UNIVERSIDAD NACIONAL AUTÓNOMA DE MÉXICO
FACULTAD DE MEDICINA
INSTITUTO NACIONAL DE ENFERMEDADES RESPIRATORIAS

COMPARACIÓN DE LA BIODISPONIBILIDAD DE DOS FORMULACIONES INHALADAS DE SALBUTAMOL EN PACIENTES CON ASMA MODERADA POR MEDIO DE LA MEDICIÓN DEL EFECTO BRONCODILATADOR

PARA OBTEBER EL TÍTULO DE NEUMÓLOGO
PRESENTA

HÉCTOR LEÓN MOLINA

INSTITUTO NACIONAL DE ENFERMEDADES RESPIRATORIAS
SET. 23 2004.
SUBDIRECCION DE ENSEÑANZA

TUTOR:
DRA. ROCÍO CHAPELA MENDOZA

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ASUNTO: Autorización del trabajo de investigación del Dr. Héctor León Molina.

DR. ISIDRO AVILA MARTINEZ
SECRETARIO DE SERVICIOS ESCOLARES
DE LA FACULTAD DE MEDICINA
Presente.

Estimado Dr. Avila Martínez:

Me permito informar a usted que el **Dr. Héctor León Molina**, alumno del curso de especialización en **Neumología** en el **Instituto Nacional de Enfermedades Respiratorias**, presenta el trabajo de investigación intitulado ***"Comparación de la biodisponibilidad de dos formulaciones inhaladas de salbutamol en pacientes con asma moderada por medio de la medición del efecto broncodilatador".***

De conformidad con el artículo 21 capítulo 5º. de las Normas Operativas del Plan Unico de Especializaciones Médicas (PUEM) se considera que cumple con los requisitos para validarlo como el trabajo formal de Investigación que le otorga el derecho de la diplomación como especialista.

Sin otro particular de momento, reciba un cordial saludo.

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LCRP*ajr.

SUBDIVISIÓN DE ESPECIALIZACIÓN
DIVISIÓN DE ESTUDIOS DE POSGRADO
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DR. LEOBARDO C. RUIZ PEREZ
JEFE DE LA SUBIDIVISION DE
ESPECIALIZACIONES MEDICAS

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

Assessment of Comparative Bioequivalence of Two Metered-Dose Inhaler Formulations of Salbutamol

Measuring Bronchodilatory Effect in Patients with Asthma

Héctor León-Molina,[1] *Francisco J. Flores-Murrieta*[1,2] and *Rocío Chapela*[1]

1 Instituto Nacional de Enfermedades Respiratorias, Secretaría de Salud, Mexico City
2 Sección de Estudios de Posgrado e Investigación, Escuela Superior de Medicina, Instituto Politécnico Nacional, Mexico City

Abstract

Objective: To compare the bioavailability of two metered-dose inhalers (MDIs) containing salbutamol (albuterol) by means of a spirometric evaluation of the time-course of bronchodilation in patients with moderate asthma.

Design and patients: 25 asthmatic patients (12 males, 13 females) participated in the study. Study participants received salbutamol 200μg (Ventolin®, GlaxoSmithKline Mexico City, or Assal®, Salus SA de CV, Mexico City) on separate days according to a double-blind, crossover design. Spirometry was performed 30 minutes before and at selected times during the 8 hours following drug administration. The time-course of changes in forced expiratory volume in 1 second (FEV_1) [transformed to individual percentage of maximal response] was used to compare the formulations. Pharmacodynamic parameters, maximal effect (E_{max}) and area under the percentage of response-time curve (AUC) were obtained and compared by analysis of variance, and ratios of AUC and E_{max} and 90% confidence limits were calculated.

