Concentration-effect relations of anti-asthma medications. Studies on inflammation markers
Derks, M.G.M.

Citation for published version (APA):

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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 hypokalemia were assessed by pharmacokinetic-pharmacodynamic (PK/PD) modelling. Following inhalation of either 120 µg of formoterol or placebo, blood samples were taken and skin tests were performed in 7 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 $E_{\text{max}}$ (the maximum obtainable effect) formula for competitive agonism, with an effect compartment, had to be employed. Fitting the wheal and flare, an apparent diurnal variation had to be taken into account by incorporating in the model rising baseline 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 $EC_{50}$ (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.

INTRODUCTION

Formoterol fumarate is a selective $\beta_2$-adrenoceptor agonist that has a rapid onset and a prolonged duration of bronchodilatory action. The latter is especially notable when formoterol is administered by inhalation. Formoterol is marketed as a racemate, consisting of the $RR$ and $SS$ enantiomers. Apparently the $RR$ enantiomer is a thousand times more potent than the $SS$ enantiomer. Lung deposition after inhalation is about 10 to 15% of the total dose. The major part of
inhaled drugs is either swallowed or exhaled. 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 following inhalation has not been described before due to low inhalation dosages, which lead to plasma concentrations in the low picogram per millimetre range.

The place of β2-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. Other actions besides this bronchodilation have been claimed, some of which may modulate the airway inflammation considered to have a causal relation to asthma. 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 response, which is characterised by local swelling of the skin surrounded by a circumscrip erythema. Early studies demonstrated that adrenaline can suppress the wheal and flare reaction in man. The adrenaline-mediated suppression could be adequately explained by elevation of intracellular cyclic adenosine monophosphate 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 β2-adrenoceptor agonists.

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. Pharmacokinetic-pharmacodynamic modelling 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 following administration of formoterol was also studied. The fall of plasma potassium due to a β2-adrenoceptor agonist can be regarded as a sensitive parameter for its β2-adrenoceptor-mediated action and can also be used as a safety parameter. Furthermore, concentration dependent hypokalemia seems to be a surrogate marker for bronchodilation.
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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 all tested for a positive skin reaction to intradermally injected grass, house dust mite or cat allergen of 30 Biological Units (B.U.) per millilitre. Any reaction to allergen that could be visualised 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 dose was titrated to find the logdose that gave a cross-section of the flare of approximately 5 cm. This dose of allergen was finally used on both experimental days.

<table>
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<th>Mean</th>
<th>SD</th>
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<tr>
<td>allergen</td>
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</tr>
<tr>
<td>conc. (B.U.)</td>
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<td>4.1</td>
<td>3.9</td>
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</tbody>
</table>

Experimental design and interventions
The study had a randomised double-blind, placebo controlled crossover design. On two experimental days separated by at least one 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 in order to minimise physical strain. An intravenous
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catheter was inserted in a forearm vein and remained in place for minimally seven hours, enabling frequent blood collection. The system was kept patent with heparinized saline (1%) solution. Before drug administration, one skin test was performed in order to obtain baseline values and blood samples were taken for baseline potassium and eosinophils measurements, and to exclude the presence of substances that could interfere with the formoterol assay.

At time \((t) = 0\), approximately thirty minutes 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 ten puffs of 12 \(\mu\)g of formoterol or ten puffs of placebo. It took the subjects three minutes to inhale the dose. One subject received only 80% of the intended dose, i.e. 96 \(\mu\)g of formoterol. During eight hours after dosing, ten blood samples for analysis of formoterol, for eosinophil counts and for potassium levels were taken, and nine pairs of skin tests were done at 15, 30, 45, 60, 90, 120, 180, 240, 330 and 450 minutes after drug administration; at 330 minutes after dosing only blood was collected. Blood samples were centrifuged for 10 minutes at 4000 rotations per minute, immediately after clotting. Plasma samples for formoterol concentration analysis were stored at -20 °C. The plasma samples for potassium measurements were analysed 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 \(\mu\)l allergen extract in the forearm.

