ative to each other and to p
emergence of low molecula
advanced stenotic atherosc

Our findings of an invers
support the hypothesis tha
*affinity for fibrin.* According
related to atherogenesis an

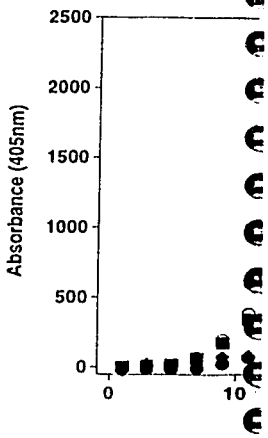
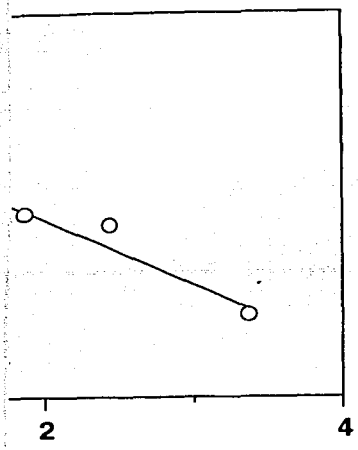



**FIGURE 7.** Inhibition of
forms. Plasminogen was activa
of disctinct Lp(a) particles co
detection of the amidolytic acti
405 nm as a function of time.
apo(a) isoforms containing
($\Delta A_{405}$/min).




tion by Lp(a). Varying concentrations of
iol/L of Glu-plasminogen (final concentra-
ion was followed at 37°C by measuring the
ubstrate (CBS 00-65, Stago). At the end of
Lp(a) was detected with a radiolabeled anti-
ity was transformed in fmol of Lp(a). Plas-
lative to the maximum of plasmin generated
amount of Lp(a) bound. Data represent the
ion ($r = 0.985$) was found between the inhi-
the amount of Lp(a) bound.

been recently reported.[64-66] Low molec-
ntly in subjects with high Lp(a) concen-
n or intermitent claudication. Thus, short
increasing the concentration of Lp(a).
n between patients at risk and controls is
nship between apo(a) size and Lp(a) con-
existing of a functional diversity among
h a high Lp(a) concentration may fail to
stion remains as to whether short apo(a)
k factor, especially when associated with
perimental and clinical evidence provide

Since the atherogenic potential of Lp(a) is related to the lysine binding properties conferred by the kringle 4 repeats of apo(a), we have explored the possibility that Lp(a) phenotypes may have different functional properties with regard to their affinity for fibrin. We demonstrated that Lp(a) particles purified from homozygous subjects containing a single, distinct isoform of apo(a) display different antifibrinolytic effect and that this heterogeneity is related to apo(a) size polymorphism. Isoforms of low molecular mass showed the highest affinity for fibrin (see FIGURE 7). This finding suggests that the variable number of kringles in apo(a) influence its ability to bind to fibrin.[67] In heterozygous subjects, representing more than 94% of the population,[62] differences in affinity for fibrin of each of the Lp(a) particles have beeen demonstrated.[13] Under these conditions, the net antifibrinolytic effect of Lp(a) is a function of the affinity of each of the Lp(a) isoforms and of their concentration relative to each other and to plasminogen.[12] This mechanism is in agreement with the emergence of low molecular weight apo(a) phenotypes as a leading risk condition of advanced stenotic atherosclerosis.[68]

Our findings of an inverse relationship between isoform size and affinity for fibrin support the hypothesis that *the real risk factor is the Lp(a) population with high affinity for fibrin.* According to this concept some Lp(a) phenotypes might fail to be related to atherogenesis and, therefore, some individuals with high Lp(a) would not

