Clinical-Pharmacokinetic Aspects of Prolonged Effect Duration as Illustrated by $\beta_2$-Agonists

BY

JOHAN ROSENBORG
ABSTRACT


Regularity is a key element of maintenance drug treatment; compliance is crucial for treatment success. Once- or twice-daily intake of a drug is always easier to comply with than regimens requiring more frequent dosing. Bronchodilating treatment was used as an example to illustrate how sustained duration of effect can be achieved by two different approaches: oral administration of the terbutaline prodrug bambuterol and inhalation of formoterol. Bioanalytical methods were employed to monitor the kinetic fate of bambuterol and formoterol in plasma, urine, or faeces. Generated terbutaline in plasma was used as a marker of effect for bambuterol. Established clinical laboratory tests were used to assess local and systemic effects of inhaled formoterol compared with salbutamol.

Recommended doses of bambuterol, 10-20 mg once daily in adults, normally produced plasma concentrations of the active moiety terbutaline within therapeutically relevant limits. Dose proportionality for terbutaline makes dosing with bambuterol predictable. Compared with adults, children should be given higher doses than indicated by their lower body weight. Pharmacokinetic analysis indicated that absorption of bambuterol was slow and multi-phasic and that slow biotransformation to terbutaline occurred both presystemically and systemically. Systemically circulating formoterol was rapidly eliminated, the inactive (S;S)-formoterol more rapidly than the active (R;R)-formoterol. An inactive phenol glucuronide was the main metabolite, and a previously unknown sulphate metabolite was discovered. Duration of systemically mediated cardiovascular or metabolic side-effects of inhaled formoterol seemed not to differ from those of an inhaled systemically equieffective dose of salbutamol. There was a trend suggesting that the magnitude of systemic side-effects may be less pronounced after inhalation of formoterol compared with a locally equieffective dose of inhaled salbutamol. Both approaches to sustaining stimulation of β₂-adrenoceptors have their pros and cons. Bambuterol can be dosed orally once daily, but full effect is reached slowly. The effect of formoterol is reached within a few minutes, but administration must occur via the lungs, often twice daily. Both treatments, however, give 24-h symptom relief during regular treatment.

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PAPERS DISCUSSED

This thesis is based on the following papers, which will be referred to in the text by their Roman numerals.


<table>
<thead>
<tr>
<th>Abbreviation</th>
<th>Definition</th>
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<tbody>
<tr>
<td>Ae</td>
<td>Amount excreted in urine during a collection interval</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
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<tr>
<td>AUC</td>
<td>Area under the curve of plasma concentration vs time</td>
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<tr>
<td>CLA</td>
<td>Apparent clearance after oral administration (dependent also on bioavailability)</td>
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<td>CLR</td>
<td>Renal clearance</td>
</tr>
<tr>
<td>ESI</td>
<td>Electrospray ionization</td>
</tr>
<tr>
<td>FEV₁</td>
<td>Forced expiratory volume (L) in the first second</td>
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<tr>
<td>$^{3}$H₂O</td>
<td>Tritiated water</td>
</tr>
<tr>
<td>F</td>
<td>Formoterol</td>
</tr>
<tr>
<td>FG1</td>
<td>Phenol glucuronide of formoterol</td>
</tr>
<tr>
<td>FG2</td>
<td>Benzyl glucuronide of formoterol</td>
</tr>
<tr>
<td>FS</td>
<td>Sulphate of formoterol</td>
</tr>
<tr>
<td>S-K⁺</td>
<td>Serum concentration of the potassium ion</td>
</tr>
<tr>
<td>LC</td>
<td>Liquid chromatography</td>
</tr>
<tr>
<td>LOQ</td>
<td>Limit of quantification</td>
</tr>
<tr>
<td>Met1</td>
<td>O-demethylated formoterol</td>
</tr>
<tr>
<td>Met1G1</td>
<td>Phenol glucuronide 1 of O-demethylated formoterol</td>
</tr>
<tr>
<td>Met1G2</td>
<td>Phenol glucuronide 2 of O-demethylated formoterol</td>
</tr>
<tr>
<td>Met2S</td>
<td>Sulphate of deformylated formoterol</td>
</tr>
<tr>
<td>MS</td>
<td>Mass spectrometry</td>
</tr>
<tr>
<td>Q-Tc</td>
<td>Q-T interval corrected for heart rate</td>
</tr>
<tr>
<td>(R;R)/(S;S)</td>
<td>Ratio of formoterol enantiomers in urine</td>
</tr>
<tr>
<td>T₁/₂</td>
<td>Terminal half-life</td>
</tr>
<tr>
<td>Total</td>
<td>Total radioactivity: the sum of formoterol plus metabolites and tritiated water excreted in urine and faeces</td>
</tr>
<tr>
<td>XTOT</td>
<td>Quantified unidentified metabolites of formoterol</td>
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INTRODUCTION

Pharmacologically mediated bronchodilatation is an important element in the treatment of asthma. The effect is primarily obtained with $\beta_2$-adrenoceptor stimulants ("$\beta_2$-agonists" in the following), normally administered by inhalation, but also by oral, subcutaneous, or intravenous routes. Inhaled short-acting $\beta_2$-agonists are used primarily as needed. Long-acting $\beta_2$-agonists are an alternative to increasing the maintenance dose of inhaled steroids (1). Three long-acting $\beta_2$-agonists are available on the market: the terbutaline prodrug bambuterol for oral administration, formoterol and salmeterol for inhalation. The dosage forms of all three compounds comprise racemic mixtures of one active and one inactive enantiomer.

Bambuterol is an amphiphilic bisdimethylcarbamate of the $\beta_2$-agonist terbutaline. Duration of action is prolonged because a therapeutically relevant plasma concentration of terbutaline, the active metabolite, is sustained. Formoterol and salmeterol are lipophilic drugs with high affinity for airway tissues. Maintained high lung concentrations of these drugs are probably important for their prolonged bronchodilation after inhalation (2). The clinical-pharmacokinetic aspects of prolonged effect duration are addressed in this thesis. Insights into the benefits conferred by different routes of administration are discussed on the basis of clinical studies of bambuterol and formoterol.

Enantiomeric mixtures were normally employed to monitor the kinetic fate of bambuterol and formoterol in plasma, urine, or faeces. Pharmacodynamic variables were used as markers of local and systemic effects of formoterol. Plasma concentration of the enantiomeric mixture of terbutaline was used as a marker of the effect potential for bambuterol. This was considered acceptable since the ratio between plasma concentrations of active and inactive enantiomers of terbutaline generated from bambuterol varies little over time, particularly during repeated oral treatment with bambuterol (AstraZeneca, data on file).

BASIC PHARMACOLOGY OF $\beta_2$-AGONISTS

Structure, activity, and selectivity

Noradrenaline and adrenaline are the two important endogenous compounds mediating $\beta$-adrenoceptor stimulation. $\beta_1$-adrenoceptors are stimulated primarily by noradrenaline released from nerve endings, $\beta_2$-adrenoceptors in blood vessels, the airways, and skeletal muscles by circulating adrenaline. Adrenaline, the unselective prototype (stimulates both $\alpha$- and $\beta$-adrenoceptors) of all $\beta$-adrenoceptor stimulating compounds, is rapidly metabolized by catechol-O-methyl transferase (COMT) and monoamine oxidase (MAO) and therefore has a short duration of action.