Drugs

Formoterol fumarate dihydrate (FORADIL® Ciba-Geigy, Basel) was used. For administration a commercially available aerosol inhalator was used. The appearance of the inhalator used for the placebo was the same as the one used for the formoterol.
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Formoterol assay
Plasma levels of formoterol were analysed using a high pressure liquid chromatography method with electrochemical detection as described previously 15. Bromo-formoterol was used as an internal standard. Reversed phase extraction was carried out using 1 ml PRS-columns. A C8 analytical column was employed in the chromatographic system. The level of quantitation of the assay was 20 pg formoterol per ml plasma. However, due to variation of the sensitivity of the electrochemical detector the limit of detection, with a signal to noise ratio of 3 to 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 Inc.) 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. Fifteen minutes following 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 on 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 minimally one week, the same procedure was repeated. On this second day the skin tests were done in identical order as on the first day.
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Data analysis
All pharmacokinetic and pharmacodynamic data were fitted to the appropriate equations using the nonlinear regression computer program PCNONLIN \(^7\). To identify outliers we looked at graphical presentation of the data and we considered the standardised 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 tri-exponential equation for a two-compartment model with first order absorption as described by Gibaldi and Perrier \(^18\), was adjusted in such a way that two different absorption rate constants were incorporated:

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

\[
C_{p1}(t) = A_1 \cdot e^{-\alpha t} + B_1 \cdot e^{-\beta t} - (A_1 + B_1) \cdot e^{-K_{a1} t}
\]

\[
C_{p2}(t) = A_2 \cdot e^{-\alpha (t-\text{tlag})} + B_2 \cdot e^{-\beta (t-\text{tlag})} - (A_2 + B_2) \cdot e^{-K_{a2} (t-\text{tlag})}
\]

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 other route with subscript 2. The PK model contains eight parameters. The parameters for the distribution rate constant (\(\alpha\)) and the elimination rate constant (\(\beta\)) are assumed to be the same for the two routes of absorption. In case of the second absorption phase, a lagtime (\(\text{tlag}\)) was incorporated; \(t\) is time after drug administration and \(\text{tlag}\) is the time between drug administration and the time that drug - via the second absorption route - starts to appear in the systemic circulation. With this model the measured formoterol plasma concentrations could be fitted adequately. From the estimated intercepts of distribution (\(A\)) and elimination (\(B\)) and from the rate constants of absorption (\(K_a\)), \(\alpha\) and \(\beta\), the area under the curve (AUC) was calculated as follows: \(\text{AUC} = \text{AUC}_1 + \text{AUC}_2\), where
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\[ AUC_1 = \frac{A_1}{\alpha + B_1/\beta} - \frac{(A_1 + B_1)}{K_{a1}} \] and \[ AUC_2 = \frac{A_2}{\alpha + B_2/\beta} - \frac{(A_2 + B_2)}{K_{a2}}; \]

\[ A_1 = \frac{(Fr*DA^1)*K_{a1}}{(K_{a1}-\alpha)/(\beta-\alpha)/(Ka_{a1}-\alpha)}; \]

\[ A_2 = \frac{((1-Fr)*DA^1)*K_{a2}}{(K_{a2}-\alpha)/(\beta-\alpha)/(Ka_{a2}-\alpha)}; \]

\[ B_1 = \frac{(Fr*DA^1)*K_{a1}}{(K_{a1}-\beta)/(\alpha-\beta)/(Ka_{a1}-\beta)}; \]

\[ B_2 = \frac{((1-Fr)*DA^1)*K_{a2}}{(K_{a2}-\beta)/(\alpha-\beta)/(Ka_{a2}-\beta)}; \]

Clearance/F (CL/F) was calculated as: \[ CL/F = D/AUC, \] where F 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.