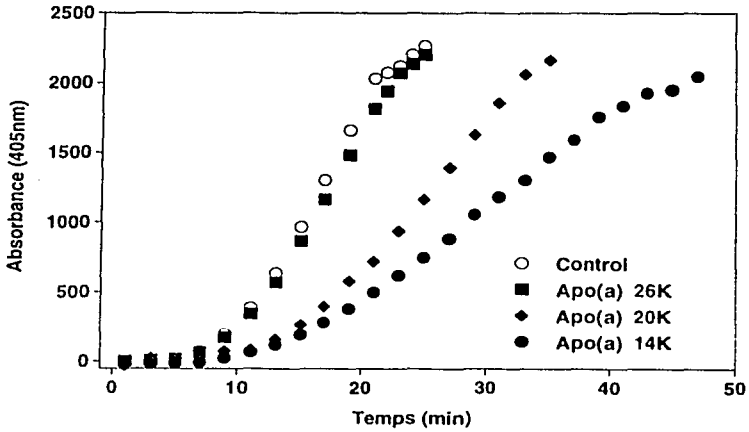



**FIGURE 7.** Inhibition of the formation of plasmin on fibrin by different Lp(a) isoforms. Plasminogen was activated by fibrin-bound t-PA in the presence of 0 or 300 nmol/L of disctinct Lp(a) particles containing either 26, 20, or 14 kringles. Chromogenic substrate detection of the amidolytic activity of plasmin is expressed by the change in absorbance at 405 nm as a function of time. The amount of plasmin formed was lower in the presence of apo(a) isoforms containing 14 and 20 kringles as indicated by lower initial velocities ($\Delta A_{405}$/min).

be at risk of coronary heart disease. Taken altogether, these results suggest that, besides the quantitative factor, an important qualitative effect must be considered in the atherothrombogenic role of Lp(a). This new concept about the functional heterogeneity of Lp(a) adds a new dimension to the evaluation of the predictive value of Lp(a) as a risk factor for cardiovascular disease.

## CONCLUSION

The similarity between plasminogen and apolipoprotein (a) allows different apo(a) isoforms to compete with plasminogen for fibrin affinity sites. The affinity of each isoform depends on its size and its plasma concentration.[19,47,67] Indeed, we have clearly shown that the influence of Lp(a) on fibrinolysis depends on the high affinity of small apo(a) isoforms for fibrin and on their concentration relative to plasminogen. Lp(a) particles containing low molecular weight apo(a) isoforms have the most profound influence on fibrinolysis by acting as a prominent competitive antagonist of plasminogen. As a result, the plasmin formed at the surface of fibrin may also vary with modifications of the concentration of Lp(a) *in vivo*.[60] An antifibrinolytic mechanism may therefore explain the pathophysiological effects of Lp(a) by relating both high concentrations of Lp(a) and small apo(a) isoforms. This mechanism is in agreement with the emergence of low molecular weight apo(a) phenotypes as a leading risk condition of advanced stenotic atherosclerosis.[68] These data indicate that, to evaluate the potential risk associated with Lp(a) levels in heterozygous subjects, it is the relative concentration of the Lp(a) subpopulation having an apo(a) isoform with the highest affinity for fibrin and not the absolute Lp(a) concentration that should be considered

## ACKNOWLEDGMENTS

This research was supported by INSERM (Project 4N001B) and by Ministère de la Recherche (Grant ACC-SV9). We gratefully acknowledge the editorial assistance of Jérôme Lechevalier.

## REFERENCES

1. BERG, K. 1963. A new serum type system in man—the Lp system. Acta Pathol. Microbiol. Scand. **59:** 369–382.
2. EATON, D.L., *et al.* 1987. Partial amino acid sequence of apolipoprotein(a) shows that is homologous to plasminogen. Proc. Natl. Acad. Sci. U.S.A. **84:** 3224–3228.
3. MCLEAN, J.W., *et al.* 1987. cDNA sequence of human apolipoprotein(a) is homologous to plasminogen. Nature **330:** 132–137.
4. ICHINOSE, A. 1992. Multiple members of the plasminogen-apolipoprotein(a) gene family associated with thrombosis. Biochemistry **31:** 3113–3118.
5. DAHLEN, G.H. 1994. Lp(a) Lipoprotein in cardiovascular disease. Atherosclerosis **108:** 111–126.
6. UTERMANN, G. 1989. The mysteries of lipoprotein(a). Science **246:** 904–910.



