Biphasic Effect-Time Courses in Man after Formoterol Inhalation: Eosinopenic and Hypokalemic Effects and Inhibition of Allergic Skin Reactions

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ABSTRACT

The kinetics of inhaled racemic formoterol and its effects on the size of the early cutaneous reaction to intradermal injection of an allergen, eosinopenia and hypokalemic were assessed by pharmacokinetic-pharmacodynamic modeling. After inhalation of either 120 µg of formoterol or placebo, blood samples were taken and skin tests were performed in seven healthy subjects. A two-compartment model was needed to describe the observed formoterol plasma concentration-time curves. To describe the observed biphasic concentration, two absorption routes with different absorption rate constants were incorporated in the model. These two phases were explained by rapid absorption via the respiratory tract together with a slower and delayed oral absorption. For the description of the concentration-effect relations, an Emax (the maximum obtainable effect) formula for competitive agonism, with an effect compartment, had to be used. Fitting the wheal and flare, an apparent diurnal variation had to be taken into account by incorporating in the model rising base-line values. For the flare responses, influence of the location on the forearm appeared to be operative. Systemic formoterol absorbed via the oral route behaved differently from the fraction absorbed via the lungs, with EC50 (steady state concentration that gives 50% of maximum effect) values for all three systemic effects being three times lower after oral absorption than after absorption via the respiratory tract. Pharmacodynamic parameters can probably only be estimated quantitatively when the kinetics of the separate enantiomers of formoterol can be taken into account.

Formoterol fumarate is a selective beta-2 adrenoceptor agonist that has a rapid onset and prolonged duration of bronchodilatory action. The latter is especially notable when formoterol is administered by inhalation (Lofdahl and Svedmyr, 1989; Wallin et al., 1993). Formoterol is marketed as a racemate, consisting of the (RR) and (SS) enantiomers. Apparently, the (RR) enantiomer is 1000 times more potent than the (SS) enantiomer (Trofast et al., 1991). Lung deposition after inhalation is ~10% to 15% of the total dose. The major part of inhaled drugs is either swallowed or exhaled (Chrystyn, 1994). Both pharmacokinetics and responses depend on the way a drug is administered; therefore, different routes of absorption can influence the extent and the time course of effects. The time course of formoterol serum concentrations in humans after inhalation has not been described before due to low inhalation dosages, which lead to plasma concentrations in the low picogram per millimeter range (van den Berg et al., 1994c).

The role of beta-2 adrenoceptor agonists in the treatment of asthma has been under discussion, not in the least because it was argued that their sole action was a reduction of bronchoconstriction (Barrett and Strom, 1995; Sears et al., 1990; Spitzer et al., 1992). Other actions besides this bronchodilation have been claimed, some of which may modulate the airway inflammation considered to have a causal relation to asthma (Barnes, 1993; Johnson, 1993; Kaliner and Austen, 1974). One of these putative anti-inflammatory actions could be the alteration of the function of mast cells. A reaction caused by an intracutaneous injection of a purified allergen in a sensitive subject is the so-called wheal and flare re-

ABBREVIATIONS: α, rate constant of distribution; β, rate constant of elimination; A, intercept of distribution; B, intercept of elimination; AUC, area under the curve; Ce, concentration in hypothetical effect compartment; Cp, plasma concentration; Cmax, maximum plasma concentration; D, dose; Emax, maximum obtainable effect; E0, base-line value; EC50, steady state concentration that gives 50% of maximum effect; Fr, fraction of the total amount of formoterol that appears in the systemic circulation, absorbed via the pulmonary route; F, bioavailability; IgE, immunoglobulin E; Kp, absorption rate constant; kst, rate constant of drug distribution from peripheral into central compartment; kSO, rate constant of elimination from effect compartment; n, sigmoid factor; PK/PD, pharmacokinetic-pharmacodynamic; t, time after dosing; Tmax, time after dosing with maximum concentration; tlag, lag time; Vc, volume of central compartment; t1/2, refers to systemic formoterol absorbed via the lungs and upper airways; t2, refers to systemic formoterol absorbed via the gastrointestinal route.
sponse, which is characterized by local swelling of the skin surrounded by a circumscript erythema. Early studies demonstrated that epinephrine can suppress the wheal and flare reaction in humans (Tuft and Brodsky, 1936). The epinephrine-mediated suppression could be adequately explained by elevation of intracellular cAMP levels, which results in inhibition of histamine release from mast cells. The extent of inhibition was found to be of such magnitude that it was assumed to contribute substantially to the therapeutic action of beta-2 adrenoceptor agonists (Lichtenstein and Margolis, 1968; Silverman et al., 1986).