As 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 even should allow for the possibility to handle 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. 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, \( K_{e01} \) and \( K_{e02} \), had to be used. The following equation describes the time course of the concentrations of formoterol (\( C_e \)) in the hypothetical effect compartment:

\[ C_e(t) = C_{e1}(t) + C_{e2}(t) \]

where
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\[ C_{e1}(t) = \frac{Fr \cdot Dose \cdot F \cdot K_{a1} \cdot K_{e01}}{V_c} \cdot \left[ \frac{(K_{21} - \alpha) \cdot e^{-\alpha \cdot t}}{(\beta - \alpha) \cdot (K_{a1} - \alpha) \cdot (K_{e01} - \alpha)} + \frac{(K_{21} - \beta) \cdot e^{-\beta \cdot t}}{(\alpha - \beta) \cdot (K_{a1} - \beta) \cdot (K_{e01} - \beta)} \right] \]

\[ + \frac{(K_{21} - K_{a1}) \cdot e^{-K_{a1} \cdot t}}{(\alpha - K_{a1}) \cdot (\beta - K_{a1}) \cdot (K_{e01} - K_{a1})} + \frac{(K_{21} - K_{e01}) \cdot e^{-K_{e01} \cdot t}}{(\alpha - K_{e01}) \cdot (\beta - K_{e01}) \cdot (K_{a1} - K_{e01})} \]

and

\[ C_{e2}(t) = \frac{(1 - Fr) \cdot Dose \cdot F \cdot K_{a2} \cdot K_{e02}}{V_c} \cdot \left[ \frac{(K_{21} - \alpha) \cdot e^{-\alpha \cdot (t - \text{lag})}}{(\beta - \alpha) \cdot (K_{a2} - \alpha) \cdot (K_{e02} - \alpha)} + \frac{(K_{21} - \beta) \cdot e^{-\beta \cdot (t - \text{lag})}}{(\alpha - \beta) \cdot (K_{a2} - \beta) \cdot (K_{e02} - \beta)} \right] \]

\[ + \frac{(K_{21} - K_{a2}) \cdot e^{-K_{a2} \cdot (t - \text{lag})}}{(\alpha - K_{a2}) \cdot (\beta - K_{a2}) \cdot (K_{e02} - K_{a2})} + \frac{(K_{21} - K_{e02}) \cdot e^{-K_{e02} \cdot (t - \text{lag})}}{(\alpha - K_{e02}) \cdot (\beta - K_{e02}) \cdot (K_{a1} - K_{e02})} \]

In the above equation \( C_{e1} \) is the effect compartment concentration resulting from the dose absorbed via the pulmonary route and \( C_{e2} \) the effect compartment concentration resulting from the orally absorbed dose. All other parameters were already defined above, apart from the following: \( V_c \) is the volume of the central compartment; \( K_{21} \) is the rate constant for transfer of the drug from the peripheral to the central compartment;
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The observed effects were related to the hypothetical effect compartment concentrations with the use of a sigmoid $E_{\text{max}}$ model derived from equations given by Ariens and Simonis \(^\text{21}\). To allow for the possibility to handle the data as if two different drugs were given a model for competitive agonism was chosen:

$$E = E_0 \left( \frac{(E_0 - E_{\text{max}}) \cdot C^n_{e_1}}{EC_{50_1} + \left( \frac{EC_{50_1} \cdot C^n_{e_2}}{EC_{50_2}} \right) + C^n_{e_1}} + \frac{(E_0 - E_{\text{max}}) \cdot C^n_{e_2}}{EC_{50_2} + \left( \frac{EC_{50_2} \cdot C^n_{e_1}}{EC_{50_1}} \right) + C^n_{e_2}} \right)$$