Synthesized analogues of adrenaline have proven useful in the pharmacological treatment of asthma (Figure 1).
The common objective in the development of such drugs was to achieve high $\beta_2$-adrenoceptor selectivity, metabolic stability, and long duration of action. Retrospectively, two strategic trends are discernable, i.e., amine and phenyl substitution of adrenaline. The former reduced inactivation by MAO and the latter inactivation by COMT. Importantly, for the intended use in treatment of asthma, both these manipulations of adrenaline increased $\beta_2$-adrenoceptor selectivity. The isopropyl derivative of adrenaline, isoprenaline (isoproterenol), was shown to be $\beta_2$-adrenoceptor-selective, and the tertiary butyl derivative, colterol, was shown to be $\beta_2$-adrenoceptor-selective (3).

Substituting the catechol structure of colterol with resorcinol gives the still more $\beta_2$-adrenoceptor-selective agonist terbutaline (4), which is not a substrate for MAO or COMT. Substituting the catechol structure of colterol with saligenin results in salbutamol, which also has increased $\beta_2$-adrenoceptor selectivity (5), and which, like terbutaline, is not a substrate for either MAO or COMT. Because of their relatively high metabolic stability, terbutaline and salbutamol can be systemically administered and

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Figure 1. Structural formulæ of $\beta_2$-adrenoreceptor stimulators mentioned in the text. Adrenaline, the endogenous prototype of all $\beta_2$-agonists, has low selectivity and short duration of action; Amine substituted adrenaline with slightly prolonged effect duration and increased selectivity: isoprenaline, the isopropyl derivative, giving increased $\beta$-adrenergic selectivity, and colterol, the tertiary butyl derivative, giving increased $\beta_2$-adrenergic selectivity; Substituted colterol giving further prolonged effect duration and selectivity: terbutaline, the resorcinol derivative, and salbutamol, the 3-methylhydroxy derivative; $\beta_2$-adrenergically inert prodrugs of terbutaline: ibuterol, the diisobutyryl ester, is rapidly bioactivated and bambuterol, the bisdimethylcarbamate, is slowly bioactivated; Formoterol, a structure analogue of the short-acting $\beta_2$-agonist fenoterol, is long-acting per se after inhalation; Salmeterol, a structure analogue of the short-acting $\beta_2$-agonist salbutamol, is also long-acting per se after inhalation. Asterisks indicate chiral centres.
their duration of action is prolonged compared with the solely amine-substituted analogues of adrenaline. Prodrugs aimed at sustaining the effect of terbutaline and formoterol, which not needs chemical modification to achieve long duration, are discussed below.

**Pulmonary pharmacology**

β₂-agonists are functional antagonists. Stimulation of β₂-adrenoceptors, located in the cell membrane of smooth muscles in the airways, decreases tone, irrespective of the cause of the constriction, i.e., the calibre of constricted airways is increased. Forced expiratory volume during the first second (FEV₁) is used as a marker of bronchodilating effect throughout this presentation. The persisting bronchodilatating effect of β₂-agonists under regular treatment was observed at an early stage {e.g. (6)}. A low inclination for development of tolerance may be explained by a large number of “spare receptors” or a high level of gene expression for the β₂-adrenoceptor (7).

Parasympathetic stimulation of the airways via cholinergic nerves increases the tone of smooth muscles and consequently reduces airway calibre. Furthermore, constrictive reactions in the airways can be triggered, e.g. by allergens, cold air, and exercise. The inhaled dose of a bronchoconstrictor giving a certain response, e.g. the dose of methacholine giving a decrease in FEV₁ of 20 %, is normally used as a measure of such reactivity.

Increased mucociliary clearance and inhibition of vascular leakage and mediator release from inflammatory cells are positive effects of β₂-agonists but their clinical role is unclear (7).

**Extrapulmonary pharmacology**

Skeletal muscle tremor, tachycardia and palpitations, increased systolic and decreased diastolic blood pressure are well known clinical side-effects of β₂-agonists.

The tremorogenic action of β₂-agonists is probably mediated via local β₂-adrenoceptors on the plasma membrane of skeletal muscle cells (8).

The cardiovascular effects of adrenoceptor agonists are mediated via α- and β-adrenoceptors. Adrenaline has a positive inotropic and chronotropic effect, mediated primarily via β₁-adrenoceptors. Cardiac β₂-adrenoceptors are less frequent, but selective β₂-agonists still have direct positive chrono- and inotropic effects. The clinical consequences are tachycardia/palpitations and increased systolic blood pressure. Diastolic blood pressure is decreased as a consequence of adrenergically mediated peripheral vasodilatation.
Metabolic effects

Reversible cellular uptake of potassium (9) and increased glycogenolysis in the liver (10) are typically seen metabolic effects of β₂-agonists. β₂-agonists are calorigenic and so increase oxygen consumption (11). Because the supply of oxygen to the muscles and capacity for glycogenesis are limited (10), plasma lactate may increase secondarily to the increase in plasma glucose. These side-effects of β₂-agonists, e.g. serum potassium suppression {severe hypokalaemia may prolong the Q-T interval (12) and be arrhythmogenic (13)}, are usually reduced during repeated treatment, because extrapulmonary β₂-adrenoceptors are down-regulated (14, 15).

The relation between pharmacokinetics and pharmacodynamics

Several pharmacodynamic predictions based on pharmacokinetics imply a direct cause-effect relationship between drug concentration and effect. This means that the effect lags negligibly behind the measured change in drug concentration. If there is a lag between concentration and effect, a pharmacokinetic-pharmacodynamic (PK/PD) model must be used that adequately takes the lag into account.

The systemically mediated bronchodilatating response to terbutaline in the average asthmatic adult has been found to be linearly correlated to systemic concentrations of 7-27 nmol/L {1.6-6 ng/mL (16-18)} and a similar relation was observed in children (19). Thus, well-treated asthmatic patients, adults and children relying on systemically administered terbutaline should usually have drug concentrations between 5 and 30 nmol/L. A concentration below this range is likely to be therapeutically inadequate, and appreciable side-effects are frequent at concentrations above the range.

There is also a cause-effect relationship between plasma concentration and effects of locally administered drugs. However, as topical effects may be elicited before any appreciable amount of drug has been absorbed into the systemic circulation, local concentrations would in this case be more relevant for prediction of therapeutic effect. Pharmacokinetic aspects of pulmonary effect of systemically administered and inhaled drugs are illustrated in Figure 2.

Pharmacokinetic qualities of long-acting β₂-agonists

Any approach to achieving long duration of effect aims at a sustained effective concentration at the site of action. The perfect drug would be 100 % systemically bioavailable via the oral route and have high efficacy with respect to the desired effect only. Because duration of action is normally dose-dependent (20), sustained effect of a drug with such selectivity could be obtained simply by having a high enough dose (21).
β₂-agonists are highly selective for β₂-adrenoceptors but their selectivity cannot be exploited for lung targeting via the systemic circulation, since typical side-effects such as tremor and palpitations are mediated via receptors outside the lungs. Thus, insufficient duration of action of systemically-administered β₂-agonists cannot be compensated for by increasing the dose, because high systemic peak concentrations make side-effects unacceptable. Any pharmacokinetic method employed to sustain the bronchodilating effect should prevent the systemic drug concentration from reaching high levels. For oral administration, this can potentially be achieved by using sustained-release formulations or prodrugs.