In the present study, formoterol serum concentrations were measured in seven healthy subjects after inhalation of a single dose via a metered-dose inhaler. Effect measurements were performed at frequent time intervals. PK/PD was used to describe the various concentration-effect relationships. As possible anti-inflammatory effect parameters, eosinophilic granulocyte counts in peripheral blood and the size of the early wheal and flare reaction were used. Effect on plasma potassium after administration of formoterol was also studied. The fall of plasma potassium due to a beta-2 adrenoceptor agonist can be regarded as a sensitive parameter for its beta-2 adrenoceptor mediated action and can also be used as a safety parameter. Furthermore, concentration-dependent hypokalemia seems to be a surrogate marker for bronchodilation (Braat et al., 1992; Jonkers et al., 1987).

Materials and Methods

The study protocol was approved by the Institutional Review Board of the Academic Medical Center.

Subjects

Twenty-three healthy male students volunteered to participate in the study. After informed consent had been obtained, they were tested for a positive skin reaction to intradermally injected grass, house dust mite or cat allergen of 30 biological units/ml. Any reaction to allergen that could be visualized by means of a distinct wheal and flare reaction was regarded as positive. Seven volunteers had a positive skin reaction; all were included in the study. Characteristics of the seven subjects are presented in table 1. When more than one allergen gave a positive skin reaction, the allergen that produced the largest wheal and flare was chosen to be used for further testing. The allergen gave a positive skin reaction, the allergen that produced the positive skin reaction; all were included in the study. Characteristics

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Subject 1</th>
<th>Subject 2</th>
<th>Subject 3</th>
<th>Subject 4</th>
<th>Subject 5</th>
<th>Subject 6</th>
<th>Subject 7</th>
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<th>S.D.</th>
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<td>Allergen</td>
<td>Mite</td>
<td>Cat</td>
<td>Mite</td>
<td>Mite</td>
<td>Mite</td>
<td>Grass</td>
<td>Cat</td>
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<td>3</td>
<td>9</td>
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<td>10</td>
<td>0.3</td>
<td>4.1</td>
<td>3.9</td>
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</table>

Experimental Design and Interventions

The study had a randomized, double-blind, placebo-controlled crossover design. On two experimental days separated by at least 1 week, the subjects arrived at 8:00 a.m. on the ward. They had been instructed not to drink any caffeine-containing beverages that day and to have used only a light breakfast in the morning. A standard lunch was provided. During the experiment, subjects were seated in a comfortable chair to minimize physical strain. An intravenous catheter was inserted in a forearm vein and remained in place for ≥7 hr, enabling frequent blood collection. The system was kept patent with heparinized saline (1%) solution. Before drug administration, one pair of skin tests was performed to obtain base-line values, and blood samples were taken for base-line potassium and eosinophil measurements and to exclude the presence of substances that could interfere with the formoterol assay.

At time t = 0, –30 min after insertion of the cannula, formoterol or placebo was administered by aerosol. Subjects had been instructed how to inhale correctly. Before the aerosol was used, the canisters were well shaken. The dose consisted of either 10 puffs of 12 μg of formoterol or 10 puffs of placebo. It took the subjects 3 min to inhale the dose. One subject received only 80% of the intended dose (i.e., 96 μg of formoterol). During 8 hr after dosing, 10 blood samples were taken for analysis of formoterol, for eosinophil counts and for potassium level determination, and nine pairs of skin tests were done at 15, 30, 45, 60, 90, 120, 180, 240, 330 and 450 min after drug administration; at 330 min after dosing, only blood was collected. Blood samples were centrifuged for 10 min at 4000 rpm immediately after clotting. Plasma samples for formoterol concentration analysis were stored at –20°C. The plasma samples for potassium measurements were analyzed immediately in the Laboratory for Clinical Pharmacology, and those for the eosinophil counts were transported as soon as possible to the Clinical Chemistry Laboratory of our hospital.

Skin tests. Allergens were obtained from ALK-Benelux (Groningen, Holland). Standard intracutaneous skin tests were performed by injection of 20 μl of allergen extract in the forearm.

Drugs. Formoterol fumarate dihydrate (FORADIL, Ciba-Geigy, Basel, Switzerland) was used. For administration, a commercially available aerosol inhalator was used. The appearance of the inhalator for the placebo was the same as the one used for the formoterol.

Formoterol Assay

Plasma levels of formoterol were analyzed using high-pressure liquid chromatography with electrochemical detection as described previously (van den Berg et al., 1994c). Bromo-formoterol was used as an internal standard. Reversed phase extraction was carried out using 1-mL propylsulfonic acid columns. A C8 analytical column was used in the chromatographic system. The level of quantification of the assay was 20 pg of formoterol/ml of plasma. However, due to variation of the sensitivity of the electrochemical detector, the limit of detection, with a signal-to-noise ratio of 3:1, could be as low as 10 pg/ml. On each day that the assay was run, a new calibration curve was made of plasma samples spiked with 0, 25, 50, 100 and 200 pg/ml of formoterol fumarate dihydrate. Whenever formoterol concentration is mentioned, this refers to the plasma concentration of formoterol fumarate dihydrate.

