Concentration-effect relations of anti-asthma medications. Studies on inflammation markers
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Urinary excretion of formoterol enantiomers: pharmacokinetics in healthy subjects after inhalation of formoterol racemate

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Chapter 6

ABSTRACT

Objective: Formoterol fumarate is a racemate of \( RR \) and \( SS \) enantiomers. Its activity resides in the \( RR \) enantiomer. Previously, a biphasic concentration- and effect-pattern was found following inhalation of formoterol. The absorption of the dose both via the gastrointestinal route and via the lungs presumably caused this. The potencies of these two absorbed fractions differed. In the present study, enantiospecific kinetics as a possible cause for these differences in potency were investigated.

Methods: Single inhaled doses of formoterol fumarate (12, 24, 48 and 96 \( \mu \)g, dry powder formulation) were given to 10 healthy male subjects. Urine was collected before dosing and between 0-1, 1-2, 2-4, 4-8 and 8-24 hours. Urinary excretion rate versus time courses of the two enantiomers were fitted to a two-compartment model with the computer program P-Pharm for population fitting.

Results: Inhaled formoterol fumarate showed linear kinetics. The amount of \( RR \) and \( SS \) enantiomers recovered in the urine was respectively 6.16% and 9.11% of the administered respective enantiomers. The \( RR/SS \) ratio increased from 0.5 in the first to 1.0 in the last sample. The kinetic parameters for \( RR \) and \( SS \) were respectively (rate constants (h\(^{-1}\)) are shown in brackets): \( T_{\text{max}} \): 1.4, 1.2 h; \( K_{a-t_1/2} \): 0.5, 0.6 h (1.4; 1.2); alpha-\( t_1/2 \): 0.5, 0.5 h (1.3; 1.3); beta-\( t_1/2 \): 11.3, 9.2 h (0.06; 0.08).

Conclusion: Differences in systemic concentration-time profiles of the enantiomers after inhalation of the racemate must possibly be taken into account when linking total formoterol plasma concentrations to effects.

INTRODUCTION

Formoterol is a long acting \( \beta_2 \)-adrenoceptor agonist. After inhalation of formoterol, rapid bronchodilation occurs and this effect is maintained for about 12 hours\(^{14} \). Four diastereomers of formoterol exist, but formoterol is marketed as a racemate, consisting only of the \( RR \) and the \( SS \) enantiomers. The pharmacodynamic activity of racemic formoterol is exerted by the \( RR \) enantiomer, whereas the \( SS \) enantiomer is almost inactive\(^5\).
Only recently, a method for the detection of formoterol in the low picogram per millilitre range became available\. This enabled us to perform pharmacokinetic/pharmacodynamic (PK/PD) analysis of results from a previous study in humans, using 'racemic' plasma concentrations of formoterol after inhalation\. In that study we found that the potency of formoterol in the systemic circulation increases with time. We suggested that this might correlate with a changing ratio of the two enantiomers in time. The changing ratio was assumed to be caused by different pharmacokinetics of the two enantiomers\. Systemic appearance of formoterol following inhalation was almost instantaneous, causing an early concentration peak that was presumably the result of rapid absorption from the lungs. A second peak in the plasma-concentration time profile occurred after a lagtime and was assumed to be caused by absorption from the gastrointestinal tract. Formoterol concentrations after the lagtime are thus the result of a summation of these two absorption processes. This dual absorption profile via the pulmonary and oral routes was presumed to give rise to the above-mentioned apparent increase of formoterol potency in time. In the present study the hypothesis of enantiospecific kinetics as a possible cause for these differences in potency was investigated. Modification of the analytical method made it possible to perform enantiospecific measurements of the enantiomers in urine\. The higher concentrations of formoterol in urine make urine a more suitable body fluid for enantiospecific analysis than plasma, especially after inhalation of formoterol\. At any time, the plasma concentration of a drug is proportional to the urinary excretion rate as long as the renal clearance is constant\. Thus the values of the pharmacokinetic parameters calculated from the excretion rates, are identical to the values for the kinetic parameters of blood concentration time curves. However, the Volume of distribution and the clearance cannot be calculated from urinary excretion rates.