The rationale for using inhaled β₂-agonists is improvement of bronchodilating dose potency and a more rapid onset of effect compared with administration by the oral route. A consequence of improved topical potency after inhalation is an improved selectivity for the desired effect, which means that a relatively higher dose can be administered to the site of action by inhalation than by oral administration. Thus, the duration of action after systemic administration can in principle be prolonged by topical administration to the lungs. The prerequisite of this approach, however, is that the β₂-agonist has a high affinity for lung tissues (or is slowly released and absorbed from the airway lumen), so that the drug will be retained in the lung after inhalation. Otherwise, the effect will rapidly disappear as the concentration difference between airways and the systemic circulation decreases with time towards the ratio obtained after oral therapy.

One of the two inhaled long-acting β₂-agonists, salmeterol, is the end product of deliberate development of a β₂-agonist with longer duration of action after inhalation than salbutamol. Lipophilic analogues of salbutamol were designed, which would more readily interact hydrophobically with the β₂-adrenoceptor and thereby prolong duration of action. The concept worked although it became clear that increased receptor affinity was not the only explanation for the sustained effect of salmeterol (22). The sustained action of inhaled formoterol, originally developed for oral administration (23), was discovered by chance (24).

Figure 2. Systemic concentration of a β₂-agonist is an important determinant for bronchodilatating effect after oral administration whereas local concentration in the lungs would be more relevant after inhalation
Prolonged systemically-mediated effect of the short-acting $\beta_2$-agonist terbutaline using prodrugs

A regular three-times-daily dosing regimen of conventional terbutaline sulphate ("terbutaline" in the following) tablets 5 mg is needed to give 24-hour symptom relief, indicating that concentrations at sites of action soon become subtherapeutic despite a lengthy terminal half-life of 17 h (25). Terbutaline’s duration of bronchodilation cannot be prolonged by a change to inhalation, probably because retention in the airways is poor, implying that the concentration in the lungs rapidly reaches a subtherapeutic level.

The original rationale for developing a systemically administered prodrug of terbutaline was to increase oral bioavailability. Initial attempts were made with acyl-substituted diesters of the parent drug. The long-chain carboxylic acids made the prodrugs more lipophilic, and the idea was that this would facilitate gastrointestinal absorption compared with terbutaline. Additionally, presystemic metabolic protection against sulphate conjugation was expected, leading to increased oral bioavailability of the parent drug (26). The diesters of terbutaline are bioactivated by pseudocholinesterase (EC 3.1.1.8) and unspecific esterases. In-vitro data suggested that the bulkier the acyl substituent the slower the bioactivation; it might therefore be possible to use a diester as an inner systemically circulating depot of terbutaline (27).

The concept above was tested with ibuterol, the diisobutyryl ester of terbutaline (Figure 1). Rate of absorption and extent of bioavailability were indeed improved with ibuterol, but a drawback was the shorter duration of action compared with terbutaline (28).

Bambuterol is the bisdimethylcarbamate prodrug of terbutaline. The adrenergically inactive bambuterol (29) has in itself a high affinity for lung tissue, but to become effective it must be administered systemically, preferably by the oral route. The reason is that biotransformation of bambuterol to terbutaline predominantly takes place outside the lungs via hydrolysis and oxidation, mediated by pseudocholinesterase and, most likely, by cytochrome P450-dependent oxidases, respectively (30-32). Intact bambuterol, the monocarbamate of bambuterol, and hydroxylated derivatives of bambuterol and the monocarbamate have been found in man after bambuterol administration (AstraZeneca, data on file). The monocarbamate is generated via both the oxidative and hydrolytic pathways, whereas the hydroxylated metabolites are generated only during oxidative degradation of bambuterol (Figure 3). Demethylated bambuterol and demethylated monocarbamate, formed from the hydroxylated compounds, are unstable and are rapidly hydrolyzed to the monocarbamate or terbutaline. Bambuterol, in contrast to ibuterol, inhibits its own hydrolysis, making it a potential inner depot from which terbutaline is slowly generated (33).
The basal pharmacokinetics of bambuterol and generated terbutaline have been evaluated in healthy subjects, by use of data after intravenous administration of bambuterol hydrochloride (“bambuterol” in the following) 30 µg per kg body weight (on a molar basis equivalent to about 20 µg of terbutaline) and terbutaline 7 µg/kg, and after single and once-daily repeated oral dosing of bambuterol 0.270 mg/kg (34). The intravenous doses were given in two equal parts separated by one hour. Mean clearance of bambuterol, 1.25 L/min, was five times higher than the clearance of terbutaline, 0.23 L/min, but mean volume of distribution, 1.6 L/kg, was similar. The AUC for terbutaline was virtually the same after intravenous bambuterol and terbutaline, giving a mean bioavailability of terbutaline of 36% after intravenous bambuterol administration.

The mean terminal half-life of bambuterol was 2.6 h after intravenous but 12 h after oral administration, implying rate-limiting gastrointestinal absorption.

The plasma concentration of intravenously infused terbutaline rapidly decreased from a very high level post-dosing to a low level during the terminal phase of elimination. The concentration of terbutaline slowly reached a lower peak after intravenous bambuterol, then decreased monophasically at a higher level than after the intravenous
administration of terbutaline as such. This obvious benefit was fortified after oral administration of bambuterol (Figure 4).

![Figure 4. Single-dose plasma concentration vs. time of terbutaline (open squares after intravenous terbutaline; open circles after intravenous bambuterol; open diamonds after oral bambuterol) (adapted from Nyberg et al (34)). Dotted horizontal lines indicate the alleged target range for effective concentrations of terbutaline (cf. text).](image)

Mean bioavailability of generated terbutaline was found to be 10% after oral bambuterol. Mean absorption of bambuterol after oral administration is about 20% (AstraZeneca data on file). Thus, approximately half the absorbed dose is ultimately transformed into active agonist, implying that first-pass metabolism makes a considerable contribution to the bioavailability of terbutaline after oral administration of bambuterol.

The mean plasma concentration peak of terbutaline during the once-daily oral regimen was 15 nmol/L and the minimum 8 nmol/L, i.e., within the therapeutic target range for systemically-mediated bronchodilation, as discussed above. Similar minimum concentrations were found in adult asthmatic patients given the same dosing regimen (35, 36).

The pharmacokinetic properties of bambuterol make it suitable for oral once-daily dosage (37), maintaining bronchodilatation around the clock (36, 38-40). The clinical efficacy of bambuterol 10 mg once daily was approximately equieffective with that of a controlled-release preparation of terbutaline 5 mg twice daily (41), whereas bambuterol 20 mg once daily was approximately equieffective with plain terbutaline tablets 5 mg three times daily (42). The side-effects with once-daily regimens of bambuterol 10-20 mg appear to be less pronounced than with equi- or less effective oral reference treatments with terbutaline and salbutamol (41-44). Regular oral once-daily regimens of bambuterol compete favourably with regular twice-daily inhalation of salmeterol (45, 46).
The recommended treatment of asthmatic adults is regular once-daily administration of bambuterol tablets or solution 10 or 20 mg. The bronchodilating effect of bambuterol was little improved by increasing the dose to more than 20 mg/day (≈0.27 mg/kg/day), but side-effects increased (35). Dosing in the evening is preferable in patients with nocturnal asthma, in whom mean FEV₁ in the early morning was improved by as much as 29 % over placebo during repeated treatment with bambuterol tablets 20 mg (39).