Results: Values obtained for E_{max} were 94.81 ± 2.19% and 84.45 ± 3.44% for Ventolin® and Assal®, respectively, whereas values for AUC were 25 278 ± 1873 %•min and 18 155 ± 1806 %•min, respectively. Ratios were 89.1 and 71.8% with 90% confidence limits of 79.6 to 98.5% and 53.9 to 89.7% for E_{max} and AUC, respectively. The probability according to the two one-sided t-test of having values lower than 80% was higher than 0.05 for both AUC and E_{max}, indicating that the formulations tested are not bioequivalent.

Conclusions: It is concluded that this method is suitable for comparing the bioavailability of MDI formulations of bronchodilatory agents and that the formulations tested were not bioequivalent.

It is widely accepted that the use of aerosols in the short- and long-term treatment of asthma is preferred over other routes of administration, since the drug directly reaches the required receptors, minimising systemic dilution and the total drug amount needed. Additionally, systemic adverse effects are diminished and the onset of action is faster.

Among the different devices used, metered-dose inhalers (MDIs) are recommended for bronchodilatory as well as anti-inflammatory medication in most situations; they are reliable and relatively inexpensive. Over the last few decades, many generic MDI bronchodilators have become available around the world, especially with salbutamol, which is one of the most frequently used drugs for the treatment of asthma and other obstructive diseases.[1]

Therefore, it is very important to have a suitable test to evaluate whether MDI formulations of bronchodilatory agents are interchangeable. Comparison of bioavailability in order to establish if two formulations are bioequivalent has been widely evaluated by measuring the plasma level time-course of the studied drug.[2] However, in the case of MDIs of bronchodilator agents, the plasma levels that are reached after a therapeutic dose are too low to be accurately determined.[3] Additionally, in order to avoid adverse effects, it is desirable that the drug does not reach the systemic circulation.[4]

Previously, several tests have been proposed for the purpose of establishing whether salbutamol formulations are bioequivalent. One of them is the measurement of salbutamol excreted in urine during a period of 30 minutes.[5] This test has two disadvantages: the first is that under these conditions it is not possible to evaluate the time-course of salbutamol plasma levels or effect, and secondly, the measurement of drug in urine is an index of drug absorption from the lung, but it is not representative of the bronchodilatory effect–time-course of the drug.[6] Another proposed test is the evaluation of plasma levels of salbutamol over a short time period;[7] however, as with other methods, this test has several disadvantages: first, salbutamol concentration can only be evaluated in a limited number of samples, and second, the amount that has to be administered is 1200μg, which is very high considering that the therapeutic dose of salbutamol is between 90 and 200μg.

Evaluation of the bronchodilatory effect may be a better method for the assessment of bioequivalence of MDI formulations of salbutamol.[8] Therefore, the purpose of this study was to evaluate the usefulness of the spirometrically determined bronchodilation in patients with moderate asthma for the comparison of bioavailability of inhaled formulations of salbutamol.

Patients and Methods

Study Participants

Twenty-five patients with moderate asthma (12 males, 13 females) with a mean age of 41.4 ± 3.3 years were enrolled for this study, which was carried out following the recommendations of the Declaration of Helsinki. The protocol was approved by the Institutional Bioethical and Scientific Committee, and in every case patients provided written informed consent.

Diagnosis of moderate asthma was made according to the guidelines of the Global Initiative for Asthma,[9] based on clinical history, frequency and severity of symptoms, and a spirometric curve that showed obstruction and reversibility of forced expiratory volume in 1 second (FEV_1) of more than 15%. We chose patients with moderate asthma so that they would have a measurable spirometric change without risk, since it was required that patients were free of any medication during the study.

Exclusion criteria were: a history of life-threatening episodes of asthma; concomitant systemic diseases at the time of the study, according to medical history, medical examination and appropriate laboratory tests; use of any medication other than inhaled bronchodilators; lack of response to salbutamol on more than 15% of FEV_1 24 hours before the beginning of the study. Additionally,

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those patients who showed a difference of 20% or more in the baseline FEV_1 value between sessions were removed from the pharmacodynamic and statistical analyses.