**PATIENTS AND METHODS**

**Study design & participants**
The study was done by Ciba-Geigy, Basel, at the facilities of the pharmaceutical division. The protocol was approved by an ethics committee. The study was
designed as a double blind, placebo controlled, randomized, single dose, 5-period crossover trial. Dosages of 12, 24, 48 and 96 μg of formoterol fumarate (Ciba-Geigy, Basel, Switzerland) or placebo were administered by using a multidose dry powder inhaler on five examination days, separated by a washout period of at least five days. Urine was collected before dosing and at the following intervals after dosing: 0-1 h, 1-2 h, 4-8 h and 8-24 hours. Some of the results of this study have been presented before 10, 14, 15.

Ten healthy male subjects participated after informed consent was obtained. Their mean ± SD height, weight and age were respectively, 185 ± 5 cm, 77 ± 8 kg, and 30 ± 6 years. The subjects came to the study site after an overnight fast. A standard breakfast, lunch and a snack together with 300 ml of caffeine-free coffee or 300 ml of water was offered respectively two, four and nine hours after dosing. In addition, 100 ml of water was given at 1, 3, 6 and 8 hours in order to maintain adequate diuresis. After completion of the 12 hours measurements, the subjects were free to go home and to have a light dinner. The next morning they returned to the laboratory after another overnight fast for the 24 hours' measurements.

Formoterol analysis
Formoterol RR and SS enantiomers were determined by HPLC with electrochemical detection as described by Butter et al 10. In short, urine sample clean-up was done by liquid-liquid extraction followed by solid phase extraction. The enantiomers were separated on an AGP-column and were detected electrochemically. The limit of detection for the RR and SS enantiomers was respectively 20.7 and 25.8 μg/L.

Pharmacokinetic analysis
From the volume of the urine, the concentration of formoterol and the time after inhalation, the urinary excretion rate of the enantiomers was calculated. (Equation 1). Effort was made to fit individual urinary excretion rates curves but insufficient data points per curve were available to obtain reliable results. Therefore, the data was described with population modelling. The disadvantage of few data points per individual excretion rate-time curve can so be balanced by the number of data sets 16, 17.
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The urinary excretion rate-midpoint time data was fitted with the two-compartment model from the model library of the population modelling program P-Pharm. The RR and SS enantiomers were fitted separately and thus in total two groups consisting of 40 RR and 40 SS excretion rate-time profiles, each consisting of 6 data points, could be analysed. The four excretion data sets obtained after administration of the four doses to a single subject were identified to belong to that subject, which would help to reduce inter-individual variations.

With either the simplex method or the stripping method we found initial parameter values for the ten individual groups of four excretion rate-time curves for both enantiomers. The second step consisted of the estimation of population parameters. Age, height and weight were introduced as covariates.

The version of P-Pharm that we used did not allow us to make changes in the library models nor could we build a model. The use of the library two-compartment model to describe urinary excretion rates gave us the opportunity to investigate a potential difference between either Volume or clearance for the two enantiomers. This can only be done if an unknown multiplication factor is introduced in the Volume of distribution, which we shall explain hereafter. Urinary excretion rate time curves and plasma concentration time curves are congruent in form, providing renal clearance is constant. A volume of distribution cannot be calculated from the urinary excretion rates without knowing renal clearance. In order to relate the urinary excretion rate (ER) (ng/h) to concentration (C\textsubscript{plasma}) (ng/L) with a two-compartment model, the urinary excretion rate must be divided by an unknown constant, U, that can be thought of as being made up of Volume of distribution, V/f (L), multiplied by a rate constant, R\textsuperscript{*} (h\textsuperscript{-1}). That gives the following equation: \[ C\textsubscript{plasma} = \frac{ER}{U} = \frac{ER}{(V/f \cdot R\textsuperscript{*})}. \] (ng/L = ng/h / L* h\textsuperscript{-1}).