Previous studies of bambuterol had given valuable information for proper dosing in adults, but there was still lack of knowledge. This dissertation has focused on gaps regarding possible mechanisms of bioactivation, dose linearity, and dosage in children.

Pharmacokinetic basis of prolonged topically-mediated effect

The physico-chemical characteristics may be important determinants of onset and duration of effect for β₂-agonists. Some possible microkinetic implications of the physico-chemical differences between short- and long-acting β₂-agonists are given in Figure 5 (2). Hydrophilic β₂-agonists such as terbutaline, salbutamol, and fenoterol, are distributed in the extracellular aqueous biophase of the whole body – within as well as outside the airways. Probably, the extracellular concentration of these drugs therefore rapidly attains high levels at the site of action in the airways after inhalation but then rapidly decline. Consequently, onset of action is rapid but duration of effect short.

Figure 5. Physico-chemical characteristics may explain why onset and duration of effect vary between different β₂-agonists. The hydrophilic salbutamol, which rapidly diffuses to the receptor and is distributed in the body via the aqueous biophase, has a rapid onset but short duration of effect. The lipophilic salmeterol, which is primarily distributed from the aqueous to a lipid biophase, such as the lipid bilayer of the cell membrane, has a slow onset but long duration of action. Formoterol, which has intermediary lipophilicity, would after inhalation be expected to have pharmacodynamic properties somewhere in between those of salbutamol and salmeterol. Small arrows indicate distribution equilibrium of drug between aqueous and lipid biophases, and large black arrows indicate major movement and interactions. The supposed fates of β₂-agonists near the receptor are adapted from Anderson et al. (2).
A lipophilic $\beta_2$-agonist such as salmeterol, however, is primarily distributed from the aqueous to a more lipid biophase, such as the lipid bilayer of the cell membrane, where it can be retained. Probably, a pool of salmeterol is slowly redistributed from the lipid biophase to the receptor either via the extracellular fluid or via the lipid bilayer. Thus, inhaled salmeterol would attain a clinically relevant concentration at the site of action in the airways slowly, but the effective concentration would then be maintained for a longer period of time than for an inhaled hydrophilic (short-acting) analogue. This could explain both the slow onset and long duration of action of inhaled salmeterol. It has also been suggested that the sustained action of salmeterol could depend on specific hydrophobic interactions with molecular regions near the actual $\beta_2$-adrenoceptor (47). Formoterol, which has intermediary lipophilicity, would after inhalation be expected to have pharmacodynamic properties somewhere in between those of salbutamol and salmeterol. Löfdahl et al. (24) first showed, in line with this hypothesis, that inhaled formoterol in fact has both a rapid onset and long duration of effect (Figure 6).

The bronchodilating effect of formoterol (formoterol as dose $=$ formoterol fumarate dihydrate) gradually increased within the dose range 4.5–18 $\mu$g after inhalation via Turbuhaler$^\circledR$ (48). Doses of formoterol up to 18 $\mu$g, delivered via Turbuhaler, are well tolerated, and 36 $\mu$g is considered clinically safe although serum potassium is occasionally low and the incidence of tremor is higher than with lower doses (Data on file, AstraZeneca R&D). The recommended dose of inhaled formoterol for regular treatment of asthmatic adults is therefore 4.5–18 $\mu$g once or twice daily via Turbuhaler. It can be mentioned as comparison, that formoterol Turbuhaler 9 $\mu$g is approximately equieffective with salmeterol xinafoate Diskhaler$^\circledR$ 50 $\mu$g both with respect to its desired bronchodilating effect and to its systemic side-effects (48, 49).
Basic pharmacokinetics of inhaled formoterol had been investigated before, but little was known about degradation of formoterol and the fate of degradation products, particularly in man after inhalation. Thus, in this dissertation, mass balance and metabolism have been studied after a dosing regimen specifically designed to mimic inhalation. Furthermore, although previous studies had shown the long-acting bronchodilating potential of inhaled formoterol, less was known about the relation between desirable and undesirable effects. Therefore, it was judged to be a safety interest to further study, in comparison with a standard short-acting alternative, the systemic effects of formoterol and the relation of side-effects to bronchodilation.

PRESENT INVESTIGATION

Objectives

1. To test a tentative pharmacokinetic model for bambuterol by use of previous data.
2. To investigate the basic pharmacokinetics of long-acting \( \beta_2 \)-agonists, and particularly regarding:
   - The proportionality between plasma exposure and dose during treatment with bambuterol in adults and children
   - The metabolism of formoterol in man
3. To investigate if an effective \( \beta_2 \)-agonist concentration can be maintained by means of systemic once-daily administration of bambuterol to different populations.
4. To assess the systemic effects of inhaled, therapeutically relevant formoterol doses, particularly regarding the duration of side-effects and the relation of side-effects to bronchodilation in comparison with a short-acting analogue.

Methods

Pharmacokinetics of bambuterol

Fitting a pharmacokinetic model to previous data on bambuterol

A pharmacokinetic model was fitted to plasma concentrations of enantiomeric mixtures of bambuterol and generated terbutaline to further explore previous data presented by Nyberg et al. (34). The model took into account the following features revealed in the non-parametric analysis: 1) the absorption of bambuterol is multi-phasic; 2) terbutaline is slowly generated from systemically circulating bambuterol via a pool of intermediate metabolites; and 3) intestinal absorption of bambuterol is slow and further levels out the plasma concentration profile of generated terbutaline. Figure 7 shows the integrated model that was fitted to plasma concentration data after intravenous and oral
administration of bambuterol and after an intravenous reference dose of terbutaline (cf. the section “Prolonged systemically mediated effect of the short-acting \( \beta_2 \)-agonist terbutaline using prodrugs”).

**Study designs**

Pharmacokinetic aspects of dosing the prodrug bambuterol were investigated in healthy adult subjects regarding time to steady state, dose proportionality, intra-individual variability, and bioequivalence between tablets and solution (Paper I), and in asthmatic children regarding dose proportionality, dosing frequency, and ethnic differences (Paper II). The recommended maintenance oral doses of bambuterol in Caucasian adults, 10 mg or 20 mg once daily, plus a supratherapeutic dose of 30 mg were investigated in the healthy subjects. The recommended doses of 10 mg or 20 mg constituted starting points also for the pharmacokinetic survey of bambuterol in children. Based on the initial results obtained in school children, regimens of reduced doses were investigated in pre-school children. Enantiomeric mixtures of bambuterol and terbutaline were used to monitor the kinetic fate in plasma and urine. The plasma concentration of enantiomeric mixture of terbutaline was used as a marker of the effect potential of bambuterol.