7. ROSENGREN, A., *et al.* 1990. Li case-control study in a gene **301:** 1248–1251.
8. JAUHIAINEN, M., *et al.* 1991. L case-control study of the He 67.
9. RIDKER, P.M., C.H. HENNEKEN protein (a) and the risk of m
10. DJUROVIC, S., *et al.* 1997. Epi rotic/thrombotic disease. Cli
11. SMITH, E.B., *et al.* 1991. Doc human atherosclerotic lesion
12. HERVIO, L., *et al.* 1995. Mul explains the effect of lipopro
13. HERVIO, L., *et al.* 1996. The subjects is modulated by th isoforms and their affinity fo
14. PATTHY, L., *et al.* 1984. Kring **171:** 131–136.
15. VALI, Z., *et al.* 1984. The fibri 13690–13694.
16. WU, T.P., *et al.* 1991. The re human plasminogen kringle
17. FLEURY, V. & E. ANGLES-CAN to fibrin surfaces: the role of
18. GUEVARA, J., *et al.* 1992. A s protein(a). Proteins **12:** 188-
19. FLESS, G.M., M.E. ZUMMAL apolipoprotein(a) and Lp(a) Biol. Chem. **261:** 8712–871
20. BRUNNER, C., *et al.* 1993. C assembly. Proc. Natl. Acad.
21. GUEVARA, J., *et al.* 1993. Co gle-like sequences in Huma
22. TULINSKY, A. 1991. The stru **66:** 16–31.
23. TREXLER, M., Z. VALI & L. binding sites of human plas
24. HOOVER, G.J., *et al.* 1993. A plasminogen that stabilize 10936–10943.
25. MATHEWS, I.I., *et al.* 1996. C human plasminogen in co 4-(aminomethyl)cyclohex
26. WU, T.P., K.P. PADMANABH plasminogen kringle 1 an 166.
27. RAMESH, V., *et al.* 1987. P kringle 4 domain of huma Biol. **198:** 481–98.
28. MOCHALKIN, I., *et al.* 1999. tein(a): structure, ligand b 1990–1998.
29. NEURATH, H. 1999. Protec 268–272.
30. BERGER, A. & I. SCHECHTE peptide substrates and in 249–264.

7. ROSENGREN, A., *et al.* 1990. Lipoprotein (a) and coronary heart disease: a prospective case-control study in a general population sample of middle aged men. Br. Med. J. **301:** 1248–1251.
8. JAUHIAINEN, M., *et al.* 1991. Lipoprotein(a) and coronary heart disease risk. A nested case-control study of the Helsinki Heart Study participants. Atherosclerosis **89:** 59–67.
9. RIDKER, P.M., C.H. HENNEKENS & M.J. STAMPFER. 1993. A prospective study of lipoprotein (a) and the risk of myocardial infarction. JAMA **270:** 2195–2199.
10. DJUROVIC, S., *et al.* 1997. Epidemiology of Lp(a) lipoprotein: its role in atherosclerotic/thrombotic disease. Clin. Genet. **52:** 281–292.
11. SMITH, E.B., *et al.* 1991. Does lipoprotein(a) (Lp(a)) compete with plasminogen in human atherosclerotic lesions and thrombi? Atherosclerosis **89:** 127–136.
12. HERVIO, L., *et al.* 1995. Multiple binding with identical linkage: a mechanism that explains the effect of lipoprotein(a) on fibrinolysis. Biochemistry **34:** 13353–13358.
13. HERVIO, L., *et al.* 1996. The antifibrinolytic effect of lipoprotein(a) in heterozygous subjects is modulated by the relative concentration of each of the apolipoprotein(a) isoforms and their affinity for fibrin. Eur. J. Clin. Invest. **26:** 414–417.
14. PATTHY, L., *et al.* 1984. Kringles: modules specialized for protein binding. FEBS Lett. **171:** 131–136.
15. VALI, Z., *et al.* 1984. The fibrin-binding site of human plasminogen. J. Biol. Chem. **25:** 13690–13694.
16. WU, T.P., *et al.* 1991. The refined structure of the e-aminocaproic acid complex of human plasminogen kringle 4. Biochemistry **30:** 10589–10594.
17. FLEURY, V. & E. ANGLES-CANO. 1991. Characterization of the binding of plasminogen to fibrin surfaces: the role of carboxy-terminal lysines. Biochemistry **30:** 7630–7638.
18. GUEVARA, J., *et al.* 1992. A structural assessment of the apo(a) protein of human lipoprotein(a). Proteins **12:** 188–199.
19. FLESS, G.M., M.E. ZUMMALLEN & S. SCANU. 1986. Physicochemical properties of apolipoprotein(a) and Lp(a) derived from the dissociation of human plasma Lp(a). J. Biol. Chem. **261:** 8712–8718.
20. BRUNNER, C., *et al.* 1993. $Cys^{4057}$ of apolipoprotein(a) is essential for lipoprotein(a) assembly. Proc. Natl. Acad. Sci. U.S.A. **90:** 11643–11647.
21. GUEVARA, J., *et al.* 1993. Comparison of ligand-binding sites of modeled Apo(a) kringle-like sequences in Human lipoprotein(a). Arterioscler.Throm. **13:** 758–770.
22. TULINSKY, A. 1991. The structures of domains of blood proteins. Thromb. Hæmost. **66:** 16–31.
23. TREXLER, M., Z. VALI & L. PATTHY. 1982. Structure of the ω-aminocarboxylic acid-binding sites of human plasminogen. J. Biol. Chem. **257:** 7401–7406.
24. HOOVER, G.J., *et al.* 1993. Amino acids of the recombinant kringle 1 domain of human plasminogen that stabilize its interaction with ω-amino acids. Biochemistry **32:** 10936–10943.
25. MATHEWS, I.I., *et al.* 1996. Crystal structures of the recombinant kringle 1 domain of human plasminogen in complexes with the ligands e-aminocaproic acid and trans-4-(aminomethyl)cyclohexane-1-carboxilic acid. Biochemistry **35:** 2567–2576.
26. WU, T.P., K.P. PADMANABHAN & A. TULINSKY. 1994. The structure of recombinant plasminogen kringle 1 and the fibrin binding site. Blood Coagul. Fibrinol. **5:** 157–166.
27. RAMESH, V., *et al.* 1987. Proton magnetic resonance study of lysine-binding to the kringle 4 domain of human plasminogen. The structure of the binding site. J. Mol. Biol. **198:** 481–98.
28. MOCHALKIN, I., *et al.* 1999. Recombinant kringle IV-10 modules of human apolipoprotein(a): structure, ligand binding modes, and biological relevance. Biochemistry **38:** 1990–1998.
29. NEURATH, H. 1999. Proteolytic processing and physiological regulation. TIBS **14:** 268–272.
30. BERGER, A. & I. SCHECHTER. 1970. Mapping the active site of papain with the aid of peptide substrates and inhibitors. Philos. Trans. R. Soc. Lond. B. Biol. Sci. **257:** 249–264.