Volume of distribution in the build in model is thus replaced by U, and will have an unity (L*h\textsuperscript{-1}), which is the unity of clearance. Therefore we refer to this constant as CI. The value of CI can be used to detect differences between clearances for the two different enantiomers, assuming that both the multiplication factor U and the bioavailability factor (f) that was incorporated in the volume are identical for both enantiomers. Likewise, the build-in parameter Clearance (V/f * R\textsuperscript{*} * \beta) now gets the unity L.h\textsuperscript{2}. We refer to this parameter as V'. Again, if one assumes that R\textsuperscript{*} does not differ between the two enantiomers and the beta does not show to be different either, this parameter allows to make comparisons for the V/f
between the two enantiomers. From $V'$ and $Cl$ we only obtain a non-quantitative indication whether or not a difference exists between volume and/or clearance for the two enantiomers.

Linearity of kinetics of formoterol enantiomers in the used dosing range was established by correlating the mean area under curve of the excreted formoterol (AUC) of each dosing group with the dose. The AUC, which is an estimation of the amount of drug excreted over the observation period, was calculated according Equation 2\textsuperscript{13}.

**Equation 1.**

Excretion rate $\text{ER}_{\text{Mpi}} = \frac{(C_{ui} \times V_{ui})}{(T_{i+1} - T_i)}$

$MP_i = \text{midpoint of } i^{th} \text{ urine collection interval} = T_i + \frac{(T_{i+1} - T_i)}{2}$

$T_i = \text{start time of } i^{th} \text{ urine collection interval}$

$C_{ui} = \text{concentration of enantiomer in } i^{th} \text{ urine collection interval}$

$V_{ui} = \text{volume of urine collected in } i^{th} \text{ urine collection interval}$

**Equation 2.**

$\text{AUC} = \sum_{i=0}^{n} (MP_{i+1} - MP_i) \times \frac{\text{ER}_{MP_i} + \text{ER}_{MP_{i+1}}}{2}$

With $MP_{i=0} = 0 \text{ (h)}$ and $\text{ER}_{MP_{i=0}} = 0 \text{ (ng/h)}$.

**Statistical analysis**

Differences between estimated mean results of the two enantiomer groups were analysed with a two-way T-test for unpaired groups. Because the standard deviation for the differences was not known, differences between both groups could not be analysed with a T-test for paired groups.

**RESULTS**

**Measured formoterol**

The concentrations of $RR$ and $SS$ formoterol in urine (mean ± SD) (ng/ml) respectively decreased from $0.88 ± 0.53$ and $1.77 ± 1.20$ in the first samples to $0.19 ± 0.17$ and $0.20 ± 0.18$ in last samples for the lowest dose of 12 µg racemic
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formoterol; from 4.92 ± 2.10 and 10.96 ± 5.91 in the first samples to 1.05 ± 0.54 and 1.11 ± 0.43 in the last samples for the highest dose of 96 µg racemic formoterol.

The percentage of the administered formoterol enantiomers recovered in the urine in relation to the dose of the racemate that was collected during 24 hours after dosing differed, being 3.34% of the dose for the RR and 4.72% of the dose for the SS enantiomer (p<0.001).

Following inhalation of formoterol fumarate the ratio of the concentration of the RR and SS enantiomers in urine changes over time from 0.50 to 0.96. (figure 1)

Linearity was established for the four doses by assessing the AUC's. The mean AUC for the dose of 12, 24, 48, and 96 µg racemic formoterol was respectively 379, 692, 1199, and 2283 ng for the RR and 499, 916, 1853, and 3499 ng for the SS. (Figure 2) The correlation coefficient ($r^2$) was 0.84 for the RR enantiomer and 0.83 for the SS enantiomer.

Table 1. Mean estimated and calculated pharmacokinetic parameters.