Study Design

Patients arrived at the hospital 24 hours before the beginning of the study and were trained in the correct use of MDIs using a spacing chamber. For the 12 hours before the administration of the study drug, subjects did not use any drug (including bronchodilatory agents). Salbutamol (200µg, two puffs of 100µg), Ventolin®[1], GlaxoSmithKline, Mexico City, or Assal®, Salus SA de CV, Mexico City, was administered using a double-blind, crossover design with a washout period of 12 hours. The MDIs used for both formulations were new and belonged to the same batch; one was issued per patient. Prior to the first administration, all the MDIs were primed by four discharges, and the sequence of administration was established randomly by means of a random numbers table.

Spirometric measurements were performed using a Sensormedics® spirometer following American Thoracic Society guidelines.[10] Baseline parameters were measured 30 minutes before drug administration at the start of each study day. The study proceeded only if the baseline FEV_1 was within 20% of that of the previous study day, as stated earlier, and spirometry was repeated at 0, 5, 15 and 30 minutes and 1, 1.5, 2, 4, 6 and 8 hours after drug administration. Additionally, symptoms, blood pressure, heart and breathing rate were determined at 0, 0.5, 1, 2 and 8 hours after drug administration.

Pharmacodynamic and Statistical Analyses

Several parameters were obtained from the spirometric curves: FEV_1, forced vital capacity (FVC), peak expiratory flow rate (PEFR), FEV_1/FVC ratio and forced expiratory flow 25-75% (FEF_{25-75}). As FEV_1 is one of the most important indices for the evaluation of respiratory function in patients with asthma, this parameter was employed in the comparison of the formulations. In order to diminish interindividual variability, FEV_1 was converted to percentage of response as stated in equation 1:

$$\% \text{ response} = \frac{FEV_{1(\text{after salbutamol})} - FEV_{1(\text{baseline})}}{FEV_{1(\text{maximum})} - FEV_{1(\text{baseline})}} \times 100$$

where FEV_1 (baseline) was the value measured at the start of each study day, FEV_1 (after salbutamol) was measured at the stated intervals after salbutamol administration and FEV_1 (maximum) was the highest FEV_1 ever recorded for that patient during either study day. This method of expressing FEV_1 reflects the degree to which a patient's expiratory flow returns towards maximal possible function as a result of the drug therapy and allows comparison of subjects independent of their body size and airway obstruction. It emphasises differences when obstruction is moderate, and there is therefore less capacity for improvement, and it adjusts for day-to-day differences in baseline pulmonary function.[11] Using the individual percentage of response-time courses, pharmacodynamic parameters were obtained. Maximal effect (E_{max}) and time to reach this maximum (t_{max}) were directly obtained from the individual plots. Area under the percentage of response-time curve (AUC) was calculated by the trapezoidal rule.[12]

In order to establish whether the formulations evaluated were bioequivalent, initially E_{max} and AUC were compared by analysis of variance for a crossover design. Then, the ratio for E_{max} and AUC [calculated by dividing values obtained with formulation A over formulation V (Assal®/Ventolin®)] and 90% confidence limits for both parameters and both formulations were calculated. Limits of acceptance for considering that the formulations tested are bioequivalent were fixed at 80 to 120%. Additionally, a Schuirmann's test (two one-sided t-test) was performed in order to calculate the probability of exceeding the limits of acceptance.[13] Bioequivalence was concluded when

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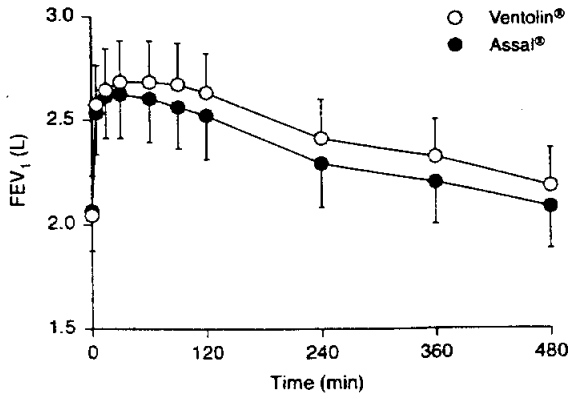

Fig. 1. Mean (± SEM) forced expiratory volume in 1 second (FEV_1) time-course obtained in 20 patients with mild persistent asthma after administration of salbutamol 200μg in two different metered-dose inhaler formulations.

