UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
FACULTAD DE MEDICINA
DIVISIÓN DE ESTUDIOS DE POSTGRADO

INSTITUTO NACIONAL DE CARDIOLOGÍA
"IGNACIO CHÁVEZ"

VASCULAR ENDOTHELIAL GROWTH FACTOR PLASMA LEVELS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS AND PRIMARY ANTIPHOSPHOLIPID SYNDROME

TESIS

PARA OBTENER EL TÍTULO DE ESPECIALISTA EN REUMATOLOGÍA

PRESENTA
DRA. DORA LILIANA CANDIA ZÚÑIGA

MÉXICO, D.F., 2002

DEDICATORIA

A ti madre mía dedico este trabajo de investigación.

Tú que siempre fuiste mi apoyo en los momentos difíciles

Tú que creíste en mí cuando todos dudaron.

Tú que compartiste mis esperanzas y mis anhelos, animándome siempre a seguir adelante con tu ejemplo de lucha contra la adversidad y la enfermedad.

Y sé que ni aún la muerte impedirá que tú sigas a mi lado cuidándome como un ángel desde el cielo, por ello te ofrendo este esfuerzo y prometo superarme para llegar a ser una digna hija tuya.

Mamá te amaré por siempre.

VASCULAR ENDOTHELIAL GROWTH FACTOR PLASMA LEVELS IN PATIENTS WITH SYSTEMIC LUPUS ERYTHEMATOSUS AND PRIMARY ANTIPHOSPHOLIPID SYNDROME.

Dra. MARY CARMEN AMIGO CASTAÑEDA

DIRECTORA DE TESIS

Dr. MANUEL MARTÍNEZ-LAVÍN GARCÍA

TITULAR DEL CURSO DE REUMATOLOGÍA

Dr. FERNANDO GUADALAJARA BOO

JEFE DE ENSEÑANZA

MEXICO
INSTITUTO NACIONAL DE CARDIOLOGIA IGNACIO CHAVEZ
Dirección de Enseñanza

PRESENTADO POR :

Dra. DORA LILIANA CANDIA ZÚÑIGA

DIVISIÓN DE ESPECIALIZACIÓN ESTUDIOS DE POSGRADO FACULTAD DE MEDICINA U. N. A. M.

MÉXICO, D. F. 2002

Lupus (2002) 11, 21–24
www.lupus-journal.com

PAPER

Vascular endothelial growth factor plasma levels in patients with systemic lupus erythematosus and primary antiphospholipid syndrome

C Navarro[1], L Candia-Zúñiga[2], LH Silveira[2], V Ruiz[1], M Gaxiola[3], MC Avila[4] and MC Amigo[2]*
[1]Molecular Biology Department, Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico; [2]Department of Rheumatology, Instituto Nacional de Cardiología Ignacio Chávez, Mexico City, Mexico; [3]Morphology Department, Instituto Nacional de Enfermedades Respiratorias, Mexico City, Mexico; and [4]Pathology Department, Instituto Nacional de Cardiología Ignacio Chávez, Mexico City, Mexico

The objective of this study was to assess the possible role of vascular endothelial growth factor (VEGF) in the pathogenesis of systemic lupus erythematosus (SLE) and primary antiphospholipid syndrome (PAPS).

We studied 28 patients with SLE, 10 patients with PAPS, and 24 healthy controls. VEGF plasma levels were measured by ELISA. Immunolocalization of VEGF was done in renal tissue from SLE patients and cadaveric controls.

Our results showed that VEGF plasma levels were increased in SLE patients compared with PAPS and controls. The correlation between clinical manifestations and VEGF levels revealed that SLE patients with renal failure had significantly increased plasma VEGF levels (134.1 + 91.0 pg/ml) compared with SLE patients with normal renal function (42.9 + 19.0 pg/ml), PAPS patients (41.9 + 26.6 pg/ml), and controls (36.2 + 27.0 pg/ml; $P < 0.01$). Immunostaining showed a strong expression of VEGF in SLE renal tissue samples. Our preliminary results indicate that VEGF is increased in plasma from patients with lupus nephritis and a moderate degree of renal failure and is overexpressed in renal tissue from these patients. *Lupus* (2002) 11, 21–24.