Measurements

A conventional flame photometer (model 143, Instrumentation Laboratory) was used for potassium measurements. A small fraction of each blood sample was used for peripheral eosinophil counting. Total blood eosinophil counts were determined with a Technicon
H6000 automated differential leukocyte counter (Technicon Instruments Co., Tarrytown, NY) using peroxidase enzyme detection were performed in the Laboratory of Clinical Chemistry in our hospital.

Skin tests were performed in duplicate, with five pairs of skin tests on each forearm. One pair of tests was performed before dosing, nine pairs thereafter. All intradermal injections were given by the same person. At 15 min after allergen injections, the outline of the wheal and flare reactions were delineated with a marker and copied to adhesive tape. The size of wheal and flare was measured by weighing the pieces that were cut out of the paper onto which the outlines were photocopied. The size of the area was then calculated by dividing the measured weight of the wheal and flare by the weight per area of the paper. The weighing of the wheals and flares was done in random order with no knowledge of the experimental day and time point. After a wash-out period of ≥1 week, the same procedure was repeated. On this second day, the skin tests were done in identical order as on the first day.

**Data analysis.** All PK/PD data were fitted to the appropriate equations using the nonlinear regression computer program PC-NONLIN (Metzler and Weiner, 1992). To identify outliers, we looked at graphic presentation of the data and considered the standardized residuals. Plasma-concentration-time curves were visually inspected. As in all curves, two concentration peaks could either be observed or presumed to be present; a dual absorption via lung and gut was postulated. The triexponential equation for a two-compartment model with first-order absorption as described by Gibaldi and Perrier (Gibaldi and Perrier, 1975) was adjusted in such a way that two different absorption rate constants were incorporated:

\[
C_p(t) = C_{p1}(t) + C_{p2}(t)
\]

\[
C_{p1}(t) = A_1 \cdot e^{-\alpha \cdot t} + B_1 \cdot e^{-\beta \cdot t} - (A_1 + B_1) \cdot e^{-k_{d1} \cdot t}
\]

\[
C_{p2}(t) = A_2 \cdot e^{-\alpha \cdot t \cdot t_{lag}} + B_2 \cdot e^{-\beta \cdot t \cdot t_{lag}} - (A_2 + B_2) \cdot e^{-k_{d2} \cdot t}
\]

This kinetic model describes the concentrations in time as if at t = 0 two doses were given of which one is absorbed via the respiratory tract (i.e., mainly during an early, first absorption phase) and the other via the gastrointestinal tract (i.e., during a second absorption phase). The intercepts related to the early pulmonary absorption are indicated with subscript 1. Those related to absorption via the second absorption route, start to appear in the systemic circulation. With this model, the measured formoterol plasma concentrations could be fitted adequately. From the estimated A, B and k values, α and β, AUC was calculated as follows: AUC = AUC1 + AUC2, where AUC1 = A1/α + B1/β - (A1 + B1)/k_{d1} and AUC2 = A2/α + B2/β - (A2 + B2)/k_{d2}. A1 = (Fr + D/V) * k_{d1} * (k_{21} - α)/α(α - β)/α(k_{a1} - α); A2 = (1 - Fr + D/V) * k_{d2} * (k_{21} - β)/β(β - α)/β(k_{a2} - β); B1 = (Fr * D/V) * k_{a1} * (k_{21} - α)/α(α - β)/α(k_{a1} - α); B2 = (1 - Fr) * D/V) * k_{a2} * (k_{21} - β)/β(β - α)/β(k_{a2} - β). Clearance/F was calculated as: clearance/F = D/AUC, where D is the parameter for total bioavailability (i.e., the part of the total dose that reaches the systemic circulation, which obviously remains unknown). Fr is the fraction of the administered dose of formoterol that entered the systemic circulation via the first, more rapid route of absorption, and 1 - Fr is the fraction of the dose that appeared in the systemic circulation via the second absorption route.

Because it was apparent from the raw data that concentrations during the early absorption phase did not have quantitatively the same effects as similar concentrations during the late absorption phase, for the descriptions of the pharmacodynamics an approach also had to be chosen that was compatible with a situation in which two doses given at t = 0 are absorbed via different routes. To account for the observed differences in activity, the models should allow for the possibility of handling the data as if two different drugs were given. To relate the calculated formoterol plasma concentrations to the observed responses, a combined PK/PD model was applied as described by Holford and Sheiner (1982, 1981). This model includes a hypothetical effect compartment to account for the time delay between peak concentration and peak effect. To describe the delay between the effects and the two formoterol plasma concentration peaks resulting from the pulmonary route and the orally absorbed dose, different rate constants for the elimination of formoterol from the hypothetical effect compartment, k_{o1} and k_{o2}, had to be used. The following equation describes the time course of the concentrations of formoterol (C_r) in the hypothetical effect compartment:

\[
C_r(t) = C_{r1}(t) + C_{r2}(t)
\]

