Concentration-effect relations of anti-asthma medications. Studies on inflammation markers
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Differential kinetics of formoterol enantiomers and the
effect on plasma potassium and pulmonary function in
asthma patients following a single inhaled dose

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ABSTRACT

The long-acting beta-2-agonist formoterol is a racemate of active RR- and inactive SS-enantiomers. The main objective of this study was to investigate whether enantiomer-specific systemic bioavailability of inhaled formoterol can explain the time dependency of formoterol EC\textsubscript{50} values, as was previously observed. Six asthma patients inhaled a single dose of 96 µg formoterol fumarate aerosol on two separate days, blocking the intestinal absorption with oral administered charcoal on one day. Urine excretion rates and plasma concentrations of formoterol were measured. Nonlinear PK/PD modelling technique was used. The observed biphasic concentration time course was described with two routes of absorption. Plasma potassium decline but also Lung function parameters (sG\textsubscript{aw} and MEF\textsubscript{50}) could be linked to RR-plasma concentrations, using effect-compartment models for all effects.

The ratio of the relative AUC of RR- and SS-plasma concentrations differed in the early absorbed fraction (p < 0.05), being 0.65 ± 0.17. The ratio of the AUC of RR and SS of the later absorbed fraction was defined by the model to be one. Charcoal reduced the systemic bioavailability of the enantiomers in the second fraction by 38 ± 20% (p < 0.05) but had no influence on either of the enantiomers in the early fraction. EC\textsubscript{50} values of RR for MEF\textsubscript{50}, sG\textsubscript{aw}, potassium were 4.0 ± 2.8, 9.1 ± 6.4 and 42 ± 31 µg/ml respectively.

In conclusion, SS appears relatively more than RR in the systemic circulation early after inhalation. Simulation suggests that the oral fraction of a low dose of inhaled formoterol contribute to the long duration of its bronchodilating action.

INTRODUCTION

Formoterol fumarate (formoterol), a racemate of RR- and SS-enantiomers, is a selective β\textsubscript{2}-agonist with effective bronchodilatory action with a fast onset and prolonged duration \textsuperscript{1}. The biological activity of formoterol resides in the RR-enantiomer \textsuperscript{2}. Knowledge about both enantiomers is important when one wants to find relations of drug concentration with either therapeutic or adverse effects \textsuperscript{3-5}. The prolonged bronchodilatory effect is thought to result in part from its
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lipophilic structure, which causes formoterol to be slowly released from fatty tissue. It is known that only 10-15% of inhaled drug actually reaches the lungs. The remainder is either swallowed or exhaled. Today's philosophy in inhalation technology embraces a maximal lung drug deposition in order to attain an optimal balance in clinical effect and systemic side effects. Dose effect relations for formoterol were described and showed a maximal bronchodilatory effect at 48 µg inhalation. Data regarding formoterol concentration-effect relations with respect to pulmonary function tests after inhalation are not available.

To ascertain a detectable plasma concentration in the present study, an inhaled dose of 96 µg was administered. The formoterol serum concentration-time course was shown to be biphasic in a previous study in healthy subjects. Presumably, this is the result of two absorption routes. From the systemically available formoterol, 68% was estimated to appear in the early phase of this biphasic concentration time course. The onset of the later absorption was seen approximately 75 minutes post-inhalation. Systemic effects following inhalation of formoterol also appeared to be biphasic. This was ascribed, at least in part, to the biphasic plasma concentration-time pattern. The relationships between 'racemic' formoterol plasma concentration and systemic effects for the two absorption routes were described separately and showed to have differential EC₅₀ values. The active RR-enantiomer was assumed to have appeared relatively less in the systemic circulation in the early absorbed fraction. At present, the two enantiomers can be measured separately in urine. The ratio of RR/SS enantiomers concentration in urine after inhalation by healthy subjects has shown a time related change after dosing from initially 0.5 to 1 after 24 hours.

The present study firstly reviews the hypothesis that after inhalation the early phase of the concentration-time curve is caused by formoterol that is systemically absorbed through the lungs. The later phase after inhalation is then caused by formoterol that entered the systemic circulation through the gastrointestinal route. To test this hypothesis, the gastrointestinal absorption is blocked by active coal on one of two sessions, and on both sessions the time courses of the enantiomers in urine and of 'racemic' formoterol in plasma are measured.

Secondly, the study tests the hypothesis that a change of the ratio of the enantiomer plasma concentrations in time can explain that the potency for a systemic effect of the 'racemic' systemic formoterol increases in the later phase,
as was previously observed. In order to investigate this change in enantiomer, the urinary excretion rates of the two enantiomers were calculated and the plasma concentration-time courses of the enantiomers were estimated. Only the plasma concentration-time course of the \( \text{R}-\)enantiomer was then linked to the hypokalemic effect.