<table>
<thead>
<tr>
<th>Parameters</th>
<th>RR</th>
<th>SD</th>
<th>SS</th>
<th>SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>V/f * F * β (L.h$^{-2}$)</td>
<td>9.7</td>
<td></td>
<td>8.4</td>
<td>2</td>
</tr>
<tr>
<td>Cl (L.h$^{-1}$)</td>
<td>22.5</td>
<td>5</td>
<td>13.5</td>
<td>1</td>
</tr>
<tr>
<td>$K_a$ (h$^{-1}$)</td>
<td>0.5</td>
<td>0.05</td>
<td>0.59</td>
<td>0.03</td>
</tr>
<tr>
<td>$K_{12}$ (h$^{-1}$)</td>
<td>0.76</td>
<td>0.16</td>
<td>0.6</td>
<td>0.07</td>
</tr>
<tr>
<td>alpha-t$_{1/2}$ (h)</td>
<td>0.53</td>
<td></td>
<td>0.53</td>
<td></td>
</tr>
<tr>
<td>beta-t$_{1/2}$ (h)</td>
<td>11.32</td>
<td></td>
<td>9.2</td>
<td></td>
</tr>
<tr>
<td>$T_{max}$ (h)</td>
<td>1.4</td>
<td></td>
<td>1.23</td>
<td></td>
</tr>
<tr>
<td>maximum likelihood</td>
<td>-1236</td>
<td></td>
<td>-1236</td>
<td></td>
</tr>
</tbody>
</table>

Estimated parameters and fits
The estimated parameters for the two enantiomers did not show any significant differences. (Cl: P>0.1; other parameters P>0.2). The kinetic parameters are presented in table 1. Cl is V/f * R* and V' is V/f * β * R* as described above; $K_a$ is absorption rate constant; $K_{12}$ is rate constant for drug transfer from the central to the peripheral compartment; alpha is the distribution rate constant; beta is the elimination rate constant. The alpha, beta, $T_{max}$, and excretion rate maximum for a given dose were calculated according the appropriate formulas $^{15}$. The
population fits for the \textit{RR} and the \textit{SS} enantiomers are presented respectively in figure 3 and figure 4.

The calculated maximum excretion rate for a dose of 96 \( \mu \)g of racemic formoterol was 559 ng/h for the \textit{RR} and 890 ng/h for the \textit{SS} enantiomers.

Body weight could explain a part of the variation of CI and \( K_{12} \) for the \textit{SS} enantiomer. This resulted in a somewhat improved description of the data; maximum likelihood changed from -1229.0 to -1235.6. The relation between CI of \textit{SS} and weight is given by the equation CI = \(-1.244 + 0.192 \times \) body weight. The relationship between \( K_{12} \) of \textit{SS} and body weight is given by the equation \( K_{12} = -0.261 + 0.0112 \times \) body weight.

\begin{figure}[h]
\centering
\includegraphics[width=0.8\textwidth]{fig1.png}
\caption{ratio of the urine concentrations of the \textit{RR} and \textit{SS} enantiomers of formoterol plotted against time.}
\end{figure}

\section*{DISCUSSION}

Following inhalation of formoterol fumarate the ratio of the concentration of the \textit{RR} and \textit{SS} enantiomers in urine changed over time from 0.5 to 1.0. Under the assumption that the urinary excretion rate constant did not change in time and was similar for both enantiomers, it can thus be concluded that the enantiomers show different kinetic profiles in plasma. As only the \textit{RR} enantiomer is active, this
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Figure 2. Area under the curve (AUC) of the measured urine concentrations of the RR- and SS enantiomers in urine.

means that the potency of the total formoterol plasma concentration will increase over time. This is in accordance with our hypothesis from a previous study in which we measured total -not enantiospecific- formoterol plasma concentrations and related them to systemic effects.\textsuperscript{7} The concentrations of formoterol in urine were 100 to 1000 times higher than what could have been expected in plasma.\textsuperscript{7} The lower urinary recovery of the RR is probably not due to an analytical problem, because the precision and accuracy did not differ between the two enantiomers, and the lower limit of detection was lower rather than higher for the RR.\textsuperscript{10} The combination of the results, -the lower recovery in the urine of RR, the changing ratio of the two enantiomers with time, identical kinetic parameters but the higher Cl of RR (although not statistically significant), can be explained by SS being relatively more absorbed into the systemic circulation than RR, early after inhalation. Although we can not rule out differences in the distribution or
clearance of the two enantiomers. The return of the ratio to approximately one in the last sample suggests that the initial enantiospecific kinetic process is overtaken by the gastrointestinal absorption of racemic formoterol that started after a lagtime. However, further study is needed to give more insight in the exact kinetic mechanisms.