where

\[
C_{r1}(t) = \frac{Fr \cdot D \cdot F \cdot k_{a1} \cdot k_{o1}}{V_c} \left[ \frac{(k_{21} - \alpha) \cdot e^{-\alpha \cdot t}}{(\beta - \alpha) (k_{21} - \alpha)} + \frac{(k_{21} - \beta) \cdot e^{-\beta \cdot t}}{(\alpha - \beta) (k_{21} - \beta)} \right] + \frac{(k_{21} - k_{a1}) \cdot e^{-k_{d1} \cdot t}}{(\alpha - k_{a1}) (k_{a1} - k_{o1})}
\]

\[
C_{r2}(t) = \left(1 - Fr\right) \cdot D \cdot F \cdot k_{a2} \cdot k_{o2} \frac{V_c}{V_c} \left[ \frac{(k_{21} - \alpha) \cdot e^{-\alpha \cdot t \cdot t_{lag}}}{(\beta - \alpha) (k_{21} - \alpha)} + \frac{(k_{21} - \beta) \cdot e^{-\beta \cdot t \cdot t_{lag}}}{(\alpha - \beta) (k_{21} - \beta)} \right] + \frac{(k_{21} - k_{a2}) \cdot e^{-k_{d2} \cdot t \cdot t_{lag}}}{(\alpha - k_{a2}) (k_{a2} - k_{o2})}
\]

In the above equation, C_r1 is the effect compartment concentration resulting from the dose absorbed via the pulmonary route, and C_r2 the effect compartment concentration resulting from the orally absorbed dose. All other parameters were already defined above, except V_c, which is the volume of the central compartment, and k_{d1}, which is the rate constant for transfer of the drug from the peripheral to the central compartment.

The observed effects were related to the hypothetical effect compartment concentrations with the use of a sigmoid E_{max} model derived from equations given by Ariens and Simonis (1964). To allow for the possibility of handling the data as if two different drugs were given, a model for competitive agonism was chosen:

\[
E = E_0 - \left(\frac{E_0 - E_{\text{max}}}{EC_{50} + \left(\frac{C_r}{EC_{50} + \frac{C_r}{EC_{50}}}ight)} + C_{S1}^n\right)
\]

\[
+ \left(\frac{E_0 - E_{\text{max}}}{EC_{50} + \left(\frac{C_r}{EC_{50} + \frac{C_r}{EC_{50}}}ight)} + C_{S2}^n\right)
\]
For both the wheal and flare reactions and the effects on eosinophil counts, the maximum obtainable effect (Emax) was fixed at 0 (cm² and mmol/liter, respectively). The Emax for the plasma potassium was entered as a parameter to be estimated. On the placebo day, the size of the wheal-and-flare reactions increased during the day. A linear relationship with a positive slope was observed between these increases and time. As described before, this rising baseline effect was therefore incorporated in the model for the wheal-and-flare reactions by multiplying a slope parameter, using the results of the placebo day as initial value (van Boxtel and Jonkers, 1992):

\[
E = E_0 + (\text{slope} \cdot t) - \frac{(E_0 + (\text{slope} \cdot t) - E_{\text{max}}) \cdot C_{\text{a}}} {EC_{50} + \left(\frac{EC_{50} \cdot C_{\text{a}}}{EC_{50}}\right)} + C_{\text{a}}
\]

\[
E_{\text{max}} + \frac{(E_0 + (\text{slope} \cdot t) - E_{\text{max}}) \cdot C_{\text{a}}} {EC_{50} + \left(\frac{EC_{50} \cdot C_{\text{a}}}{EC_{50}}\right)} + C_{\text{a}}
\]

Emax values were assumed to be the same for the two fractions of the dose of formoterol absorbed via different routes. However, to account for the observed differences between the effects caused by these two fractions, different EC50 parameters for the two fractions were incorporated in the model.

Statistical analysis

All pairwise comparisons between the first systemically absorbed fraction of formoterol and the second systemically absorbed fraction of formoterol were made with the Wilcoxon signed rank test (two-tailed for matched pairs).

Results

The high single inhaled dose of 120 μg formoterol was reasonably well tolerated. Practically all of the subjects had, when questioned, some complaints of palpitations, tremor and feelings of agitation, but these side effects were never graded as serious. They started within 10 min after dosing and gradually disappeared during the next 3 to 5 hr. On the 2 (formoterol, placebo) experimental days, the mean ± S.D. base-line levels of plasma potassium were 3.96 ± 1.41 and 3.86 ± 1.37 mmol/liter, respectively. Base-line levels of blood eosinophil counts on the 2 days were 229 ± 146 and 221 ± 138 × 10⁶/liter. Base-line values for the size of the wheals on the 2 days were 1.29 ± 0.24 and 1.27 ± 0.70 cm². There were no statistically significant differences for base-line levels of blood eosinophil counts, plasma potassium or size of wheal (P = .58, .08 and 1.00, respectively).