Thirdly, the study attempts to investigate the relation between formoterol plasma concentration and lung function, following a single dose inhalation. We postulate that systemically available formoterol sorts a direct effect on lung function\(^{13}\). Inhaled formoterol demonstrated a more prolonged bronchodilator effect compared with oral administration\(^{1}\). Following our postulate it can be presumed that the prolonged bronchodilator effect following inhalation is not induced exclusively by formoterol arriving directly at the effector organ (i.e. bronchial smooth muscle of the lung). The delayed gastrointestinal absorption of formoterol into the systemic circulation may contribute to the prolonged bronchodilator effect.

**MATERIAL AND METHODS**

**Patients**

Six adult outpatients with mild to moderate, stable, allergic asthma, 3 males and 3 females, were included. Their mean age was 33.8 (range: 20-41) years. The last historical pulmonary function tests showed a 15% reversibility and a mild to moderate bronchial obstruction (FEV\(_1\) between 60 and 80% of predicted). The disease state was retested before the patients were included. The values (mean ± SD) for reversibility was 16.2 ± 5.7%, FEV\(_1\) as percentage of predicted was 80.7 ± 6.5% and FEV\(_1:\text{VC}_{\text{max}}\) was 69.5 ± 9.4%. A history of any major disease, disease with fever for the last 2 weeks and use of any other drugs apart from asthma medication or hormonal contraceptives, were exclusion criteria. Patients were not childbearing or lactating. The physical examination did not show abnormalities. ECG and laboratory values were within normal range.

After written informed consent was obtained, ipratropium was supplied as rescue medication on the week before the trial. The patient was asked to record the time and amount of ipratropium that was needed. However, none of the patients needed
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to use any rescue medication. Oral glucocorticosteroids were not used for the last six weeks. All patients inhaled steroids equivalent to 800 µg budesonide daily. Oral beta-agonists and theophylline were not prescribed. Long-acting beta-agonists, if prescribed, were stopped 7 days before the start of the test day, while short-acting beta-agonists were stopped 24 hours before the start of the test day. The study was approved by the Institutional Review Board (IRB) of the Academic Medical Center and the IRB of the Reinier de Graaf Groep. The amended Helsinki II declaration was fully observed.

Study design
Patients were invited for two sessions, lasting for ten hours each. The two test days were at least seven days apart. At the beginning of each session starting at 7:00 am, thirty minutes before the inhalation (7:30), a pulmonary function test was performed. All patients were asked to empty their bladder before formoterol inhalation. Patients had been instructed on a previous occasion how to inhale correctly. In total 8 puffs of formoterol aerosol (96 µg) were administered (at time t = 0) without an aerochamber. Inhalation was completed within ninety seconds, holding the breath at the end of each inspiration for 6 seconds. At set intervals, 10 plasma samples (-0.5, 0.25, 0.5, 1, 1.5, 2, 3, 4, 6, and 9 h) were collected for formoterol and potassium determination and twelve pulmonary function tests (-0.5, 0.5, 1, 2, 2.5, 3.5, 4.5, 6.5, 7.5, 8.5, 9, and 9.5 h) were performed. The patients were asked to drink 1000 ml of water before dosing and 500 ml in each of the first two hours after dosing and after that at free will. We asked the patients to urinate if possible every half hour in the first two hours and thirty minutes after inhalation and thereafter every one and a half hour. During the test days all urine was collected for formoterol detection in separate portions of which the volumes were measured and 20 ml was sampled. On one of the two sessions the patients were in random order pre-treated with active coal to bind the formoterol that was not deposited in the lungs. Fifty gram of active coal was solved in 200 ml of water. Two min before the start and 2 min after the end of the inhalation the subjects drank 20 ml of a solution of coal; at 1, 2, 3 and 4 hour after inhalation the subjects were given another 40 ml, which they had to swirl in the mouth before swallowing. The mouth had not to be rinsed with water before the inhalation. Plasma potassium was used as a systemic effect parameter, which has proven to
be a sensitive marker for β₂-receptor agonist action \(^{16, 17}\). As peripheral effect parameter maximum expiratory flow at 50% of the Flow Volume Curve (MEF\textsubscript{50}) and specific airway conductance (sG\textsubscript{aw}) were measured.

**Test drug**
Formoterol fumarate dihydrate (Foradil, Ciba-Geigy AG, Basel), a racemate of RR- and SS-formoterol was administered via inhalator dosage-aerosol of 12 µg.

**MEASUREMENTS**

**Lung function**
Pulmonary function tests were performed with a Jaeger Masterlab (Erich JAEGER GmbH, Hoechberg, Germany). Static values were established at the start, dynamic values (FEV\textsubscript{1}, sG\textsubscript{aw} and MEF\textsubscript{50}) were serially measured.