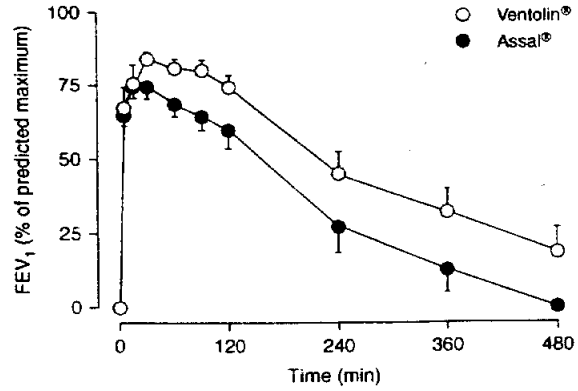

Fig. 2. Percentage of response based on forced expiratory volume in 1 second (FEV_1) time-curve after the administration of 200μg of salbutamol in two metered-dose inhaler formulations to patients with mild persistent asthma. Data are expressed as the mean of 20 patients ± SEM.

the p-value of exceeding the limits of acceptance was ≤0.05.

Results

Twenty-five patients participated in this study. One of them was removed from the study because he developed an asthmatic exacerbation in the washout period between sessions. Four other subjects were removed from the analysis due to a change in their baseline FEV_1 between sessions greater than 20%. Therefore, pharmacodynamic and statistical analyses were carried out on 20 study participants. Figure 1 shows FEV_1 time-curves obtained after administration of the two salbutamol formulations. It can be seen that Ventolin® showed higher values of FEV_1 than those obtained with Assal®. In order to establish if the formulations tested were bioequivalent, FEV_1 was transformed as a percentage of response, as described in equation 1. Figure 2 shows the percentage of response–time-course obtained with the two formulations. The pharmacodynamic parameters obtained are shown in table I. It is clear that Ventolin® produced a greater bronchodilatory response than Assal®.

Statistical comparison by analysis of variance indicated that the formulations evaluated were

Table I. Pharmacodynamic parameters obtained after the administration of salbutamol 200μg in two MDI formulations, Ventolin® (formulation V) and Assal® (formulation A). Data correspond to the mean of 20 patients ± SEM

Parameter	Formulation V	Formulation A
E_{max} (%)	94.81 ± 2.19	84.45 ± 3.44
t_{max} (min)	72 ± 22.91	43 ± 8.43
AUC (%•min)	25 278.3 ± 1872.5	18 155.4 ± 1806.3

AUC = area under the percentage of response-time curve; E_{max} = maximal effect; **MDI** = metered-dose inhaler; t_{max} = time to reach E_{max}.

Table II. Statistical comparison by analysis of variance of pharmacodynamic parameters, AUC and E_{max}, obtained after administration of salbutamol 200μg in two MDI formulations, Ventolin® and Assal®, using a crossover design

Parameter	Effect	F	Probability
AUC	Sequence	3.08	0.0963
AUC	Subject (sequence)	0.92	0.5724
AUC	Period	0.12	0.7351
AUC	Formulation	7.43	0.0138
E_{max}	Sequence	0.27	0.607
E_{max}	Subject (sequence)	0.31	0.9915
E_{max}	Period	0.01	0.915
E_{max}	Formulation	4.05	0.0594

AUC = area under the percentage of response-time curve; E_{max} = maximal effect; **MDI** = metered-dose inhaler.