Pharmacokinetics. The individual pharmacokinetic parameters are presented in table 2. Formoterol plasma concentrations showed a biphasic time course in all subjects. The two mean ± S.D. values for the peak serum-concentrations (Cmax), as calculated by the fitting procedure, were 51.8 ± 11.6 pg/ml for the first peak and 40.5 ± 7.8 pg/ml for the second peak at Tmax values (mean ± S.D.) of 0.25 ± 0.11 and 1.58 ± 0.71 hr, respectively, after inhalation. Because the first peak concentration was without exception observed within the tlag, which was calculated for the second absorption phase, the first observed peak concentration consisted exclusively of the first absorbed fraction of the dose, which was assumed to take place via the pulmonary route. The second peak, however, was a summation of drug concentrations belonging to the first absorbed fraction as well as the second orally absorbed fraction of the dose. The two absorption rate constants (ka) were significantly different from each other (P = .016). The calculated mean ± S.D. formoterol peak concentration of the second fraction was 15.7 ± 6.3 pg/ml, and this peak occurred at a calculated time (mean ± S.D.) of 2.00 ± 0.74 hr after dosing. The time courses of the measured and estimated formoterol serum concentration of a representative subject are shown in figure 1.

Pharmacodynamics. The majority of the effect data showed a biphasic time course. When the effects were plotted against the corresponding concentrations, anticalciclyc/hysteresis was observed. Describing the effects with a PK/PD model was only possible if different parameters for the two absorbed fractions of the dose were used. Furthermore, the model had to be adapted to various aspects of the three observed effects.

To correct for a diurnal variation of the dermal response to allergens, a base-line effect had to be incorporated in the model for the wheal-and-flare reactions. However, our efforts

<table>
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<th>4</th>
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<td>474*</td>
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* These values are corrected for the lower dose of 96 μg that subject 1 received by multiplying the data with 1.25.

** P < .05.
to relate drug concentrations to the flare response still did not give reliable results, and thus no drug effect could be described for the flare reactions. The variation of flare responses found was large. The four pairs of skin tests done in the first hour of the placebo days, a time frame in which diurnal variation of the reactions can be ignored, showed a clear location dependency for the flare but not for the wheal reactions. The slope of the base-line on the formoterol day was estimated by PCNONLIN. In one subject, the slope of the base-line effect was estimated to be negative, which was also observed in this single subject on the placebo day. The slopes did not differ significantly between the placebo and formoterol days (P = .578). The time courses of the measured and estimated wheal size of a representative subject are shown in figure 2.

The maximum attainable effect, E_{max}, for formoterol-induced eosinopenia and for the inhibition of the wheal response were not estimated as a parameter but could be fixed at 0 (i.e., a complete disappearance of the number of eosinophils and inhibition of the wheal response, respectively). The mean ± S.D. of the largest deviations from the base line that were observed in this study for hypokalemia, eosinopenia and the inhibition of the wheal response were 34% ± 17, 50% ± 11 and 38% ± 12, respectively. The individual pharmacodynamic data are presented in table 3. Mean maximum attainable hypokalemic effect was calculated to be 1.5 mmol/liter. The lowest potassium value that was observed in the study was 3.0 mmol/liter. Mean EC_{50} values (± S.D.) for the eosinopenic effect of formoterol absorbed via the lung, the first absorption phase, and of formoterol absorbed via the digestive tract, during the second absorption phase, were 39.3 ± 7.0 and 12.5 ± 5.4 pg/ml, respectively. For the inhibition of the wheal reaction, these values were, respectively, 47.7 ± 4.1 and 17.5 ± 5.8, and for the hypokalemic effect, these values were 66.1 ± 24.2 and 19.8 ± 5.7. Thus, EC_{50} values for each of the two absorbed fractions of the dose differed by a factor of 3. For all three effects, the differences between the two EC_{50} values reached significance (P = .016 for all three effects). These differences are illustrated in the calculated concentration-response relations in figure 3. The k_{so} values estimated for the three different effects of the two systemically absorbed fractions of formoterol appeared to be statistically significant different for the formoterol-induced eosinopenia and hypokalemia (P = .016 for both effects) but not for the inhibiting effect on the size of the wheal (P = .297).