**Formoterol in urine**
During the test days all urine produced was collected in separate portions. As soon as possible, the total volume of each portion of urine was measured, and 20 ml was centrifuged at 3000 rounds per minute for 5 minutes. This was stored at -70 °C in plastic tubes. The formoterol urine concentration was analysed according to the method as has been described by Butter et al.\(^{14}\).

**Formoterol in plasma**
Blood (15 ml) was collected in plain glass tubes. After clotting the blood was centrifuged and plasma was stored at -70 °C in plastic tubes. 'Racemic' formoterol was analysed according the method as has been described by van den Berg et al.\(^{18}\). For estimating V\textsubscript{c} only data points from each concentration-time curve were used which were calculated from chromatograms not showing any interfering peaks. All other kinetic parameters that describe the plasma concentration curve were obtained from the urinary excretion rate curve.

**Potassium in plasma**
Blood (5 ml) was collected in heparinized tubes. The plasma was immediately
centrifuged and stored at -70 °C. A conventional flame photometer (model 143, instrumentation laboratory Inc.) was used for potassium measurements.

DATA ANALYSIS

All pharmacokinetic and pharmacodynamic data were fitted to the equations of the various models, using the curve fitting program Scientist 19.

Pharmacokinetics
Formoterol concentrations were measured both in urine and in plasma. As the profile of the plasma concentration time curves is identical to the time profile of urinary excretion rates, providing that the urinary elimination rate is constant. All kinetic parameters for the plasma concentration, apart from the volume of distribution, were estimated from the urinary excretion rates. Plasma formoterol concentrations were used to estimate the volume of distribution by fitting the sum of the RR and SS data to the 'racemic' plasma formoterol curve.

A two-compartment model with first order absorption as described by Gibaldi and Perrier 20 was used to describe the plasma and urine data. Urinary excretion-time curves, plasma concentration-time curves for both enantiomers and the time courses of the cumulative excreted amounts were visually inspected. Although the urinary excretion rate data consisted of fewer points and varying time intervals due to less control over time of collection than plasma sampling, the excretion rates again indicated a double absorption route as was observed in a previous study 13. Therefore, we added an extra parameter (Fr) to the standard two compartment model in order to describe the amount of formoterol that reached the systemic circulation via the lungs (Fr) and the amount of formoterol that reached the systemic circulation via the gastrointestinal tract (1-Fr). The first fraction will appear into the systemic circulation immediately after dosing at $t = 0$, the second fraction at $t = t_{lag}$. As the sum of these fractions will exist of an unknown part of the dose that will be available for systemic absorption, the calculated $V_c/f$ will be the Volume of the central compartment divided by an unknown bioavailability factor. The relative bioavailability of both enantiomers in each of the two fractions was described by relative bioavailability parameters. As it was
previously found that the ratio of the two enantiomers was one for that part of the concentration-time curve where only the second fraction could still be present, it was assumed in the model that the bioavailability in the second fraction was identical for both enantiomers. The systemic bioavailability at each absorption site can thus be calculated for each enantiomer separately by multiplying half of the fraction of the dose, which is available for systemic absorption (f) with a relative bioavailability factor. The estimated values of the relative bioavailability parameters had to be between 0 and 1. The model allowed for different V_c/f values for the days with and without active coal to account for variation of the amount of the dose that was actually deposited at the absorption sites. Therefore, if the relative bioavailability of the enantiomers between two test days is tested, the difference in estimated Volumes has to be taken into account also.

The kinetic data from the two experimental days were modelled simultaneously, in order to improve the precision of the estimation. For the two enantiomers common parameter values were so estimated for: the distribution rate constant from the central to the peripheral compartment: alpha; elimination rate constant from the central compartment out of the body: beta; the rate constant for transfer of the drug from the peripheral to the central compartment: K_{21}; the urinary excretion rate constant: K^u. The following parameters were estimated separately for the two days: the Volume of the central compartment divided by an unknown availability parameter: V_c/f; the absorption rate constants for the first and the second fraction: Ka_R1 and Ka_R2 for the RR-enantiomers, Ka_S1 and Ka_S2 for the SS-enantiomers, respectively; the relative bioavailability of the enantiomers in each fraction: F_R1 for the RR in the first fraction, F_S1 for the SS in the first fraction, and F_2 for both the RR- and SS-enantiomers in the second fraction; the lagtime between the inhalation of the drug and the start of the absorption of the second fraction: T_{lag}. The RR-formoterol plasma concentration time curve was described by the following equation^{20}.

\[ C_{RR}(t) = C_{R1}(t) + C_{S2}(t) \]
The formoterol enantiomer excretion rate (ER) was calculated for each time point that lies halfway the time of the urine sample and the sample before. The excretion rate is calculated from the total volume of the urine collection multiplied by its formoterol enantiomer concentration and divided by the time interval between the urine sample and the sample before. The urinary excretion rate ($K_{\text{urine}}$) determines the proportionality of the urinary excretion rate to the plasma concentration. It was assumed that the $K_{\text{urine}}$ did not differ for the two enantiomers. The urinary excretion rate curve of the $RR$-formoterol is described by the following equation: $ER_{RR} = C_{RR}(t) \cdot V_c/f \cdot K_{\text{urine}}^{20}$. 