Key words: angiogenesis; VEGF; lupus nephropathy; primary antiphospholipid syndrome

Introduction

Angiogenesis is an essential physiologic process and plays a role in diseases such as rheumatoid arthritis, neoplasias and atherosclerosis. Vascular endothelial growth factor (VEGF) and angiogenin (Ang) promote angiogenesis *in vitro* and *in vivo*. New vessel formation seems limited to tissues with some degree of inflammation or hypoxia.[1–3] Systemic lupus erythematosus (SLE) and primary antiphospholipid syndrome (PAPS) feature endothelial damage; however, angiogenesis has not been fully explored in these diseases.[4] In this study, we have investigated a possible role of VEGF and Ang in the pathogenesis of SLE and PAPS.

*Correspondence: MC Amigo, Instituto Nacional de Cardiología Ignacio Chávez, Juan Badiano no. 1, Tlalpan, Mexico City, 14080, Mexico.
E-mail: mary-carmenamigo@hotmail.com
Received 25 August 2001; accepted 5 September 2001

Patients and methods

We studied 28 consecutive patients fulfilling ACR criteria for the classification of SLE seen at the Instituto Nacional de Cardiología Ignacio Chávez. In addition, 10 PAPS patients (Sapporo criteria), and 24 matched healthy controls from the same center were studied. Plasma was obtained from all patients and controls. Kidney samples were obtained by biopsy for diagnostic purposes. Autopsy kidney tissue from patients without renal disease was used as control.

VEGF and Ang plasma levels were performed using a sensitive and specific enzyme-linked immunoabsorbent assay (ELISA) commercial kit following the instructions of the manufacturers (R&D Systems, MN, USA).

Renal tissue obtained by percutaneous biopsy or autopsy was formalin-fixed and paraffin-embedded for examination by conventional light microscopy. Renal sections were immunostained for VEGF with the

10.1191/0961203302lu131oa

Biotin/streptavidin complex technique using the Vectastain Universal Quick Kit (Vector Laboratories, Inc. Burlingame, CA). Polyclonal anti-VEGF antibodies (R&D Systems Inc., MN, USA) were used. All the experimental assays were performed at the Instituto Nacional de Enfermedades Respiratorias. The study was approved by the ethics committee in both medical institutions.

Statistics

Comparisons between groups were analyzed by the non-parametric Kruskal–Wallis test and VEGF and Ang levels were compared among groups with the Dunn's multiple comparison test. The unpaired *t*-test was used to assess differences between SLE subgroups.

Results

Demographic data of the patients are shown in Table 1. PAPS patients were free of acute thrombosis at the time of the study. VEGF plasma levels were increased in SLE patients compared with PAPS patients and healthy controls (Table 1). The correlation between clinical manifestations and VEGF levels showed that SLE patients with renal failure had significantly increased plasma VEGF levels (134.1 ± 91.0 pg/ml) compared with SLE patients with normal renal function (42.9 ± 19.0 pg/ml), PAPS patients (41.9 ± 26.6 pg/ml) and controls (36.2 ± 27.0 pg/ml; $P < 0.01$; Figure 1).

There were no significant differences in Ang levels between groups (data not shown). VEGF immunolocalization in SLE tissue occurred mostly in distal tubular ducts and collecting-duct epithelia, as well as some podocytes. The number of positive cells was much lower in normal samples (Figure 2).