**Paper I:** Twenty-six healthy Caucasian subjects were included and 23 (12 women) completed an open, randomized, crossover study. Bambuterol tablets, 10, 20, and 10+20 mg, and a solution, 20 mg (dose given as bambuterol hydrochloride), were given.
orally once daily for 2 weeks at about 7 p.m. Plasma concentrations and urinary recoveries of bambuterol and terbutaline were measured after single doses and during repeated treatments.

**Paper II:** Forty-eight asthmatic children in four different studies each completed two double-blind bambuterol 1- or 2-week treatments (daily doses of bambuterol hydrochloride): 12 pre-school (5 mg×2 vs 10 mg) and 12 school (10 mg vs 20 mg) Caucasians plus 12 pre-school (2.5 mg vs 5 mg) and 12 school Orientals (10 mg vs 20 mg). Plasma concentrations and urinary recoveries of bambuterol and terbutaline were measured during repeated treatments.

Demographic data of subjects and patients participating in the studies with bambuterol are summarized in Table 1.

**Table 1: Demographic data on subjects and patients in prospective bambuterol studies**

<table>
<thead>
<tr>
<th>Paper</th>
<th>Ethnic Origin</th>
<th>Group</th>
<th>Number of Subjects (male/female)</th>
<th>Bambuterol Hydrochloride (mg/day)</th>
<th>Demographics</th>
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<td>9/3</td>
<td>10, 20</td>
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<td>School Asthmatics</td>
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1 Two men who discontinued after one treatment were replaced. One man discontinued after two treatments and was not replaced
2 Pre-school children were given bambuterol solution
3 Administered twice daily
4 School children were given bambuterol tablets

**Bioanalysis**

Bambuterol was analysed using non-chiral gas chromatography plus mass spectrometry (50). At the limits of quantification (LOQ), 1 nmol/L in plasma and 8 nmol/L in urine, the within-day coefficient of variation (CV) was 4% and 5%, respectively.

Terbutaline was analysed by use of non-chiral coupled-column high performance liquid chromatography with electrochemical detection, according to a modification of a
previously described method (51). At 8 nmol/L in plasma, the CV was 2.1% within a day and 3.6% between days. The applied LOQ was 4 nmol/L. The same method was used for terbutaline in urine. The assay calibration curve was then extended up to 5000 nmol/L to allow for measurement of the higher concentrations in urine. At 80 nmol/L (the LOQ) the within-day variation was 3.7% in the urine analysis.

Calculations and primary statistical analyses

A non-linear mixed effect model was used to evaluate previous data on bambuterol parametrically. The analysis was performed using the ADVAN5 subroutine and conventional first order estimation method in the NONMEM V 1.1 package (52). The final model was selected on the basis of the objective function value and graphical analysis of predicted vs observed values and residual plots. Parameters describing elimination of bambuterol and terbutaline (K10, K40), systemic irreversible biotransformation from bambuterol to terbutaline (K17, K74), oral bioavailability of bambuterol (F8, F9), and the fraction of orally administered bambuterol that is presystemically transformed to terbutaline (F10) were assumed to be normally distributed random variables within the group of subjects; other parameters were not assumed to vary between subjects. Intraindividual variability in pharmacokinetic parameters was not regarded. The model was fitted to log-transformed data.

The elimination rate constants of bambuterol and terbutaline were calculated from urinary excretion data, ln (dAe/dt) regressed on midpoint of collection period. Terminal half-life (T½) is ln2 divided by the elimination rate constant. Area under the curve of plasma concentration vs time (AUC) was calculated using the trapezoidal rule. Extrapolation of AUC to infinity was done assuming monoexponential decline after the last measurable concentration according to the estimated terminal half-life. Apparent clearance after oral administration, CLA, and renal clearance, CLR, were calculated as dose and urinary recovery, respectively, divided by AUC.

Treatments were statistically compared and described using multiplicative or additive (T½) ANOVA models normally with the factors subject, period, and treatment, plus dose number in the healthy adults.

Time to steady state for terbutaline was investigated in the healthy adults by comparing AUC_{0-24h} after dose 15 and dose 8 of bambuterol tablets 20 mg and by comparing Ae_{0-24h} after dose 15 and doses 8-14 of bambuterol tablets and solution 20 mg - it was assumed that bambuterol had reached steady state after dose 8 and terbutaline after dose 15 (Paper I).

Dose proportionality was addressed by use of AUC/dose or Ae/dose (Papers I and II).

Steady-state intraindividual variability in systemic exposure was assessed indirectly from Ae_{0-24h} after doses 8-15 of bambuterol tablets and solution 20 mg, and directly from AUC_{0-24h} after dose 8 and dose 15 of bambuterol tablets 20 mg - the assumption of this analysis was that steady state was reached at dose 8 (Paper I).
Bioequivalence of generated terbutaline between tablets and solution was evaluated by use of the ratios of AUC$_{0-24h}$ and Ae$_{0-24h}$ after dose no. 15 of bambuterol tablets and solution 20 mg. The criterion for bioequivalence was a 90% confidence interval contained within 80-125% (Paper I).

**Pharmacokinetics and effects of formoterol**

**Study designs**

Pharmacokinetic aspects of formoterol dosing and systemic dose response after inhalation were studied in healthy subjects (Papers III and IV). A modelling approach for assessing a relative therapeutic index – the relation between therapeutic ratios of drugs exerting similar effects – was developed comparing inhaled formoterol with inhaled salbutamol (Paper V). A supratherapeutic single or cumulative formoterol dose of 54 µg was used to investigate basic pharmacokinetics, metabolism, tolerability, and systemic potency. Single formoterol doses covering the recommended range of 4.5-18 µg via Turbuhaler, plus the supratherapeutic dose of 54 µg were used to relate systemic to local effects. The effects of formoterol were referred to those of inhaled salbutamol in doses up to 3600 µg via a pressurized metered dose inhaler. Enantiomeric mixtures were used to monitor the kinetic fate of formoterol in plasma, urine, and faeces (Papers III and IV). Terminal elimination was followed enantioselectively in urine (Paper IV). Pharmacodynamic variables were used as markers of local and systemic effects of formoterol (Papers IV and V).

**Paper III**: Mass balance and metabolism of $^3$H-formoterol were investigated in six healthy men in an open fashion. Simultaneous oral (mean dose 88.6 nmol, 49.3 MBq) and intravenous (mean dose 38.2 nmol, 21.4 MBq) doses of tritium-labelled formoterol were administered. The combination of these two administrations aimed at simulating the fate of inhaled formoterol. Total radioactivity was monitored for 24 h in blood plasma and for at least 4 days in urine and faeces.

**Paper IV**: Relative systemic dose potency and tolerability of inhaled formoterol and salbutamol were compared in β$_2$-agonist naïve and accustomed subjects and patients, respectively, and pharmacokinetic enantioselectivity was described. Twelve healthy subjects completed three open study days, and eleven asthmatic patients completed four double-blind study days in randomized, placebo-controlled, and crossover fashion. The healthy subjects inhaled 13.5+13.5+27 µg of formoterol fumarate dihydrate (“formoterol”, Oxis®) via Turbuhaler® and 300+300+600 µg of salbutamol (Ventolin®) via a pressurised metered dose inhaler (pMDI) on two separate days. The asthmatics, who were on formoterol 9 µg twice daily via Turbuhaler during the study, inhaled the same single doses of formoterol or salbutamol as the healthy subjects on two separate days, and on an additional day 900+900+1800 µg of salbutamol via pMDI. Doses were given cumulatively 30 min apart. Placebo was a day of no treatment in the healthy subjects and double dummies were used for the asthmatics. Cardiovascular and
metabolic effects were evaluated. Plasma concentrations and urinary excretion of formoterol were monitored in the healthy subjects.