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The cumulative amount of formoterol that was excreted in the urine at the time of a urine sampling was calculated from the formoterol concentration in that sample multiplied by the sample volume. This amount is added to the sum of previous excreted formoterol. The cumulative excreted \( RR \)-formoterol time curve is described by the differential of the ER\(_{RR} \): \( RR \) cumulative = \( \frac{d(ER_{RR})}{dt} \).²⁰

**Pharmacodynamics**

To relate the calculated \( RR \)-formoterol plasma concentrations to the observed hypokalemic and lung function responses, a combined PK/PD model was applied as described by Holford and Sheiner.²¹²². This model includes a hypothetical effect compartment. To describe the delay between the effects and the formoterol \( RR \)-plasma concentration a rate constant for the elimination of formoterol from this hypothetical effect compartment, \( K_{e0} \) is used. The following equation describes the time course of the concentrations of \( RR \)-formoterol (\( C_e \)) in the hypothetical effect compartment:

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

where

\[
C_{e1}(t) = \frac{F_r \cdot \frac{1}{2} \cdot \text{Dose} \cdot F_{1R} \cdot K_{aR} \cdot K_{e0}}{V_e \cdot \tau} \cdot \frac{1}{f} \left[ \frac{(K_{21} - \alpha) \cdot e^{-\alpha t}}{(\beta - \alpha) \cdot (K_{aR} - \alpha) \cdot (K_{e0} - \alpha) + (\alpha - \beta) \cdot (K_{aR} - \beta) \cdot (K_{e0} - \beta)} + \frac{(K_{21} - \beta) \cdot e^{-\beta t}}{(\alpha - K_{aR}) \cdot (\beta - K_{aR}) \cdot (K_{e0} - K_{aR}) + (\alpha - K_{e0}) \cdot (\beta - K_{e0}) \cdot (K_{aR} - K_{e0})} \right]
\]

and

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\[ C_{e_1}(t) = \frac{(1 - Fr) \cdot \frac{1}{2} \cdot Dose \cdot F_2 \cdot K_{a_2} \cdot K_{e_0}}{V_e} \cdot f \]

\[
\left[ \frac{(K_{21} - \alpha) \cdot e^{-\alpha \cdot (t - \text{lag})}}{(\beta - \alpha) \cdot (K_{a_2} - \alpha) \cdot (K_{e_0} - \alpha)} + \frac{(K_{21} - \beta) \cdot e^{-\beta \cdot (t - \text{lag})}}{(\alpha - \beta) \cdot (K_{a_2} - \beta) \cdot (K_{e_0} - \beta)} \right] \\
\left[ \frac{(K_{21} - K_{a_2}) \cdot e^{-K_{a_2} \cdot (t - \text{lag})}}{(\alpha - K_{a_2}) \cdot (\beta - K_{a_2}) \cdot (K_{e_0} - K_{a_2})} + \frac{(K_{21} - K_{e_0}) \cdot e^{-K_{e_0} \cdot (t - \text{lag})}}{(\alpha - K_{e_0}) \cdot (\beta - K_{e_0}) \cdot (K_{a_2} - K_{e_0})} \right]
\]

In the above equation \( C_{e_1} \) is the effect compartment concentration resulting from the RR fraction of the dose absorbed via the pulmonary route and \( C_{e_2} \) the effect compartment concentration resulting from the orally absorbed RR fraction of the dose.

All concentration-effect relations were described with a sigmoid E\(_{\text{max}}\) model. The baseline values for the potassium concentration was estimated for every test day separately, because physiological variations can be expected. Although the patients were stable, variation in the lung way obstruction could clearly be observed both from varying E\(_0\) and E\(_{\text{max}}\) values. Therefore, both the baseline values and the maximum obtainable effect were estimated separately for the sG\(_{aw}\) and MEF\(_{50}\) on the two days. As it was clear that on all test days plateaux in the lung functions were obtained, the E\(_{\text{max}}\) values were estimated within a small range of these observed maximal values. The maximum achievable effect (E\(_{\text{max}}\)) for the potassium was estimated for the two days with a common parameter, since a difference was not observed nor to be expected.