For both the wheal and flare reactions and the effects on eosinophil counts, the maximum obtainable effect ($E_{\text{max}}$) was fixed at 0 (cm\(^2\) and mmol/l, respectively). The $E_{\text{max}}$ 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 time with a slope (si) parameter, using the results of the placebo day as initial value \(^\text{22}\):

$$E = E_0 + (sli) \left( \frac{(E_0 + (sli) - E_{\text{max}}) \cdot C^n_{e_1}}{EC_{50_1} + \left( \frac{EC_{50_1} \cdot C^n_{e_2}}{EC_{50_2}} \right) + C^n_{e_1}} + \frac{(E_0 + (sli) - E_{\text{max}}) \cdot C^n_{e_2}}{EC_{50_2} + \left( \frac{EC_{50_2} \cdot C^n_{e_1}}{EC_{50_1}} \right) + C^n_{e_2}} \right)$$

$E_{\text{max}}$ 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 $EC_{50}$ parameters for the two fractions were incorporated in the model.

Statistics

All pair-wise comparisons between the first systemically absorbed fraction of

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formoterol and the second systemically absorbed fraction of formoterol were made with the Wilcoxon signed-ranks 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. However, these side effects were never graded as serious. They started within 10 min after dosing and gradually disappeared during the next 3-5 hours. On the two (formoterol, placebo) experimental days the mean (± SD) baseline levels of plasma potassium were respectively 3.96 ± 1.41 mmol/l and 3.86 ± 1.37 mmol/l. Baseline levels of blood eosinophil counts on the two days were respectively 229 ± 146 x 10⁶/l and 221 ± 138 x 10⁶/l. Baseline values for the size of the wheals on the two days were respectively 1.29 ± 0.24 and 1.27 ± 0.70 cm². There were no statistically significant differences for baseline levels of blood eosinophil counts, plasma potassium, nor size of wheal (p values respectively 0.58, 0.08, 1.00).

Pharmacokinetics

The individual pharmacokinetic parameters are presented in Table 2. Formoterol plasma concentrations showed a biphasic time-course in all subjects. The two means (± SD) for the peak serum-concentrations (C_{max}), as calculated by the fitting procedure, were 51.8 pg.ml⁻¹ ± 11.6 for the first peak and 40.5 pg.ml⁻¹ ± 7.8 for the second peak at T_{max} values (mean ± SD) of respectively 0.25 hours ± 0.11 and 1.58 ± 0.71 hours after inhalation. As the first peak concentration was without exception observed within the lagtime 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 (k_{a}) were significantly different from each other (p = 0.016). The calculated mean (± SD) formoterol peak concentration of the second fraction was 15.7 pg.ml⁻¹ ± 6.3 and this peak occurred at a calculated time (mean ± SD) of 2.00 hours ± 0.74 after
dosing. The time courses of the measured and estimated formoterol serum concentration of a representative subject are shown in Fig. 1.

Table 3. Individual dynamic parameters of the eosinopenic, hypokalemic, and wheal size inhibiting effects after inhalation of 120 μg of formoterol.

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<tr>
<th>Parameter</th>
<th>Subject</th>
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*p<0.05
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**Pharmacodynamics**

The majority of the effect data showed a biphasic time-course. When the effects were plotted against the corresponding concentrations anti-clockwise hysteresis was observed. Describing the effects with a pharmacokinetic/pharmacodynamic 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 baseline effect had to be incorporated in the model for the wheal and flare reactions. However, our efforts 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 baseline on the formoterol day was estimated by PCNONLIN. In one subject the slope of the baseline 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-day and the formoterol-day (p = 0.578). The time courses of the measured and estimated wheal size of a representative subject are shown in fig.2.

The maximum attainable effect, \( E_{\text{max}} \), for formoterol induced eosinopenia and for the inhibition of the wheal response had not to be estimated as a parameter but could be fixed at 0, i.e. a complete disappearance of respectively the number of eosinophils and inhibition of the wheal response. The mean (± SD) of the largest deviations from the baseline 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.l\(^{-1}\). The lowest potassium value that was observed in the study was 3.0 mmol.l\(^{-1}\). Mean \( EC_{50} \) values (± SD) for the eosinopenic effect of formoterol absorbed via the lung, the first absorption phase, and of formoterol absorbed via the digestive tract, i.e. during the second absorption phase, were 39.3 pg.ml\(^{-1}\) ± 7.0 and 12.5 pg.ml\(^{-1}\) ± 5.4 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
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$_{50}$'s reached significance (p = 0.016 for all three effects). These differences are illustrated in the calculated concentration-response relations in figure 3. The Ke$_0$'s 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 = 0.016 for both effects), but not for the inhibiting effect on the size of the wheal (p = 0.297).