**Paper V:** The bronchodilating and serum-potassium-suppressing effects of inhaled formoterol and salbutamol were measured in twenty-eight stable asthmatic patients who completed a double-blind, randomized crossover study. Baseline FEV\(_1\) (mean 2.08 L) was 49–93\% of predicted and reversibility was 16–82\% after inhalation of 200-400 µg salbutamol. Patients inhaled three single doses of formoterol (Oxis, delivered doses of 4.5, 18, and 54 µg) via Turbuhaler, two single doses of salbutamol (200 and 1800 µg) via a pressurised metered dose inhaler (pMDI), and placebo at intervals of 48 h or more. Maximum local and systemic effects were assessed. A classic sigmoid model of log-dose response was used to discriminate pharmacologically between formoterol and salbutamol. Relative local and systemic dose potencies and their ratio - the relative therapeutic index - were estimated simultaneously using a non-linear mixed effect modelling approach.

Demographic data of subjects and patients participating in the studies of formoterol are summarized in Table 2.

Table 2: Subjects and patients in formoterol studies

<table>
<thead>
<tr>
<th>Paper</th>
<th>Topic</th>
<th>Number of Subjects</th>
<th>Administration</th>
<th>Formoterol Fumarate2 H(_2)O (µg)</th>
<th>Comparator</th>
<th>Demographics</th>
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</thead>
<tbody>
<tr>
<td>III</td>
<td>Pharmacokinetics, Mass balance</td>
<td>6/0</td>
<td>I.V. + Oral</td>
<td>16.2 ± 37.8</td>
<td>-</td>
<td>Age (years)</td>
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<tr>
<td>IV</td>
<td>Tolerability, Pharmacokinetics</td>
<td>6/6</td>
<td>Inhalation</td>
<td>13.5+13.5+27</td>
<td>Salbutamol</td>
<td>Age (years)</td>
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<td>Weight (kg)</td>
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<tr>
<td>IV(^1)</td>
<td>Tolerability</td>
<td>5/6</td>
<td>Inhalation</td>
<td>13.5+13.5+27</td>
<td>Salbutamol</td>
<td>Age (years)</td>
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<td>Weight (kg)</td>
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<tr>
<td>V</td>
<td>Therapeutic Ratio</td>
<td>14/14</td>
<td>Inhalation</td>
<td>4.5, 18, 54</td>
<td>Salbutamol</td>
<td>Age (years)</td>
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<td>Weight (kg)</td>
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</table>

\(^1\)Single dosing on top of regular inhalation of formoterol 9 µg twice daily via Turbuhaler

**Effect measurements and bioanalysis**

Pulse rate and blood pressure were measured after a 5-minute rest with subjects or patients in the sitting position using standard techniques. The electrocardiograph automatically generated Q-Tc, i.e., Q-T interval corrected for heart rate. Reported adverse events and vital sign measurements were used to evaluate clinical tolerability (Papers IV and V).
Clinical laboratory tests were performed in plasma or serum routinely prepared from venous blood samples. An ion-selective electrode was used to measure serum potassium (Papers IV and V). Reagent kits were used to measure plasma glucose and plasma lactate (Paper IV). The maximum coefficient of variation in analyses of quality control samples was 1.0% for serum potassium, 5.1% for plasma glucose, and 6.9% for plasma lactate. Pulse (bpm, beats per minute) was measured via the radial artery. Forced expiratory volume in the first second (FEV₁) was measured with the patient sitting in an upright position, using a Vitalograph Alpha spirometer (Vitalograph Ltd., U.K.) according to well established standards defined by the American Thoracic Society (53).

³H-formoterol and metabolites were determined using LC techniques plus radiodetection, directly after centrifugation in urine and after sample work-up in blood plasma and faeces. Metabolites were identified in urine, sampled from two subjects, using LC-ESIMS/MS techniques.

Inhaled formoterol was determined in urine and plasma by use of non-chiral coupled-column liquid chromatography with electrochemical detection (Paper IV). The limit of quantification was 0.50 nmol/L in urine and 0.05 nmol/L in plasma. The between-day variation in authentic samples was 4.8% at 5.0 nmol/L of formoterol in urine and 5.4% at 0.18 nmol/L of formoterol in plasma. The enantiomeric ratio of formoterol was determined after chiral separation on an α₁-acid glycoprotein column with electrochemical detection. The range for determination of enantiomeric ratio was 0.50 - 2.0 and the lower limit for determination of the ratio was 1.0 nmol/L of the enantiomeric mixture of formoterol. The between-day variation in authentic samples was 3.5% at an enantiomeric ratio of 0.60 at 37 nmol/L of total formoterol.

Calculations and primary statistical analyses

The elimination rate constant of formoterol was calculated from urinary excretion data, ln (dAe/dt) regressed on midpoint of collection period. Terminal half-life (T½) is ln2 divided by the elimination rate constant. Area under the curve of plasma concentration vs time (AUC) was calculated using the trapezoidal rule.

Treatments were statistically compared with respect to maximum effects (Paper V) or measurements after cumulative dose increments (Paper IV) using ANOVA models with factors subject or patient, treatment, and period (Papers IV and V), plus dose number and the interaction between treatment and dose number at cumulative dosing (Paper IV). The pre-drug administration measurement (a measurement made in the morning before administration of study treatment) was used as covariate.

Formoterol and salbutamol were assumed to have similar modes of action, implying that their log-dose response curves would be parallel. The possible device-related impact on the relation between local and systemic effects was not regarded. Relative dose potencies were estimated on this principle as the horizontal shift between parallel lines fitted to adjusted means, plotted against logarithms of doses (Paper IV), or using a
bivariate non-linear mixed effect model fitted simultaneously to all individual measurements (Paper V). Fieller’s method (54) was used to calculate confidence intervals for the estimates of relative dose potency in Paper IV. The asymptotic normality of the estimates was used to calculate confidence intervals for relative dose potencies and relative therapeutic index in Paper V.

Results

Pharmacokinetic modelling of previous data on bambuterol

The applied model adequately described the fate of bambuterol after intravenous and oral administration and terbutaline after intravenous administration as such. Predicted plasma levels of terbutaline generated from bambuterol fairly well agreed with observations, although the terminal rate of decline was slightly overestimated (Figure 8). The program code used in the model analysis and goodness of fit plots are given in the Appendix.

The outcome of the model analysis is summarized in Table 3. Clearance of bambuterol, calculated to 0.96 L/min (K10 × VC), was considerably higher than the clearance of terbutaline, calculated to 0.23 L/min (K40 × VC). The total rate of elimination of bambuterol was modelled as K10 (elimination not generating terbutaline) plus K17 (irreversible biotransformation to terbutaline via a systemic intermediary pool).
The fraction of terbutaline systemically transformed from intravenously administered bambuterol was estimated at about 35% using the values for these parameters \( \{K_{17}/(K_{17}+K_{10})\} \). Furthermore, oral bioavailability was calculated to about 10% for bambuterol \( (F_8 + F_9) \) and 9.5% for terbutaline \( \{F_{10} + (F_{8} + F_{9}) \times K_{17}/(K_{17}+K_{10})\} \). None of these values contradicted the outcome of the previous non-parametric analysis (34). The slowly absorbed fraction of bambuterol \( (F_9) \) was estimated to be about twice the more rapidly absorbed one \( (F_8) \).