31. ROBBINS, K.C., *et al.* 1967. The peptide chains of human plasmin. Mechanism of activation of human plasminogen to plasmin. J. Biol. Chem. **242:** 2333–2342.
32. LINDE, V., *et al.* 1998. Elimination of the Cys558-Cys566 bond in Lys78-plasminogen—effect on activation and fibrin interaction. Eur. J. Biochem. **251:** 472–479.
33. BUSBY, S.J., *et al.* 1991. Expression of recombinant human plasminogen in mammalian cells is augmented by suppression of plasmin activity. J. Biol. Chem. **266:** 15286–15292.
34. HERVIO, L., *et al.* 1999. Effect of plasminogen activators on human recombinant apolipoprotein(a) having the plasminogen activation cleavage site. Biochim. Biophys. Acta **1434:** 124–134.
35. COOMBS, G.S., *et al.* 1996. Distinct mechanisms contribute to stringent substrate specifity of tissue-type plasminogen activator. J. Biol. Chem. **271:** 4461–4467.
36. MILES, L.A., *et al.* 1989. A potential basis for the thrombotic risks associated with lipoprotein(a). Nature **339:** 301–303.
37. HAJJAR, K.A., *et al.* 1989. Lipoprotein(a) modulation of endothelial cell surface fibrinolysis and its potential role in atherosclerosis. Nature **339:** 303–305.
38. HOYLAERTS, M., *et al.* 1982. Kinetics of the activation of plasminogen by human tissue plasminogen activator. J. Biol. Chem. **257:** 2912–2919.
39. SUENSON, E., *et al.* 1984. Initial plasmin-degradation of fibrin as the basis of a positive feedback mechanism in fibrinolysis. Eur. J. Biochem. **140:** 513–522.
40. VARADI, A. & L. PATTHY. 1983. Location of plasminogen—binding sites in human fibrin (ogen). Biochemistry **22:** 2440–2446.
41. PLOW, E.F., K. ALLAMPALLAM & V. REDLITZ. 1997. The plasma carboxypeptidases and the regulation. Trends Cardiovasc. Med. **7:** 71–75.
42. SUGIYAMA, N., M. IWAMOTO & Y. ABIKO. 1987. Effects of kringles derived from human plasminogen on fibrinolysis *in vitro*. Thromb. Res. **47:** 459–468.
43. LOSCALZO, J., *et al.* 1990. Lipoprotein(a), fibrin binding, and plasminogen activation. Atherosclerosis **10:** 240–245.
44. ROUY, D., *et al.* 1991. Lipoprotein (a) impairs generation of plasmin by fibrin-bound tissue-type plasminogen activator. Arterioscler. Thromb. **11:** 629–638.
45. MILES, L.A., *et al.* 1995. Interaction of Lp(a) with plasminogen binding sites on cells. Thromb. Hæmost. **73:** 458–465.
46. HARPEL, P.C. & W. BORTH. 1992. Fibrin, lipoprotein(a), plasmin interactions: a model linking thrombosis and atherogenesis. Ann. N.Y. Acad. Sci. **667:** 233–238
47. ANGLÉS-CANO, E., *et al.* 1993. Effects of lipoprotein(a) on the binding of plasminogen to fibrin and its activation by fibrin-bound tissue-type plasminogen activator. Chem. Phys. Lipids **67/68:** 369–380.
48. ROUY, D., *et al.* 1992. Apolipoprotein(a) and plasminogen interactions with fibrin: a study with recombinant apolipoprotein(a) and isolated plasminogen fragments. Biochemistry **31:** 6333–6339.
49. EZRALTY, A., D.I. SIMON & J. LOSCALZO. 1993. Lipoprotein(a) binds to human platelets and attenuates plasminogen binding and activation. Biochemistry **32:** 4628–4633.
50. ETINGIN, O., *et al.* 1991. Lipoprotein(a) regulates plasminogen activator inhibitor-1 expression in endothelial cells. J. Biol. Chem. **266:** 2459–2465.
51. LEVIN, E.G., *et al.* 1994. Lipoproteins inhibit the secretion of tissue plasminogen activator from human endothelial cells. Arterioscler. Thromb. **14:** 438–442.
52. KOJIMA, S., P.C. HARPEL & D.B. RIFKIN. 1991. Lipoprotein(a) inhibits the generation of transforming growth factor b: an endogenous inhibitor of smooth muscle cell migration. J. Cell Biol. **113:** 1439–1445.
53. GRAINGER, D.J., *et al.* 1994. Activation of transforming growth factor is inhibited in transgenic apolipoprotein(a) mice. Nature **370:** 460–462.
54. WILLIAMS, J.K., *et al.* 1993. Occlusive arterial thrombosis in cynomologous monkeys with varying plasma concentrations of lipoprotein(a). Arterioscler. Thromb. **13:** 548–554.
55. BIEMOND, B.J., *et al.* 1997. Àpolipoprotein(a) attenuates endogenous fibrinolysis in vivo in a rabbit jugular vein thrombosis model. Circulation **96:** 1612–1615.