**Discussion**

After inhalation, formoterol serum concentrations showed a biphasic course. As soon as 10 to 15 min after formoterol inhalation, a peak serum concentration was observed. The first blood sample after inhalation was obtained not before 15
min after dosing. Because the highest concentration of formoterol was measured in this first sample for all except one subject, the calculated early serum peak was estimated on the basis of only one data point. This is also reflected by the large confidence interval of the estimation of the first absorption rate constant. A mean concentration of first serum peak was 51.8 pg/ml and occurred at 0.25 hr. The mean serum concentration of the second peak was 40.5 pg/ml and occurred at 0.25 hr. After oral dosing of capsules, peak concentrations do exist, from a purely theoretical point of view the above assumption is not altogether correct. Because formoterol is a racemate of two enantiomers, the measured concentrations should then be regarded as the summation of two absorbed fractions of formoterol with probably different enantiomer ratios; thus, there are actually two different drugs with their own kinetic characteristics (Ariens, 1984). It has been shown that during and/or after absorption, there is a change in enantiomer ratios of formoterol (Butter et al., 1996). Studies of enantioselective metabolism of other adrenergic drugs also support the assumption that relatively large changes in enantiomer ratios can be expected during oral and pulmonary absorption (Boullon and Fawcett, 1996; Eaton et al., 1996). In the present study, kinetic parameters could only be approximated for the total sum of the two fractions because an enantiomer specific assay for formoterol in plasma does not exist. However, it is very important in this respect to make a distinction between the difference observed for the two absorption routes and the kinetic differences between the two enantiomers. By describing the concentration-time data for the two absorption routes without having the actual information about enantiomer ratios, estimates are provided for hybrid rate constants for both the oral and pulmonary routes. With these hybrid rate constants, we could adequately describe the biphasic concentration-time data and therefore use these constants for the equations for

In the kinetic model, it is assumed that the dose of formoterol consisted of two different fractions with different routes of absorption; therefore, the model allowed for two different absorption rate constants. The rate constants \( \alpha \) and \( \beta \) had to be kept the same because we did not have sufficient information to do otherwise. Although pulmonary and oral absorptions do exist, from a purely theoretical point of view the above assumption is not altogether correct. Because formoterol is a racemate of two enantiomers, the measured concentrations should then be regarded as the summation of two absorbed fractions of formoterol with probably different enantiomer ratios; thus, there are actually two different drugs with their own kinetic characteristics (Ariens, 1984). It has been shown that during and/or after absorption, there is a change in enantiomer ratios of formoterol (Butter et al., 1996). Studies of enantioselective metabolism of other adrenergic drugs also support the assumption that relatively large changes in enantiomer ratios can be expected during oral and pulmonary absorption (Boullon and Fawcett, 1996; Eaton et al., 1996). In the present study, kinetic parameters could only be approximated for the total sum of the two fractions because an enantiomer specific assay for formoterol in plasma does not exist. However, it is very important in this respect to make a distinction between the difference observed for the two absorption routes and the kinetic differences between the two enantiomers. By describing the concentration-time data for the two absorption routes without having the actual information about enantiomer ratios, estimates are provided for hybrid rate constants for both the oral and pulmonary routes. With these hybrid rate constants, we could adequately describe the biphasic concentration-time data and therefore use these constants for the equations for

### TABLE 3

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Subject</th>
<th>Mean</th>
<th>S.D.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Eosinophils</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( K_{0\alpha} \cdot t^{1/2} ) (min)</td>
<td>9</td>
<td>13</td>
<td>35</td>
</tr>
<tr>
<td>( K_{0\gamma} \cdot t^{1/2} ) (min)</td>
<td>64</td>
<td>59</td>
<td>92</td>
</tr>
<tr>
<td>( E_{50,1} \cdot a ) (ng/liter)</td>
<td>33</td>
<td>45</td>
<td>40</td>
</tr>
<tr>
<td>( E_{50,2} \cdot a ) (ng/liter)</td>
<td>4</td>
<td>9</td>
<td>16</td>
</tr>
<tr>
<td>( E_{50} \cdot a ) (10^11/liter)</td>
<td>186</td>
<td>440</td>
<td>139</td>
</tr>
<tr>
<td>n</td>
<td>3</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>r</td>
<td>0.96</td>
<td>0.99</td>
<td>0.84</td>
</tr>
<tr>
<td>Potassium</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( K_{0\alpha} \cdot t^{1/2} ) (min)</td>
<td>5</td>
<td>18</td>
<td>14</td>
</tr>
<tr>
<td>( K_{0\beta} \cdot t^{1/2} ) (min)</td>
<td>10</td>
<td>27</td>
<td>17</td>
</tr>
<tr>
<td>( E_{50,1} \cdot a ) (ng/liter)</td>
<td>68</td>
<td>56</td>
<td>61</td>
</tr>
<tr>
<td>( E_{50,2} \cdot a ) (ng/liter)</td>
<td>20</td>
<td>20</td>
<td>16</td>
</tr>
<tr>
<td>( E_{50} \cdot a ) (mmol/liter)</td>
<td>0.8</td>
<td>0.8</td>
<td>2.5</td>
</tr>
<tr>
<td>n</td>
<td>2</td>
<td>3</td>
<td>2</td>
</tr>
<tr>
<td>r</td>
<td>0.96</td>
<td>0.99</td>
<td>0.98</td>
</tr>
<tr>
<td>Wheal</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>( K_{0\alpha} \cdot t^{1/2} ) (min)</td>
<td>15</td>
<td>40</td>
<td>23</td>
</tr>
<tr>
<td>( K_{0\beta} \cdot t^{1/2} ) (min)</td>
<td>48</td>
<td>18</td>
<td>176</td>
</tr>
<tr>
<td>( E_{50,1} \cdot a ) (ng/liter)</td>
<td>49</td>
<td>43</td>
<td>47</td>
</tr>
<tr>
<td>( E_{50,2} \cdot a ) (ng/liter)</td>
<td>15</td>
<td>18</td>
<td>13</td>
</tr>
<tr>
<td>Slope (cm²/liter)</td>
<td>0.40</td>
<td>0.14</td>
<td>0.57</td>
</tr>
<tr>
<td>( E_{50} ) (cm²)</td>
<td>1.3</td>
<td>1.8</td>
<td>1.2</td>
</tr>
<tr>
<td>n</td>
<td>4</td>
<td>4</td>
<td>3</td>
</tr>
<tr>
<td>r</td>
<td>0.81</td>
<td>0.86</td>
<td>0.94</td>
</tr>
</tbody>
</table>