Table 3: Pharmacokinetic model analysis of literature data (34).

<table>
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<th>Variable</th>
<th>Parameter</th>
<th>Mean</th>
<th>SEM</th>
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<td>Bambuterol</td>
<td>( K_{10} ), h(^{-1} )</td>
<td>5.07</td>
<td>0.396</td>
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<tr>
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<td>( K_{12} ), h(^{-1} )</td>
<td>5.05</td>
<td>0.744</td>
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<td>( K_{21} ), h(^{-1} )</td>
<td>1.64</td>
<td>0.112</td>
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<td>( K_{13} ), h(^{-1} )</td>
<td>2.92</td>
<td>0.560</td>
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<td>( K_{31} ), h(^{-1} )</td>
<td>0.352</td>
<td>0.0344</td>
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<tr>
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<td>( K_{17} ), h(^{-1} )</td>
<td>2.78</td>
<td>0.209</td>
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<tr>
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<td>( K_{81} ), h(^{-1} )</td>
<td>0.474</td>
<td>0.110</td>
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<tr>
<td></td>
<td>( F_8 )</td>
<td>0.0386</td>
<td>0.00898</td>
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<tr>
<td></td>
<td>( F_9 )</td>
<td>0.0658</td>
<td>0.00900</td>
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<tr>
<td>Terbutaline</td>
<td>( K_{40} ), h(^{-1} )</td>
<td>1.22</td>
<td>0.100</td>
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<td>( K_{45} ), h(^{-1} )</td>
<td>0.882</td>
<td>0.114</td>
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<td>( K_{54} ), h(^{-1} )</td>
<td>0.0924</td>
<td>0.0111</td>
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<td>( K_{46} ), h(^{-1} )</td>
<td>2.39</td>
<td>0.327</td>
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<td>( K_{64} ), h(^{-1} )</td>
<td>1.51</td>
<td>0.228</td>
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<td></td>
<td>( K_{74} ), h(^{-1} )</td>
<td>0.311</td>
<td>0.0451</td>
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<tr>
<td></td>
<td>( F_{10} )</td>
<td>0.0578</td>
<td>0.00408</td>
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<tr>
<td>Bambuterol/Terbutaline</td>
<td>( \sum_{i=1,\ldots,4} K_i )</td>
<td>11.4</td>
<td>0.646</td>
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<tr>
<td></td>
<td>( K_{91}, K_{104}, h^{-1} )</td>
<td>0.0477</td>
<td>0.00583</td>
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</table>

Interindividual variability was considered for underlined parameters

Pharmacokinetics of bambuterol tablets and solution (Papers I and II)

Mean plasma concentrations of bambuterol and its active metabolite terbutaline after administration of bambuterol tablets, 10 and 30 mg to healthy adults, are shown in Figure 9. The initial absorption rate could not be assessed directly, but it was faster than during a second phase where absorption was rate-limiting for elimination, resulting in a mean terminal half-life of 16 h. The mean terminal half-life of terbutaline was 22 h and steady-state was reached within one week of bambuterol treatment.

Steady-state plasma exposure \( (AUC_{0-24h}) \) of bambuterol was not linearly correlated to dose, but the overall pharmacokinetics of terbutaline indicated dose linearity. The mean minimum plasma concentration of terbutaline was 5–17 nmol/L within the studied range of bambuterol doses, and the corresponding mean maximum 12–31 nmol/L. Steady-state for terbutaline was reached after 8 days. Intraindividual variation between day 8 and day 15 in terbutaline \( AUC_{0-24h} \), 15% with the tablets, was half that of bambuterol. Urine data indicated that intraindividual variability was slightly lower with the solution.
Tablets were bioequivalent with the solution regarding terbutaline (90% confidence interval: 87-100%).

As in adults, plasma exposure of terbutaline but not of bambuterol was linearly related to the dose of bambuterol in asthmatic children. Mean plasma concentrations of generated terbutaline during repeated dosing of bambuterol are shown in Figure 10.

Twice-daily dosing ($2 \times \text{AUC}_{0-12h}$) could not be shown to differ from once-daily dosing ($\text{AUC}_{0-24h}$) in the pre-school Caucasians, mean AUC for terbutaline being 128 and 242 h×nmol/L (5 mg/12h; 10 mg/24h). With once-daily dosing, terbutaline AUC was 213 and 406 h×nmol/L in the Caucasian school children (10 mg; 20 mg), 87.4 and 202 h×nmol/L in the Oriental pre-school children (2.5 mg; 5 mg), and 356 and 640 h×nmol/L in the Oriental school children (10 mg; 20 mg). Oriental school children had higher plasma concentrations of bambuterol and terbutaline than Caucasian school children. The strictly ethnical implication of the difference could not be elucidated, because demographic data were not perfectly matched. Terbutaline AUC was only moderately increased in the Caucasian school children compared with Caucasian adults during administration of bambuterol tablets 10 and 20 mg. With a once-daily dose of 10 or 20 mg, the increase was more pronounced in some pre-school Caucasians and generally in Oriental children. The highest concentration of terbutaline, 58 nmol/L, was seen in an Oriental school child after a 20-mg dose. The range of mean plasma concentrations for the applied regimens was otherwise fairly well within the previously discussed range of 5–30 nmol/L (cf. section “The relation between pharmacokinetics and dynamics”), except in the pre-school Orientals after 2.5 mg, where very low concentrations were found.
Clearance of both bambuterol and terbutaline was related to body weight. Individual values in adults and children are plotted in Figure 11. Note that the relation is blurred for bambuterol because of the non-linear pharmacokinetics. Body weight correction levels out much of the clearance difference for bambuterol between children and adults, whereas such an adjustment was not an effective means to scale clearance of terbutaline (Figure 12); clearance of terbutaline corrected for body weight was higher in the children, particularly in those weighing less than 40 kg (Table 4). Dosing according to body weight could therefore lead to undertreatment of the children.

Table 4. Normalized apparent (CLA/weight) and renal (CLR/weight) clearance of bambuterol and generated terbutaline during oral once-daily administration of bambuterol

<table>
<thead>
<tr>
<th>Variable</th>
<th>Ethnic origin</th>
<th>Age group</th>
<th>Dose (mg)</th>
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<th>CLA/Weight (mL/min/kg)</th>
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Mass balance and metabolism of $^3$H-formoterol in healthy men (Paper III)

The plasma peak of $^3$H-formoterol after intravenous (16.2 µg) and oral (37.8 µg) co-administration was attained within minutes, after which the concentration rapidly decreased in a poly-exponential manner. The metabolite peaks were lower and appeared later than the peak of formoterol (Figure 13).