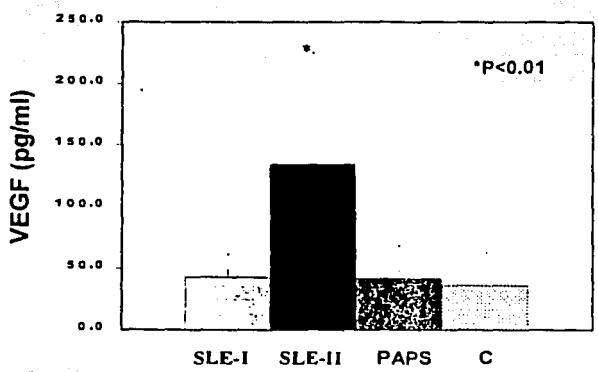
Table 1 Demographic data and VEGF levels

Group	*Gender (female/male)*	*Age (years)*	*VEGF (pg/ml) (media/range)*
SLE	24/4	36.6 ± 16.1	70.25 (4.69–341.2)*
PAPS	8/2	36.2 ± 8.0	34.1 (6.91–83.78)
Healthy controls	19/5	29.2 ± 8.5	23.48 (0–307.4)

*$P < 0.01$.

Discussion

In this study we found that VEGF plasma levels were increased in SLE patients compared to PAPS patients and controls. The correlation between clinical manifestations and VEGF showed that SLE patients with moderate renal failure (endogenous creatinine clearance of 55 ml/min or less) had significantly increased plasma VEGF compared with SLE patients with normal renal function. VEGF is a multifunctional cytokine that contributes to angiogenesis both directly and indirectly. Many cell types including endothelial cells, platelets, macrophages, smooth muscle cells/pericytes and podocytes upregulate its expression in response to several cytokines or hypoxia. VEGF activities are mediated by high affinity receptor tyrosine kinases (RTKs).[5,6] Vascular conditions such



SLE-I = Systemic lupus erythematosus patients with normal renal function
SLE-II= Systemic lupus erythematosus patients with moderate renal failure

Figure 1 VEGF plasma levels were significantly increased in SLE patients with renal failure compared with SLE patients with normal renal function. PAPS patients and controls. Values are expressed as average ± s.d.

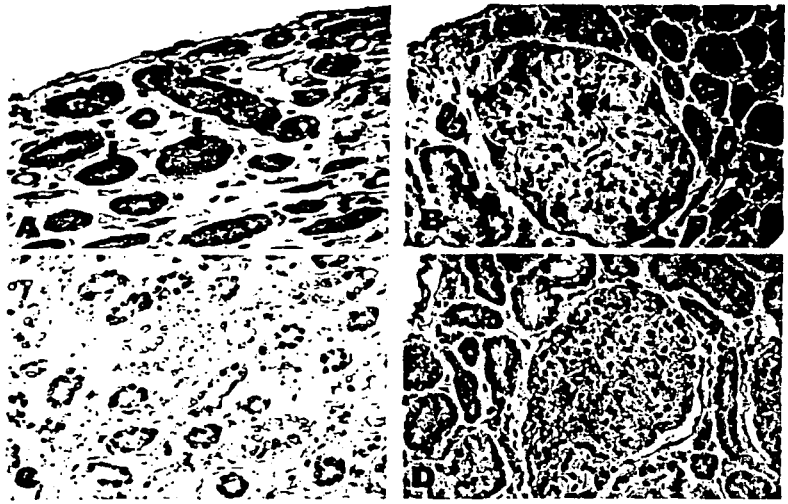


Figure 2 Immunolocalization of VEGF in SLE and normal kidney (20×). (A) Kidney sample of an SLE patient showing most of the distal tubular ducts and collecting-ducts epithelia positive for VEGF (arrows). (B) Kidney sample of another SLE patient exhibiting some podocytes (head arrows) with strong expression of VEGF. (C) and (D) Normal tissue with low immunohistochemical staining of VEGF.

as those occurring in SLE and PAPS, which include inflammation, vessel occlusion or thickening of the vascular wall, might be a strong stimulus for angiogenic factor production. Since VEGF is the main angiogenic factor we decided to study its expression in these two diseases.