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Table III. Statistical analysis of E_{max} and AUC in order to establish bioequivalence or bioinequivalence of the salbutamol formulations, Ventolin® (formulation V) and Assal® (formulation A)

Parameter	Ratio A/V (%)	90% confidence limits	p < 80%	p > 120%
E_{max}	89.1	79.6 – 98.5	0.056	0.007
AUC	71.8	53.9 – 89.7	0.780	0.000

AUC = area under the percentage of response-time curve; E_{max} = maximal effect.

different, since a statistical difference was observed in AUC (table II). Moreover, when ratios between formulations for E_{max} and AUC, as well as confidence limits were calculated, it was observed that values exceeded the limits of acceptance. Additionally, when probabilities of exceeding the limits of acceptance were calculated we observed that probabilities of having confidence limits lower than 80% in both AUC and E_{max} were higher than 0.05 (table III), indicating that the formulations were bioinequivalent.

Figure 3 shows a comparison of FEF_{25-75}, a test that measures small airway resistance, obtained after the administration of the two formulations. It can be seen that this parameter is more variable than FEV_1, as has been previously stated.[14] However, it is clear that a similar profile to that observed with FEV_1 is shown (Ventolin® shows a greater effect than that observed with Assal®). Although Ventolin® produced a greater response in FEV_1 and FEF_{25-75}, no statistical differences were observed in raw data due to wide variability. Therefore, in order to adequately evaluate if the formulations were bioequivalent, we decided to transform the data according to equation 1 to diminish interindividual variability. Using this transformation, we were able to observe an important difference between formulations (see figure 2).

Both treatments were well tolerated, and expected adverse effects such as tremor, palpitations or nervousness were not reported. No important changes in blood pressure, respiratory and heart rates or additional symptoms were found. Furthermore, when patients were asked blindly about efficacy of treatment, 14 of 20 showed a preference for Ventolin® action.

Discussion

In recent years, the treatment of asthma has relied mainly on the use of MDI formulations of medications. Therefore, it is very important to have methods for evaluation of generic MDI formulations of bronchodilatory and anti-inflammatory drugs. Unfortunately, there is not an accurately predictive model to relate *in vitro* characteristics with efficacy and tolerability *in vivo*.[15]

In this paper we compared the bioavailability of two MDI formulations of salbutamol by comparing the time-course of the bronchodilator response in asthmatic patients. Several tests have been proposed in order to establish whether MDI formulations of bronchodilatory agents are bioequivalent, such as measurement of the particle size of suspension,[16] evaluation of salbutamol plasma levels after a high dose of the drug,[7] and the amount of salbutamol excreted in urine during a short time period.[5] However, none of these tests provided

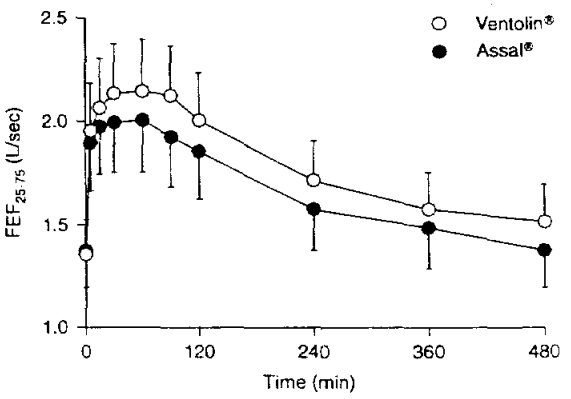

Fig. 3. Forced expiratory flow rate 25-75% (FEF_{25-75}) time-course obtained after administration of two metered-dose inhaler formulations of salbutamol to 20 patients with moderate persistent asthma. Data are expressed as mean ± SEM.