Sigmoidal E\(_{\text{max}}\) formula:

\[
E = E_0 - \left( \frac{(E_0 - E_{\text{max}}) \cdot C_e^n}{EC_{50}^n + C_e^n} \right)
\]
Figure 1. Representative of fitted curves of urinary excretion rate of SS formoterol (solid lines and circles) and RR formoterol (striped lines and squares) after inhalation of aerosol of 96 μg of formoterol fumarate. (data from patient 6).
Figure 2. Representative of fitted curve of potassium after inhalation of aerosol of 96 µg of formoterol fumarate. (data from patient 6).
Figure 3. Simulation of curves of MEF$_{50}$ after inhalation of aerosol of 12 µg of formoterol fumarate; parameters with geomean values calculated from the days without coal. The simulated MEF$_{50}$ without blocking the oral absorption is represented by the striped line; the simulated MEF$_{50}$ with blockage of the oral absorption is represented by the solid line.
Figure 4. Simulation of curves of $sG_{aw}$ after inhalation of aerosol of 12 $\mu$g of formoterol fumarate; parameters with geomean values calculated from the days without coal. The simulated $sG_{aw}$ without blocking the oral absorption is represented by the striped line; the simulated $sG_{aw}$ with blockage of the oral absorption is represented by the solid line.
Statistical analysis
Statistical differences between the test day with and without charcoal, and
differences within a test day between \( RR \) and \( SS \), or differences between
pulmonary and oral fractions were all analysed with the two-tailed Wilcoxon
Signed Ranks test.
The goodness of fit for the kinetic and dynamic curves is expressed by the
coefficient of determination as calculated by Scientist. These are shown in the
appropriate tables.

RESULTS
Pharmacokinetics
As in our previous study, the urinary excretion rates showed a biphasic pattern,
and the data could be fitted nicely to the kinetic model. Urinary excretion rate
curves of a representative subject (subj. 6) on the day without coal are shown in
figure 1. The mean and standard deviation of the common kinetic parameters are
shown in table 1. The mean and standard deviation of the estimated kinetic
parameters, and the coefficients of variation as measure for the goodness of fit of
the urinary excretion rate curves are given in table 2 for the day with and the day
without coal.
The \( RR \) and \( SS \) in the second fraction were changed significantly by coal with -38
\( \pm \) 20\% (\( p < 0.05 \)), whereas the enantiomers in the first fraction were unchanged,
with 4.3 \( \pm \) 28\% for \( RR \) and 0.6 \( \pm \) 58 for \( SS \). The AUC of \( RR \)- and \( SS \)-plasma
concentrations differed in the early absorbed fraction on both days (\( p < 0.05 \)), the
ratio of \( RR/SS \) being 0.65 \( \pm \) 0.17 on the day without coal, and 0.71 \( \pm \) 0.20 on the
day with coal. For comparison, we expressed as percentages of the sum of \( RR \) and
\( SS \) in the pulmonary fraction, the areas under the time-courses as calculated as
follows: \( RR = 100 \times (1-Fr) \times F_{1R} / (1-Fr) \times (F_{1R} + F_{1S}) \) and \( SS = 100 \times (1-Fr) \times F_{1S} / (1-Fr) \times (F_{1S} + F_{1R}) \). To estimate the effect of active coal we expressed the orally
absorbed fraction as a percentage of the total amount of formoterol that was
systemically absorbed, as follows: 2 \times 100 \times Fr \times F_2. The contributions of the two
enantiomers in each of the absorbed fractions relative to the total area under the

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curve (AUC) of formoterol on the day without administration of coal are shown
in table 6.
On the day without coal, RR and SS were excreted on average with 305 ± 85 ng.h⁻¹
and 457 ± 150 ng.h⁻¹; on the day with coal, RR and SS were excreted on average
with 263 ± 44 ng.h⁻¹ and 332 ± 85 ng.h⁻¹.
If the estimated Vc/f is divided by the outcome of the relative bioavailability
factor multiplied by the calculated fraction, separate Vc's that incorporate the total
bioavailability factor (f*) can then be calculated for both enantiomers in each of
the two fractions of the dose. A mean of the four Vc's, thus corrected for the
relative contribution of each enantiomer to the total AUC, gives a single Vc/f* that
makes comparison possible with the mean Vc/f (1300 L) and eventually the mean
EC₅₀ found in the previous study (Derks et al, 1997) with inhaled formoterol in
which the enantiomers were not measured separately. The Vc/f* thus calculated
for the day without active coal is (geomean) 282 L.