Our results showed that patients with PAPS had VEGF plasma levels that were similar to those found in controls. This could be explained by the clinical characteristics of the PAPS patients, who were free of clinical arterial thrombosis at the time of the study. This is in agreement with the study by Williams *et al*,[4] who found high plasma levels of VEGF and tissue factor in patients with PAPS with arterial thrombosis, but not in those with venous thrombosis. In that study, VEGF plasma levels in patients with PAPS with venous thrombosis were similar to those in our study. In contrast, we found that SLE patients with renal failure had significantly raised plasma levels and overexpression of VEGF in renal tissue. To our knowledge, angiogenesis has seldom been explored in lupus nephritis. Nishitani *et al*[7] measured VEGF mRNA in peripheral blood mononuclear cells in 34 patients with lupus nephritis. They were unable to find significant differences between patients with lupus nephritis and healthy volunteers. Their negative results could be explained if other cell types within the kidney were the main site of VEGF production. In support of this hypothesis, we found by immunolocalization upregulation of VEGF protein in epithelial cells and podocytes.

Several groups studying nephropathies such as diabetic, thrombotic microangiopathy and renal transplant rejection have demonstrated high VEGF plasma levels in these conditions, suggesting that this factor is involved in glomerular endothelial repair.[8–10] In addition, urinary excretion of VEGF has been found decreased in idiopathic membranous glomerulonephritis and it has been proposed as an indicator of disease activity in this condition.[11] Studies are underway in our laboratory to assess VEGF urinary excretion in SLE and other conditions.

Our preliminary results indicate that VEGF is increased in plasma from patients with lupus nephritis and a moderate degree of renal failure and is overexpressed in renal tissue from these patients. It is therefore possible that this angiogenic factor is related to endothelial repair in this condition. However, decreased VEGF excretion could also explain the findings. Other angiogenic factors, or an imbalance between inductor and inhibitory angiogenic factors, may be involved in the characteristic vasculopathy of PAPS.

References

1 Colville-Nash PR, Willoughby DA. Growth factors in angiogenesis: current interest and therapeutic potential. *Mol Med Today* 1997; Jan: 14–23.

2 Koch AE. The role of angiogenesis in rheumatoid arthritis: recent developments. *Ann Rheum Dis* 2000; 59: 165–171.
3 Thompson WD, Li WW, Maragoudakis M. The clinical manioulation of angiogenesis: pathology, side-effects, susprises and opportunities with novel human therapies. *J Pathol* 1999; 187: 503–510.
4 Williams FM, Parmar K, Hughes GR, Hunt BJ. Systemic endothelial cell markers in primary aniphospholipid syndrome. *Thromb Haemost* 2000; 84: 742–746.
5 Blau HM, Banfi A. The well-tempered vessel. *Nature Med* 2001; 7: 532–534.
6 Thomas KA. Vascular endothelial growth factor, a potent ans selective angiogenic agent. *J Biol Chem* 1996; 271: 603–606.
7 Nishitani Y, Kubo A, Iwano M *et al*. Imblance between interleukin-6 and adrenomedullin mRNA levels in peripheral blood mononuclear cells of patients with lupus nephritis. *Clin Exp Immunol* 2001; 124: 330–336.
8 Ostendorf T, Kunter U, Eitner F *et al*. VEGF mediates glomerular endothelial repair. *J Clin Invest* 1999; 104: 913–923.
9 Nitta K, Uchida K, Kimata N *et al*. Increased serum levels of vascular endothelial growth factor in human crescentic glomerulonephritis. *Clin Nephrol* 1999; 52: 76–82.
10 Del Prete D, Anglani F, Ceol M *et al*. Molecular biology of diabetic glomerulosclerosis. *Nephrol Dial Transplant* 1998; 13(Suppl 8): 20–25.
11 Honkanen EO, Teppo AM, Gronhagen-Riska C. Decreased urinary excretion of vascular endothelial growth factor in idiopathic membranous glomeulonephritis. *Kidney Int* 2000; 57: 2343–2349.