DISCUSSION

After inhalation, formoterol serum concentrations showed a biphasic course. As soon as ten to fifteen minutes after formoterol inhalation, a peak serum concentration was observed. The first blood sample following inhalation was obtained not before fifteen minutes after dosing. Since for all but one subject the highest concentration of formoterol was measured in this first sample, 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 h. The mean serum concentration of the second peak was 40.5 pg/ml and occurred at 1.58 h. Following oral dosing of capsules, peak concentrations of formoterol are found after approximately one hour$^{23,24}$. In the present study, a lagtime was used for the second absorption phase. This lagtime was considerably larger than the lagtime found after oral dosing in earlier studies$^{23,24}$. The slower passage of small droplets, compared to the swallowed tablets flushed with a glass of water after intake can explain this difference. These findings are very much in agreement with the assumption that the first serum peak was a result of very rapid systemic absorption via the lungs and mucous membranes (pulmonary fraction), and that the second peak was a result of a much slower absorption of formoterol from the aerosol via the gastrointestinal tract (oral fraction). Our results show that seventy percent of all formoterol in the systemic circulation appeared very rapidly and that the remainder, i.e. 30% of
systemic available formoterol, was absorbed more slowly and after a mean lagtime of more than one hour. The pharmacokinetic behaviour of formoterol was described with a two-compartment open model. When a one-compartment model was used, no reliable fits were obtained. Even after oral administration van den Berg et al also needed a two-compartment model to describe the kinetics of formoterol. 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 absorption do exist, from a purely theoretical point of view the above assumption is not altogether correct. As 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. It has been shown that during and/or after absorption there is a change in enantiomer ratios of formoterol. Studies of enantioselective metabolism of other adrenergic drugs also support the assumption that relatively large changes in enantiomer ratios can be expected during oral absorption and during pulmonary absorption. 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, very important in this respect, is to make a distinction between the difference observed for the two absorption routes and 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 provides estimates for hybrid rate constants for both the oral and the pulmonary routes. With these hybrid rate constants, we could adequately describe the biphasic concentration-time data, and therefore could 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
Fig. 1. Reproducible curves describing biphasic pattern of formoterol plasma concentration in time (bold line) and the calculated fractions of pulmonary and orally absorbed formoterol (the first and second thin line, respectively) after inhalation of 120 μg formoterol fumarate. (Subject 6)
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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 at t=0 two different drugs were given.

Because of the presence of anti-clockwise hysteresis, for a proper description of the effect-time curves models were used with an hypothetical effect compartment as described by Holford and Sheiner. Good fits were only obtained when the values for $K_{e0}$ and EC$_{50}$ differed between both fractions.

Diurnal variations for histamine dependent phenomena have been described. To correct for such diurnal variation of the dermal response to allergens a baseline effect was incorporated in the model for the wheal reactions. The performed wheal measurements are not the most precise effect measurements possible; six out of 72 of these measurements had to be considered as outliers. Flare responses could still not be modelled due to large variation and an apparent location dependency. It is also possible that formoterol reduces permeability but not vasodilatation. This different behaviour of the wheal and flare for reproducibility and location dependency for the size of the reaction has been described before. The eosinophils on the placebo day showed little diurnal variation, which has been observed before in non asthmatic individuals. In our experience also plasma potassium does not show variation over the day of any importance. So, in the models for eosinopenia and hypokalemia we did not use corrections for baseline effects.