Table 1. Common kinetic parameters on both days.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Subject</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>K₂1-t₁/₂ (h)</td>
<td>1 2 3 4 5 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>alpha- (h)</td>
<td>0 0 0.18 0.19 0.12 0.14</td>
<td></td>
<td></td>
</tr>
<tr>
<td>beta-t₁/₂ (h)</td>
<td>5 13 9.8 7.9 13.9 6</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Kₘicr-t₁/₂ (h)</td>
<td>8 2 5.7 5.3 1.9 3.2</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Pharmacodynamics
The hypokalemic effect and lung functions could be well described by the
dynamic model. The fit of the hypokalemic effect of a representative subject
(subj. 6) on the day without coal is shown in figure 2. The mean and standard
deviation of the estimated parameters for the hypokalemic effect, the sGₚw, and
MEF₅₀ of the day with and without coal and the coefficient of variations as
measure for the goodness of fit are given in table 3, 4, and 5, respectively.
The EC₅₀ values of RR for MEF₅₀ and sGₚw responses and for the hypokalemic
effect were 4.0 ± 2.8, 9.1 ± 6.4 and 42 ± 31 pg/ml respectively. The concentration-
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effect delay for MEF$_{50}$ and sG$_{aw}$ differed approximately five folds, being 2.4 and 0.5 hour, respectively. To compare the estimated EC$_{50}$ values for the hypokalemic effect with the values found in the previous study, each EC$_{50}$ was divided by the factor that the $V_{cif}$ (calculated as described in the pharmacokinetics section above) differed from the average $V_{cif}$ found previously. The thus corrected RR-EC$_{50}$ for the effect on potassium was (geomean) 7.6 pg/ml. For racemic formoterol this would result in 15 pg/ml; the corrected EC$_{50}$ of 'racemic' formoterol of the fraction absorbed via the pulmonary route is calculated to be 40.0 pg/ml.

When the MEF$_{50}$ and sG$_{aw}$ curves are simulated with the estimated geomean parameters, it can be seen that after inhalation of 12 µg formoterol the oral fraction of the systemic concentration contributes substantially to the long duration of the effects. (figures 3 and 4) As especially the MEF$_{50}$ is at or near maximum effect after inhalation of 96 µg formoterol for almost twelve hours, the contribution of the oral fraction to the duration of this effect is then no longer important.

Table 2. Kinetic parameters that differed on the two test days.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean with coal</th>
<th>SD with coal</th>
<th>Mean without coal</th>
<th>SD without coal</th>
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</thead>
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<tr>
<td>V$_{cif}$ (L)</td>
<td>95</td>
<td>63</td>
<td>129</td>
<td>158</td>
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<tr>
<td>Ka$<em>{R1}$-t$</em>{1/2}$ (h)</td>
<td>0.42</td>
<td>0.16</td>
<td>0.62</td>
<td>0.84</td>
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<td>Ka$<em>{S1}$-t$</em>{1/2}$ (h)</td>
<td>0.5</td>
<td>0.51</td>
<td>0.62</td>
<td>0.87</td>
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<td>Ka$<em>{R2}$-t$</em>{1/2}$ (h)</td>
<td>0.84</td>
<td>0.66</td>
<td>0.43</td>
<td>0.34</td>
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<tr>
<td>Ka$<em>{S2}$-t$</em>{1/2}$ (h)</td>
<td>0.45</td>
<td>0.36</td>
<td>0.21</td>
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<td>Fr</td>
<td>0.36</td>
<td>0.13</td>
<td>0.34</td>
<td>0.2</td>
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<tr>
<td>t$_{lag}$ (h)</td>
<td>2</td>
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<td>1.7</td>
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<td>F$_{IR}$</td>
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<td>0.59</td>
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<td>F$_{IS}$</td>
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<td>0.16</td>
<td>0.91</td>
<td>0.09</td>
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<td>F$_{2}$</td>
<td>0.5</td>
<td>0.31</td>
<td>0.86</td>
<td>0.16</td>
</tr>
<tr>
<td>CoD RR</td>
<td>0.95</td>
<td>0.05</td>
<td>0.96</td>
<td>0.03</td>
</tr>
<tr>
<td>CoD SS</td>
<td>0.97</td>
<td>0.02</td>
<td>0.97</td>
<td>0.03</td>
</tr>
</tbody>
</table>
**Table 3.** Dynamic parameters for hypokalemic effect.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_0$ coal</td>
<td>4</td>
<td>0.2</td>
</tr>
<tr>
<td>$E_0$ no coal</td>
<td>3.9</td>
<td>0.2</td>
</tr>
<tr>
<td>$E_{\text{max}}$</td>
<td>2</td>
<td>0.9</td>
</tr>
<tr>
<td>$E_{C50}$</td>
<td>42</td>
<td>31</td>
</tr>
<tr>
<td>$K_{e_0-t_{1/2}}$</td>
<td>1.6</td>
<td>1</td>
</tr>
<tr>
<td>$n$</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>CoD coal</td>
<td>0.72</td>
<td>0.34</td>
</tr>
<tr>
<td>CoD no coal</td>
<td>0.78</td>
<td>0.16</td>
</tr>
</tbody>
</table>