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information about the therapeutic effect of the drug. It is important to note that salbutamol produces its effect directly in the bronchi, therefore, the drug concentration in this site is more important than that in the systemic circulation. Moreover, most of the adverse effects produced by salbutamol are a consequence of the absorption of the drug into the systemic circulation; it is therefore desirable that there is no absorption of the drug into the systemic circulation. In this study we evaluated the bronchodilatory effect–time-course of salbutamol by spirometry. Data were transformed into a percentage of individual response in order to minimise the intersubject variability as well as that between study days, as described by Blake et al.[11] This transformation has been shown to be useful in the evaluation of bronchodilatory drugs, allowing the quantitative characterisation of the bronchodilator activity of different β-adrenergic agonists.[17]

In order to be able to reach an adequate conclusion on the bioequivalence of bronchodilatory drugs using this data transformation, it is necessary to demonstrate that the bronchodilator effect is not at its maximum with the dose used. Therefore, we evaluated the percentage of predicted maximum achieved during this study, as described by Mitchell et al.[18] It can be clearly seen in figure 4 that the predicted maximum reached during the study is about 60%, suggesting that a comparison of formulations using this method is adequate.

Asthma affects airways of all sizes, and small airways account for a large part of the overall obstruction; unfortunately, FEF_{25-75}, which tests their function, has a variability of over 30%. On the other hand, FEV_1 reflects the sum of airways airflow, and with a 10% variability it is the best spirometric parameter for the evaluation of bronchodilatory effect.[14] FEF_{25-75} and FEV_1 curves showed similar profiles: a higher effect for Ventolin® than that observed with Assal®.

Pharmacodynamic parameters were calculated in order to compare them by statistical analysis as described by Schuirmann.[13] Limits of acceptance were fixed at 80 to 120% for both E_{max} and AUC. These limits have been commonly used in

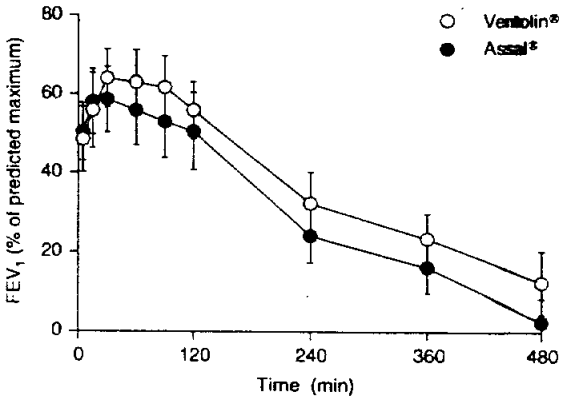

Fig. 4. Bronchodilatory response [expressed as % predicted maximum forced expiratory volume in 1 second (FEV_1)] time-curve after administration of salbutamol 200μg in two different formulations to 20 patients with moderate asthma. Data are expressed as mean ± SEM.

bioequivalence studies; however, when changes versus baseline are considered, as was the case in this study, wider limits of acceptance (67 to 150%) have been suggested.[19] If we had considered these limits of acceptance for concluding bioequivalence, the conclusion would be similar: the formulations were not bioequivalent, since confidence limits for AUC were much lower than 67%, which means that the effect of Ventolin® is higher than that observed with Assal®.

Both formulations contained the same quantity of salbutamol; however, Assal® produced a lower effect than that observed with Ventolin®. This difference may be due to pharmaceutical factors that we did not evaluate in this study, such as particle size.

Conclusion

In conclusion, the evaluation of the time-course of spirometry in patients with moderate asthma is an accurate, well tolerated, easy to perform and inexpensive method for the establishment of bioequivalence of MDI formulations for bronchodilatory agents. The formulations tested in this study were bioinequivalent and they cannot be considered interchangeable.

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Correspondence and offprints: Dra. *Rocío Chapela*, Instituto Nacional de Enfermedades Respiratorias, Tlalpan 4502, CP 14080, México D.F., México.
E-mail: rociochm@diego.iner.gob.mx

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