56. PALABRICA, T.M., *et al.* 1
human apolipoprotein(a
tor-mediated thrombol
57. BOONMARK, N.W., *et al.*
reduces atherosclerosis
58. KRAFT H.G., *et al.* 1989.
liver transplantation. J.
59. KRONENBERG, F., *et al.* 19
1–25.
60. SOULAT, T., *et al.* 1999.
formation on fibrin—a s
Lp(a). Thromb. Hæmost
61. SOULAT, T., *et al.* 2000.
on its competition with
using plasma from c
Thromb. Vasc. Biol. **20**
62. GAW, A., *et al.* 1994. Co
and plasma concentrati
mon "null" allele at the
63. UTERMANN, G., *et al.* 198
Lp(a)-lipoprotein conce
64. MØLGAARD, J., *et al.* 1992
lipoprotein (a) isoform
895–901.
65. SEED, M., *et al.* 1990. R
type to coronary heart
Engl. J. Med. **322:** 1494
66. SANDHOLZER, C., *et al.* 1
A study in six populatio
67. HERVIO, L., *et al.* 1993.
(a) effects on fibrinoly
68. KRONENBERG, F., *et al.* 1
in atherogenesis—pros
1154–1160.

ains of human plasmin. Mechanism of acti-
. J. Biol. Chem. **242:** 2333–2342.
s558-Cys566 bond in Lys78-plasminogen—
Eur. J. Biochem. **251:** 472–479.
combinant human plasminogen in mamma-
of plasmin activity. J. Biol. Chem. **266:**

gen activators on human recombinant apoli-
tivation cleavage site. Biochim. Biophys.