Inhaled formoterol induced an approximately 40% reduction of 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. 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. 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.
Chapter 5

As described before, also in this study 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 as a redistribution of eosinophils towards the lung compartment can thus far not 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.l⁻¹ 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_{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. If one does so its mechanistic meaning will explicitly be 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 modelling of the observed systemic effects of the pulmonary and orally formoterol apparent mean EC_{50} values were found of respectively 39.3 pg.ml⁻¹ and 12.5 pg.ml⁻¹ for the drug induced eosinopenia, 47.7 pg.ml⁻¹ and 17.5 pg.ml⁻¹ for the inhibition of wheal reactions and 66.1 pg.ml⁻¹ and 19.8 pg.ml⁻¹ for the hypokalemic 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 three fold difference between the two EC_{50}'s for each of the three studied effects is certainly in agreement with this hypothesis. Enantioselective disposition of β₂-adrenoceptor agonists following oral and intravenous administration is a known phenomenon. Furthermore, a large first pass metabolism in the lung of the active RR enantiomer of formoterol is a real possibility as an analogous finding was described for salbutamol.
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Fig. 2. Reproducible curve describing the effect on wheal size after inhalation of 120 μg formoterol. The measured wheal values (filled rectangles) and the calculated curve (broad line) are shown next to the calculated formoterol plasma concentration (straight line) and calculated $C_c$ of the inhaled and orally absorbed fractions (thin lines). The dotted line is the regression line of the wheal values during the placebo day. (Subject 6)
Fig. 3. Curves reflecting the concentrations in the effect compartments ($C_e$) of the pulmonary (pul) and the orally (oral) absorbed formoterol related to the calculated effects (as percentage of $E_0-E_{\text{max}}$) on eosinophils (eos), potassium (pot) and wheal (whl) size. (Subject 6)
Table 2. Kinetic parameters of the individual subjects after inhalation of 120 µg of formoterol.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>Mean</th>
<th>SD</th>
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<tr>
<td>Fr</td>
<td>0.80</td>
<td>0.68</td>
<td>0.62</td>
<td>0.77</td>
<td>0.70</td>
<td>0.52</td>
<td>0.66</td>
<td>0.68</td>
<td>0.09</td>
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<tr>
<td>α-t₁/₂ (min)</td>
<td>15.9</td>
<td>18.9</td>
<td>35.0</td>
<td>32.0</td>
<td>29.7</td>
<td>46.7</td>
<td>22.0</td>
<td>28.6</td>
<td>10.6</td>
</tr>
<tr>
<td>β-t₁/₂ (h)</td>
<td>4.5</td>
<td>8.1</td>
<td>3.9</td>
<td>7.5</td>
<td>4.5</td>
<td>7.5</td>
<td>9.9</td>
<td>6.5</td>
<td>2.3</td>
</tr>
<tr>
<td>Ka₁-t₁/₂* (min)</td>
<td>3.2</td>
<td>3.8</td>
<td>2.1</td>
<td>3.0</td>
<td>8.7</td>
<td>2.1</td>
<td>4.3</td>
<td>3.9</td>
<td>2.3</td>
</tr>
<tr>
<td>Ka₂-t₁/₂ (min)</td>
<td>14</td>
<td>16</td>
<td>33</td>
<td>31</td>
<td>30</td>
<td>40</td>
<td>22</td>
<td>27</td>
<td>10</td>
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<tr>
<td>tlag (min)</td>
<td>31</td>
<td>52</td>
<td>56</td>
<td>124</td>
<td>58</td>
<td>75</td>
<td>44</td>
<td>63</td>
<td>30</td>
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<td>Vc/F (l)</td>
<td>1443</td>
<td>1179</td>
<td>868</td>
<td>1647</td>
<td>1700</td>
<td>1130</td>
<td>1131</td>
<td>1300</td>
<td>305</td>
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<tr>
<td>CL/F (l.min⁻¹)</td>
<td>7.1</td>
<td>4.2</td>
<td>6.1</td>
<td>5.1</td>
<td>8.3</td>
<td>5.8</td>
<td>3.5</td>
<td>5.7</td>
<td>1.6</td>
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<td>AUC (ng.l⁻¹.h)</td>
<td>283</td>
<td>474</td>
<td>329</td>
<td>390</td>
<td>241</td>
<td>347</td>
<td>575</td>
<td>377</td>
<td>115</td>
</tr>
<tr>
<td>Cₘₐₓ 1 (ng.l⁻¹)</td>
<td>52²</td>
<td>52²</td>
<td>74²</td>
<td>48²</td>
<td>35²</td>
<td>49²</td>
<td>52²</td>
<td>52²</td>
<td>12²</td>
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<tr>
<td>Tₘₐₓ 1 (h)</td>
<td>0.20</td>
<td>0.23</td>
<td>0.17</td>
<td>0.23</td>
<td>0.48</td>
<td>0.17</td>
<td>0.25</td>
<td>0.25</td>
<td>0.11</td>
</tr>
<tr>
<td>Cₘₐₓ 2 (ng.l⁻¹)</td>
<td>9²</td>
<td>17²</td>
<td>24²</td>
<td>9²</td>
<td>11²</td>
<td>23²</td>
<td>18²</td>
<td>16²</td>
<td>6²</td>
</tr>
<tr>
<td>Tₘₐₓ 2 (h)</td>
<td>1.1</td>
<td>1.5</td>
<td>2.0</td>
<td>3.3</td>
<td>2.1</td>
<td>2.5</td>
<td>1.5</td>
<td>2.0</td>
<td>0.7</td>
</tr>
<tr>
<td>Cₘₐₓ 1 + 2 (ng.l⁻¹)</td>
<td>43²</td>
<td>43²</td>
<td>52²</td>
<td>29²</td>
<td>33²</td>
<td>39²</td>
<td>45²</td>
<td>40²</td>
<td>8²</td>
</tr>
<tr>
<td>Tₘₐₓ 1 + 2 (h)</td>
<td>0.7</td>
<td>1.3</td>
<td>1.5</td>
<td>2.9</td>
<td>1.4</td>
<td>2.1</td>
<td>1.2</td>
<td>1.6</td>
<td>0.7</td>
</tr>
<tr>
<td>r</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
<td>0.99</td>
<td>0.98</td>
<td>1.00</td>
<td>0.97</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*These values are corrected for the lower dose of 96 microgram that subject 1 received by multiplying the data with 1.25.