*P < .05.*

In the kinetic model, it is assumed that the dose of formoterol consisted of two different fractions with different routes of absorption; therefore, the model allowed for two different absorption rate constants. The rate constants \( \alpha \) and \( \beta \) had to be kept the same because we did not have sufficient information to do otherwise. Although pulmonary and oral absorptions do exist, from a purely theoretical point of view the above assumption is not altogether correct. Because formoterol is a racemate of two enantiomers, the measured concentrations should then be regarded as the summation of two absorbed fractions of formoterol with probably different enantiomer ratios; thus, there are actually two different drugs with their own kinetic characteristics (Ariens, 1984). It has been shown that during and/or after absorption, there is a change in enantiomer ratios of formoterol (Butter et al., 1996). Studies of enantioselective metabolism of other adrenergic drugs also support the assumption that relatively large changes in enantiomer ratios can be expected during oral and pulmonary absorption (Boullon and Fawcett, 1996; Eaton et al., 1996). In the present study, kinetic parameters could only be approximated for the total sum of the two fractions because an enantiomer specific assay for formoterol in plasma does not exist. However, it is very important in this respect to make a distinction between the difference observed for the two absorption routes and the kinetic differences between the two enantiomers. By describing the concentration-time data for the two absorption routes without having the actual information about enantiomer ratios, estimates are provided for hybrid rate constants for both the oral and pulmonary routes. With these hybrid rate constants, we could adequately describe the biphasic concentration-time data and therefore use these constants for the equations for
the two-effect compartments for the different routes of absorption.

Most of the observed systemic effects showed a similar biphasic pattern as was seen in the formoterol concentration-time curves. However, concentrations during the early pulmonary absorption phase did not seem to have the same effect as similar concentrations during the late absorption phase. To account for these observed differences in activity, pharmacodynamic models were chosen that could handle the data as if two different drugs were given at $t = 0$. Because of the presence of anticlockwise hysteresis, for a proper description of the effect-time curves, models were used with an hypothetical effect compartment as described by Holford and Sheiner (1982, 1981). Good fits were only obtained when the hypothetic effect compartment as described by Holford and Sheiner of choice (van Boxtel and Jonkers, 1992). If one does so, its mechanistic meaning will be explicitly denied, and there is no doubt that intersubject variability for this parameter and thus for the estimates of $EC_{50}$ will be found.

Diurnal variations for histamine-dependent phenomena have been described before (Reinberg et al., 1978). To correct for such diurnal variation of the dermal response to allergens, a base-line effect was incorporated in the model for the wheal reactions. The performed wheal measurements are not the most precise effect measurements possible; 6 of 72 of these measurements had to be considered as outliers. Flare responses could still not be modeled due to large variation and an apparent location dependency. It is also possible that formoterol reduces permeability but not vasodilatation. These different behaviors of the wheal and flare for reproducibility and location dependency for the size of the reaction have been described before (Bowman, 1935; Clarke et al., 1982; Swain and Becker, 1952). The eosinophils on the placebo day showed little diurnal variation, which has been observed before in nonasthmatic individuals (Dahl et al., 1978). In our experience, plasma potassium does not show variation over the day of any importance (Braat et al., 1992; Koopmans et al., 1995). Therefore, in the models for eosinopenia and hypokalemia, we did not use corrections for base-line effects.

Inhaled formoterol induced a ~40% reduction in wheal response after intradermal injections of an allergen. It seems safe to assume that the intradermal formoterol concentrations were much lower than the concentrations in the airways after inhalation and therefore much stronger effects on mast cell activation could be expected in the lung. An explanation for the previously described relatively high doses of intradermally injected formoterol needed to inhibit acute cutaneous reactions provoked with anti-IgE could be a rapid distribution of formoterol from the injection site causing low formoterol levels at the moment that the skin tests were done (Gronneberg and Zetterstrom, 1990). It has been demonstrated that at least part of the inhibition of the cutaneous response could be explained by the effect of a beta agonist on cutaneous vasculature instead of on dermal mast cells (Lamkin et al., 1976). Still, this does not change the potential meaningfulness of this particular action of formoterol, which could be a beneficial contribution to the treatment of asthma.