The kinetic parameters differed only substantial -although not significantly- for the CI. It must be noted that clearance should be interpreted with knowledge of the volumes of distribution. It cannot be solved whether the difference between the CI for the enantiomers is caused by either a difference between the relative (i.e. different bioavailability) or absolute Volumes or possibly by a difference between clearance. The absolute Volume of the central compartment of the formoterol enantiomers has not been reported in humans. Although CI of the RR was 60% larger than CI of the SS, statistical differences could not be shown, as could neither for any of the other estimated kinetic parameters. However, the statistical differences for the two relatively small groups of ten kinetic parameter sets could only be tested with a T-test for unpaired groups, because the standard deviation for the difference of the means could not be obtained by P-Pharm.

The four different doses showed proportional increases of AUC. The linear kinetics in the studied dose range of 12 to 96 μg of racemic formoterol fumarate agrees with recently described linear lung function changes with changing doses. A two-compartment model was applied to fit the data as this was found to describe the data best, and agrees with earlier modelling studies with formoterol concentration-time data in plasma. An absorption model with two fractions that differentiates between presumably an early pulmonary and a late gastrointestinal absorption has been shown to describe formoterol plasma concentrations following inhalation adequately. Due to the few samples and the times of urine collection, information was not available on a possible dual systemic appearance of formoterol in the present study. Therefore, no possible differences in site specific absorption rates for the two enantiomers, or a lagtime for gastrointestinal absorption could be established.
Figure 3. Population fit of $RR$-enantiomer excretion rate (ng/l) in urine after inhalation of different doses of formoterol. Fitted curve (line) and observed points.
Figure 4. Population fit of SS-enantiomer excretion rate (ng/L) in urine after inhalation of different doses of formoterol. Fitted curve (line) and observed points.
As the amount of 'racemic' formoterol absorbed via this second absorption was previously estimated to be substantial, being approximately 30% of the total absorbed amount of formoterol, this will compromise the parameter estimations. Kinetic differences between the two enantiomers after absorption, distribution or elimination rates can thus only be regarded as an approximation of the reality, and all parameter values should be regarded as hybrid values. In the present study we also find a beta half-life for formoterol which appears longer than the approximately 6-9 hours, which has been found in previous studies. A longer beta half-life of 10 hours was also suggested recently by Lecaillon. However, several points have to be considered when comparing these beta half-lives estimations. Firstly, the longer half-lives were found following inhalation, but the second slow absorption of 30% of the total systemically absorbed amount, which begins only after a lagtime of an hour was not taken into account. Furthermore, simple linear analysis disregarding the absorption and distribution phases was used to calculate the longer beta-half-life in one of the studies. Since the calculations were based on the interval between 8 to 24 hours after dosing, an overestimation of the beta is not likely, because the absorption is completed five hours after dosing, and the distribution from the drug to the peripheral compartment is completed two to three hours after absorption, as can be concluded from the present and a previous study. Moreover, one can argue that those longer beta-half-lives were based on urinary data up to 16 hours following inhalation, which are more reliable because of the higher concentrations of formoterol. The studies on formoterol kinetics in which shorter beta half-lives were found, did not last more than eight hours, and the relatively low concentrations in the final plasma samples could have lead to underestimation of the beta.

Population modelling allows for detecting correlations between parameter values and covariates. Correlations could only be detected between Clearance and $K_{12}$ for SS with body weight, and correction resulted in a little improvement of the description of the data. As only a group of ten subjects was studied, which was homogenous regarding these covariates, strong correlations were not to be expected. The grouping of the data from a single subject, which leads to a single set of pharmacokinetic parameter values, reducing intra-individual variations and the Bayesian method that takes into account the data of all subjects, leads to better
parameter estimations of urinary excretion curves with only five observations after dosing. The results must be validated in a study with a formoterol enantiospecific assay and sufficient data points that are measured for a sufficiently long period after dosing to fit to a model that accounts for the dual absorption sites following inhalation. It is necessary to know the absolute Volume of distribution for both enantiomers in order to explain the different kinetics. After inhalation of formoterol, different pharmacokinetics for the two enantiomers must be taken into consideration when linking formoterol concentrations to systemic or peripheral effects. This can have implications for our understanding of formoterol concerning duration of effect, efficacy, or beta--receptor down-regulation.

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