**Table 4.** Dynamic parameters for $sG_{aw}$.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_0$ coal</td>
<td>0.6</td>
<td>0.32</td>
</tr>
<tr>
<td>$E_0$ no coal</td>
<td>0.6</td>
<td>0.35</td>
</tr>
<tr>
<td>$E_{\text{max}}$ coal</td>
<td>2.47</td>
<td>1.05</td>
</tr>
<tr>
<td>$E_{\text{max}}$ no coal</td>
<td>2.7</td>
<td>1.35</td>
</tr>
<tr>
<td>$E_{C50}$</td>
<td>9.1</td>
<td>6.4</td>
</tr>
<tr>
<td>$K_{e_0-t_{1/2}}$</td>
<td>0.5</td>
<td>0.2</td>
</tr>
<tr>
<td>$n$</td>
<td>1.6</td>
<td>1.8</td>
</tr>
<tr>
<td>CoD coal</td>
<td>0.65</td>
<td>0.17</td>
</tr>
<tr>
<td>CoD no coal</td>
<td>0.72</td>
<td>0.17</td>
</tr>
</tbody>
</table>

**Table 5.** Dynamic parameters for $MEF_{50}$.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Mean</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>$E_0$ coal</td>
<td>2.32</td>
<td>0.6</td>
</tr>
<tr>
<td>$E_0$ no coal</td>
<td>2.3</td>
<td>0.85</td>
</tr>
<tr>
<td>$E_{\text{max}}$ coal</td>
<td>4.07</td>
<td>1.11</td>
</tr>
<tr>
<td>$E_{\text{max}}$ no coal</td>
<td>4.32</td>
<td>1.34</td>
</tr>
<tr>
<td>$E_{C50}$</td>
<td>4.4</td>
<td>2.4</td>
</tr>
<tr>
<td>$K_{e_0-t_{1/2}}$</td>
<td>2.39</td>
<td>2.94</td>
</tr>
<tr>
<td>$n$</td>
<td>3.5</td>
<td>5.2</td>
</tr>
<tr>
<td>CoD coal</td>
<td>0.86</td>
<td>0.09</td>
</tr>
<tr>
<td>CoD no coal</td>
<td>0.84</td>
<td>0.15</td>
</tr>
</tbody>
</table>
Table 6. The contributions of the systemically available formoterol via the pulmonary and oral route and the two enantiomers in each of the absorbed fractions, relative to the total area under the curve (AUC) of formoterol on the day without administration of coal.

<table>
<thead>
<tr>
<th>Total AUC without coal</th>
<th>100%</th>
<th>Pulm. AUC without coal</th>
<th>64%</th>
<th>Oral AUC without coal</th>
<th>36%*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RR 0.39* x 64% = 25.0%</td>
<td></td>
<td>RR 0.5 x 36% = 18%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SS 0.61* x 64% = 39.0%</td>
<td></td>
<td>SS 0.5 x 36% = 18%</td>
<td></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Total AUC with coal</th>
<th>79%</th>
<th>Pulm. AUC with coal</th>
<th>58%</th>
<th>Oral AUC with coal</th>
<th>21%*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>RR 0.41* x 58% = 23.8%</td>
<td></td>
<td>RR 0.5 x 21% = 11%</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>SS 0.59* x 58% = 34.2%</td>
<td></td>
<td>SS 0.5 x 21% = 11%</td>
<td></td>
</tr>
</tbody>
</table>

* p < 0.05

DISCUSSION

Previously the kinetics of inhaled racemic formoterol after a single dose in healthy volunteers were studied by us \textsuperscript{13}. For the data analysis in that study the following two assumptions were made. Firstly, the biological availability was considered to be equal for the two enantiomers and also independent of the absorption route. Secondly, kinetic rate constants of the two enantiomers were considered to be identical. The used mathematical formulas permitted an accurate empirical description of the observed kinetics, however any meaningful estimation of the kinetic parameters of the two enantiomers required two important parameters to be known: the differential bioavailability of the two fractions absorbed respectively via the lungs and via the gastrointestinal tract, and the ratio between the active and inactive enantiomers in both fractions. Likewise,
PK/PD modelling of formoterol enantiomers in asthma patients

the observed biphasic effects in that study were empirically described with the used formulas, but provided information on the values of the dynamic parameters that were strongly influenced by the above mentioned assumptions. Furthermore, it was noted that the effect curves could only be fitted with the concentration curve, when two rate constants ($K_{e0}$) for describing the kinetics were used for each of the two routes of systemic absorption of inhaled formoterol into a single effect compartment. Additionally, the fraction of the dose that appeared in the systemic circulation after inhalation through the lung was 2 to 3 times less potent in comparison with the systemically absorbed formoterol through the oropharyngeal/gastrointestinal route.