*p<0.05
Finally, in the urine of healthy subjects the mean ratio of the $RR$ and $SS$ enantiomers steadily and consistently increased from 0.49 (SD ± 0.019) in the first urine samples to 0.95 (SD ± 0.016) in the urine samples collected over the last time period after single inhaled doses of 12, 24, 48 and 96 μg formoterol fumarate dry powder. Probably both different systemic appearances of the two enantiomers as well as different elimination half-lives are responsible for the observed changes in 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 $EC_{50}$'s between studies is not possible. If only kinetic information is available about the racemate, then the $EC_{50}$'s found with PK/PD modelling should be considered as hybrid parameters, which can be influenced substantially by competitive interactions between enantiomers, especially if these enantiomers have different affinities for the receptor. Within study comparisons of $EC_{50}$'s for different effects are of course allowed. It is remarkable though, that the ratio of the $EC_{50}$ of the hypokalemic effect over the $EC_{50}$ of the eosinopenic effect is in the same order of magnitude as the ratio of 1.4 that was found in studies with oral formoterol, the ratio being 1.7 and 1.6 respectively for the first and second fraction of formoterol. Furthermore, this ratio of $EC_{50}$'s stays practically the same for the first and second fraction 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. Particularly 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 three fold less potent drug than orally absorbed
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formoterol regarding peripheral effects. It can very well be argued that these pharmacodynamic differences are caused by different kinetics of the two enantiomers of formoterol.

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