As described before (Koopmans et al., 1995; van den Berg et al., 1994a), formoterol had a considerable eosinopenic effect. The mechanism of this effect is poorly understood, but most likely redistribution plays an important role. It seems likely that the lowering of the peripheral eosinophils can be considered an anti-inflammatory effect in asthma, but this is not certain because a redistribution of eosinophils toward the lung compartment cannot be excluded.

In terms of hypokalemia, inhalation of single doses of 120 µg of formoterol seems safe in young healthy men. Plasma potassium did not fall below 3.0 mmol/liter because of the fact that the higher concentrations caused by pulmonary absorption were less active than the lower concentrations of orally absorbed formoterol, which appeared slower in the blood.

For our dynamic models, we used sigmoid $E_{\text{max}}$ models in combination with an effect compartment model approach. The sigmoid factor $n$ was estimated between predefined integer values of 0 and 5. To make the determination of the exponent part of the fitting procedure is in essence a matter of choice (van Boxtel and Jonkers, 1992). If one does so, its mechanistic meaning will be explicitly denied, and there is no doubt that intersubject variability for this parameter and thus for the estimates of $EC_{50}$ will be found.

With PK/PD modeling of the observed systemic effects of the pulmonary and orally formoterol, apparent mean $EC_{50}$ values were found of respectively 39.3 and 12.5 µg/ml for the drug-induced eosinopenia, 47.7 and 17.5 µg/ml for the inhibition of wheal reactions and 66.1 and 19.8 µg/ml for the hypokalemie effect. Thus, in this way, calculated potency of formoterol in the systemic circulation absorbed via the alimentary tract appears to be on average three times higher than that of formoterol absorbed via the pulmonary route. We postulate that the explanation for this substantial difference in potency can be found in changes of enantiomer ratios, depending on the route via which formoterol enters the body.
The consistency of the 3-fold difference between the two EC$_{50}$ values for each of the three studied effects is certainly in agreement with this hypothesis. Enantioselective disposition of beta-2 adrenoceptor agonists after oral and inhaled administration is a known phenomenon (Boulton and Fawcett, 1996). Furthermore, a large first-pass metabolism in the lungs of the active (RR) enantiomer of formoterol is a real possibility because an analogous finding was described for salbutamol (Eaton et al., 1996). Finally, in the urine of healthy subjects, the mean ratio of the (RR) and (SS) enantiomers steadily and consistently increased from 0.49 (S.D. = 0.019) in the first urine samples to 0.95 (S.D. = 0.016) in the urine samples collected over the last time period after single inhaled doses of 12, 24, 48 and 96 µg of formoterol fumarate dry powder (Butter et al., 1996). Probably both the different systemic appearance of the two enantiomers and different elimination half-lives are responsible for the observed changes in the ratio. However, the low (RR/SS) ratio in the first urine samples strongly indicates that via pulmonary absorption, preferentially the inactive enantiomer (SS) reaches the systemic circulation. It should be emphatically stated that because of this continuously changing ratio of the enantiomers, in vivo comparisons of EC$_{50}$ values between studies were not possible. If only kinetic information is available about the racemate, then the EC$_{50}$ value determined with PK/PD modeling should be considered a hybrid parameter, which can be influenced substantially by competitive interactions between enantiomers, especially if these enantiomers have different affinities for the receptor (van Bokxel and Jonkers, 1992). Within-study comparisons of EC$_{50}$ values for different effects are, of course, allowed. It is remarkable, though, that the ratio of the EC$_{50}$ value of the hypokalemic effect to the EC$_{50}$ value of the eosinopenic effect is in the same order of magnitude as the ratio of 1.4 that was found in EC$_{50}$ values stays practically the same for the first and second fractions of formoterol, which is to be expected when a change of enantiomer ratio is the cause of the different observed dynamic properties of the two fractions.

In conclusion, inhalation of a high dose of formoterol by healthy young men did not cause any serious side effects. In particular, potassium appears not to be lowered to a potentially dangerous degree. Formoterol is capable to sort an effect on parameters such as peripheral eosinophil counts and end results of mast cell activation, which are thought to be of considerable importance in the inflammatory processes in asthma. When formoterol is administered by inhalation, a biphasic plasma concentration-time curve is observed, which is most likely due to different absorption sites. The first concentration peak must then be a result of formoterol absorption via the lung and the second peak of oral absorption. Formoterol absorbed via the lungs in the systemic circulation is a 3-fold less potent drug than orally absorbed formoterol regarding peripheral effects. It can be argued that these pharmacodynamic differences are caused by different kinetics of the two enantiomers of formoterol.

References


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