isms contribute to stringent substrate speci-
J. Biol. Chem. **271:** 4461–4467.
is for the thrombotic risks associated with

odulation of endothelial cell surface fibrin-
osis. Nature **339:** 303–305.
activation of plasminogen by human tissue
**7:** 2912–2919.
gradation of fibrin as the basis of a positive
J. Biochem. **140:** 513–522.
of plasminogen—binding sites in human
16.
Z. 1997. The plasma carboxypeptidases and
**7:** 71–75.
. 1987. Effects of kringles derived from
*ro.* Thromb. Res. **47:** 459–468.
fibrin binding, and plasminogen activation.

airs generation of plasmin by fibrin-bound
oscler. Thromb. **11:** 629–638.
a) with plasminogen binding sites on cells.

poprotein(a), plasmin interactions: a model
n. N.Y. Acad. Sci. **667:** 233–238
poprotein(a) on the binding of plasminogen
d tissue-type plasminogen activator. Chem.

nd plasminogen interactions with fibrin: a
) and isolated plasminogen fragments. Bio-

1993. Lipoprotein(a) binds to human plate-
and activation. Biochemistry **32:** 4628–

egulates plasminogen activator inhibitor-1
hem. **266:** 2459–2465.
bit the secretion of tissue plasminogen acti-
rioscler. Thromb. **14:** 438–442.
1991. Lipoprotein(a) inhibits the generation
dogenous inhibitor of smooth muscle cell

transforming growth factor is inhibited in
e **370:** 460–462.
erial thrombosis in cynomologous monkeys
lipoprotein(a). Arterioscler. Thromb. **13:**

n(a) attenuates endogenous fibrinolysis in
nodel. Circulation **96:** 1612–1615.

56. PALABRICA, T.M., *et al.* 1995. Antifibrinolytic acitivity of apolipoprotein(a) *in vivo:* human apolipoprotein(a) transgenic mice are resistant for tissue plasminogen activator-mediated thrombolysis. Nat. Med. **1:** 256–259.
57. BOONMARK, N.W., *et al.* 1997. Modification of apolipoprotein(a) lysine binding site reduces atherosclerosis in transgenic mice. J. Clin. Invest. **100:** 558–564.
58. KRAFT H.G., *et al.* 1989. Changes of genetic apolipoprotein phenotypes caused by liver transplantation. J. Clin. Invest **83:** 137–142.
59. KRONENBERG, F., *et al.* 1996. Lipoprotein(a) in Renal Disease. Am. J. Kidney Diss. **27:** 1–25.
60. SOULAT, T., *et al.* 1999. Evidence that modifications of Lp(a) *in vivo* inhibit plasmin formation on fibrin—a study with individual plasmas presenting natural variations of Lp(a). Thromb. Hæmost. **82:** 121–127.
61. SOULAT, T., *et al.* 2000. Effect of individual plasma lipoprotein(A) variations in vivo on its competition with plasminogen for fibrin and cell binding—an *in vitro* study using plasma from children with idiopathic nephrotic syndrome. Arterioscler. Thromb. Vasc. Biol. **20:** 575–584.
62. GAW, A., *et al.* 1994. Comparative analysis of the apo(a) gene, apo(a) glycoprotein, and plasma concentrations of Lp(a) in three ethnic groups. Evidence for non common "null" allele at the apo(a) locus. J. Clin. Invest. **93:** 2526–2534.
63. UTERMANN, G., *et al.* 1987. Lp(a) glycoprotein phenotypes: inheritance and relation to Lp(a)-lipoprotein concentations in plasma. J. Clin. Invest. **80:** 458–465.
64. MÖLGAARD, J., *et al.* 1992. Significant association between low molecular weight apolipoprotein (a) isoforms and intermittent claudication. Arterioscler. Thromb. **12:** 895–901.
65. SEED, M., *et al.* 1990. Relation of serum lipoprotein(a) and apolipoprotein(a) phenotype to coronary heart disease in patients with familial hypercholesterolemia. N. Engl. J. Med. **322:** 1494–1499.
66. SANDHOLZER, C., *et al.* 1992. Apo(a) isoforms predict risk for coronary heart disease. A study in six populations. Arterioscler. Thromb. **12:** 1214-1226.
67. HERVIO, L., *et al.* 1993. Does apolipoprotein (a) heterogeneity influence lipoprotein (a) effects on fibrinolysis? Blood **82:** 392–397.
68. KRONENBERG, F., *et al.* 1999. Role of lipoprotein(A) and apolipoprotein(A) phenotype in atherogenesis—prospective results from the Bruneck study. Circulation **100:** 1154–1160.