In the present study, we were able to estimate with the used kinetic model, the different kinetic profiles of the RR- and SS-formoterol enantiomers following an early and late absorption phase. In short, the kinetic model describes the concentration-time curves of two sub-fractions of the systemically available fraction of the dose that are derived from the lungs and the gastrointestinal tract. The relative bioavailability of the separate enantiomers of each of these two sub-fractions is expressed by a bioavailability factor. The number of parameters ($n=22$) that had to be estimated was large. However, in all but one subject the total number of data points of urinary excretion was much larger (range: 24-38). Information about one important kinetic parameter, $V_c/f$, was obtained from the measured plasma formoterol concentrations. Moreover, the simultaneous fitting of the four obtained urinary excretion rate curves per individual with alpha, beta and $K_{21}$ as common parameters improved the precision. As is reflected in the good coefficients of determinations. In order to reduce the number of parameters, the relative bioavailability of the RR- and SS-enantiomers was not estimated differentially in the second absorbed fraction. It is not proven that the ratio of the two enantiomers indeed do not differ in the second systemically absorbed fraction of the dose. However, we feel that this assumption explains best the findings in the present and a previous study $^{14}$ where the ratio of $RR/SS$ is smaller than one immediately after inhalation and changes to one over time. The observed differences between peak heights of the second fraction is mainly caused by the difference between the SS and RR of the first peak. The estimated beta-half-life agreed with previous found value, although in one study in which urine and
plasma samples were measured with an sensitive assay for over 72 hours after dosing, the beta-half-life was larger.\textsuperscript{23, 13, 24}

Charcoal only changed the amount of formoterol that reached the systemic circulation via the second absorption. This is in agreement with the idea that the second formoterol concentration peak is caused by gastrointestinal absorption. The first formoterol concentration peak is then absorbed via the lungs, although it is still possible that a part of it reaches the systemic circulation via the oropharynx. With charcoal still 27\% of the systemic formoterol is estimated to be absorbed via the gastrointestinal tract. In other studies charcoal was able to block absorption completely.\textsuperscript{15, 25, 26} It is unlikely that the lower but still relatively high and well measurable concentrations of formoterol in the urine caused unreliable data. The dilution of the charcoal sludge as a result of the amount of water that the patients had to drink will certainly have diminished its effectiveness to some extend.

The amount of RR was smaller than SS in the pulmonary fraction. This agrees with our hypothesis that a change of the enantiomer ratio is the explanation for the observation that the EC\textsubscript{50} of 'racemic' formoterol will become smaller over time after inhalation. If one corrects the EC\textsubscript{50} of the hypokalemic effect for the estimated V\textsubscript{c}/f, the calculated EC\textsubscript{50} of the second absorbed 'racemic' formoterol agrees with the previous findings. Previously we estimated the EC\textsubscript{50} for the hypokalemic effect of racemic formoterol for the second fraction to be 20 ± 6 pg/ml and this agrees reasonably with the present finding of 15 pg/ml. The corrected EC\textsubscript{50} of 'racemic' formoterol of the fraction absorbed via the pulmonary route is calculated to be 40.0 pg/ml and was previously found to be 66 ± 24 pg/ml. The observed or inferred ratio of RR/SS in previous studies\textsuperscript{14, 13} was approximately identical to the ratio seen in the present study and not as was argued before, to be caused by a different sensitivity of the assay for either of the two enantiomers\textsuperscript{14}. Both mentioned inhalation studies differed from the present in that they were done in healthy subjects instead of asthma patients, using dry powder in one and aerosol in the other\textsuperscript{14, 13}.

As the concentration time curves of the RR-enantiomer were biphasic, the fitted effect curves of the potassium showed a similar profile. On the days without coal, the MEF\textsubscript{50} and the sG\textsubscript{aw} curves were at maximum effect in most patients before the second fraction was being absorbed and thus no biphasic profile could be
discerned then. On the days with coal, when plasma concentrations did not result in maximal response, both lung function parameters showed a biphasic profiles. It is surprising that plasma formoterol time-curves could be linked to the lung function time-curves, as a part of the inhaled drug is deposited directly to effector organ. As the lung functions were reasonably well described, with no systematic outliers of the fitted data in the early or late part of the curves, it can be concluded that a very rapid equilibration occurs of the formoterol concentration in the lungs after inhalation and the plasma formoterol concentration. However, the very low apparent EC$_{50}$ values found for these lung function parameters, which were -in contrast to previous studies$^{27,28}$- much lower than the EC$_{50}$ value for the hypokalemic effect, indicate that the concentrations in the lung were much higher than in plasma. When the lung parameters are simulated for a low dose of inhaled formoterol with the mean parameter values, the results suggest that the systemically available formoterol via the gastrointestinal tract contribute to the long duration of the bronchodilating action.

In conclusion, the fraction of racemic formoterol that is systemically absorbed via the lungs after inhalation contains more SS than RR formoterol. This study suggests that systemically available formoterol absorbed via the gastrointestinal route after inhalation of a low dose, contributes to the long acting effect on bronchodilation.

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