![Figure 13. Mean plasma concentration time curves of formoterol and its metabolites when inhalation of formoterol was mimicked. Linear (left) and logarithmic (right) y-axis scale. The horizontal line in the right graph indicates limit of quantification.](image)

Mean total recovery was 86% of the administered formoterol dose, 62% in urine and 24% in faeces. Tritiated water was generated and because its *in vivo* turnover is slow, the terminal decline of total radioactivity was slow and dose recovery was incomplete during the sampling period. Formoterol was conjugated to inactive glucuronides and a previously unidentified sulphate. The phenol glucuronide of formoterol (FG1) was the main metabolite in urine (Figure 14).

Formoterol was also O-demethylated (Met1) and deformylated (Met2). Plasma exposure to these pharmacologically active metabolites was low. O-demethylated formoterol was seen mainly as inactive glucuronide conjugates and deformylated formoterol only as an inactive sulphate conjugate. Intact formoterol and O-demethylated formoterol dominated the recovery in faeces. Mean recovery of unidentified metabolites was 7.0% in urine and 2.0% in faeces.
Systemically, formoterol was estimated to be 28-109 times as potent as salbutamol, depending on the measured variable; potency relations with respect to serum potassium suppression and the decrease in diastolic blood pressure are illustrated in Figure 15. The duration of systemic action seemed to differ marginally at approximately equieffective doses of formoterol and salbutamol. Systemic effects were well tolerated and tended to be more pronounced in the healthy subjects than in the asthmatic patients. Half-life of the pharmacologically active (R;R)-formoterol was longer than half-life of the inactive (S;S)-formoterol, 7.8 vs 5.5 h.

Assessment of a relative therapeutic index between inhaled formoterol and salbutamol in asthma patients (Paper V)

A linear approximation was considered sufficient to describe bronchodilation whereas a sigmoid approximation was more apt to describe serum potassium suppression. A bivariate non-linear mixed effect model based on these principles was fitted simultaneously to all individual measurements of maximum FEV₁ and minimum S-K⁺ in order to estimate the relative therapeutic index between formoterol and salbutamol. The fitted model is presented graphically superimposed on mean maximum values of FEV₁ and mean minimum values of serum potassium concentration in Figure 16. The mean relative therapeutic index between formoterol given via Turbuhaler and salbutamol given via pMDI was estimated at 2.5. This ratio in favour of formoterol was not statistically significant, the 95% confidence interval for the estimate being 0.9–6.5.
Figure 15. Mean serum potassium concentration and mean diastolic blood pressure 30 min after each subdivided dose fraction vs. the cumulative dose in healthy subjects and asthmatic patients. Dose fractions of formoterol were 13.5+13.5+27 \( \mu \)g (filled circles), and dose fractions of salbutamol were 300+300+600 \( \mu \)g (open squares), or 900+900+1800 (filled squares). Dotted lines indicate the dose of salbutamol shown to be equipotent with half the cumulative dose of formoterol. The solid line, parallel to the x-axis, indicates the 95% confidence interval for the estimate of equipotency.

Figure 16. Mean maximum FEV\(_1\) and minimum serum potassium concentration together with approximations of the dose response relationships. Dashed lines indicate the dose of salbutamol that is equipotent to formoterol 9 \( \mu \)g. The solid line, parallel to the x-axis, indicates the 95% confidence interval for that estimate. Note that relative increases in maximum FEV\(_1\) compared with placebo, not differences, are plotted against dose. The reason is that log transformed data were used in the statistical analysis.
Discussion

Clinical pharmacokinetics of bambuterol

Terminal elimination of orally administered bambuterol was shown to be slow and most likely absorption-rate limited (Paper I). This flip-flop behaviour of bambuterol was less clear during the first few hours after dosing (Figure 9) when a fraction was more rapidly absorbed than it was eliminated. Indeed, the model simultaneously fitted to literature data provided some evidence that absorption of bambuterol be multi-phasic; the predicted half-life of rapid absorption, using a biphasic absorption model, was only about 1.5 h (ln2/K81, Table 3), whereas the predicted half-life of slow absorption, about 14.5 h (ln2/K91), was in close agreement with the mean estimate of terminal half-life in Paper I. The slow absorption is probably a rate-limiting factor for elimination of the major bambuterol fraction.

The mean T½ of terbutaline generated from oral bambuterol was estimated at 22 h (Paper I). The mean after oral administration of plain terbutaline tablets as such is about 17 h (25). The model analysis indicated that elimination of terbutaline generated from bambuterol was slowed down after oral dosing of the prodrug, because biotransformation is sustained systemically and further more by first-pass mechanisms. The fitted model described well the terminal elimination rate of terbutaline after intravenous administration as such, but tended to overestimate that rate after administration of bambuterol. This is likely due to overestimation of terbutaline formation rate by the model, since the elimination rate of terbutaline derived from bambuterol seems to be governed by its formation rate.

A comparison of dosing-interval AUCs showed that terbutaline was in steady state after one week during treatment with bambuterol (Paper I). T½ of terbutaline as such was similar in Caucasian children and adults (55). Therefore, it is reasonable to assume that steady state for terbutaline is reached after one week of a once-daily bambuterol regimen in asthmatic children, too.

Dose proportionality was shown for terbutaline, i.e., the plasma exposure (AUC) of terbutaline was linearly correlated to the administered dose of bambuterol, but this was not the case for bambuterol (Papers I and II). Hydrolysis of bambuterol is to a great extent catalyzed by pseudocholinesterase. Bambuterol is a potent inhibitor of pseudocholinesterase and therefore partly inhibits its own metabolism (33); this is a conceivable reason for the observed pharmacokinetic non-linearity. The outcome of the modelling exercise suggested that about 60 % of systemically bioavailable terbutaline generated from a moderate oral dose of bambuterol was produced by slow presystemic biotransformation and that the rest was generated systemically. Considering the found dose linearity for terbutaline, any loss of presystemic capacity to generate terbutaline should have been compensated for systemically. Previous data in subjects with low pseudocholinesterase activity support this, since mean AUC0-24h of terbutaline after bambuterol 20 mg in that group was similar to the mean in subjects with normal pseudocholinesterase activity after the same dose (56).
It is often recommended that drug dosing to children, compared with adults, should be reduced in proportion to the children’s lower weight. This routine dose-adjusting principle in paediatric practice is mainly based on the assumption that the clearance of the drug be linearly proportional to body weight, whereas bioavailability is assumed to be independent of body weight. However, oral dosing of bambuterol based on body weight adjustment was shown not to be an effective strategy in children (Figure 12), primarily because weight-normalized clearance was higher in the children (Table 4). In fact, dosing on the basis of body weight could lead to undertreatment of children.

**Clinical pharmacokinetics of formoterol**

Systemic absorption of inhaled formoterol is rapid; the plasma peak due to substance overflow into the systemic circulation is seen within 15 min after dosing, after which the concentration rapidly declines (57). The plasma profile after simultaneously administered oral and intravenous dosing of formoterol, was shown to fairly well serve its purpose - to simulate inhalation (Paper III).

Glucuronidation of formoterol occurred mainly at the phenolic position, but also the benzylic OH position was conjugated (Paper III). This is similar to the adrenoceptor antagonist labetalol, where monoglucuronides were found at the benzylic or the phenolic positions (58, 59). In conformity with terbutaline (60), but in contrast to salbutamol (61), elimination of the pharmacologically active (R;R)- formoterol (62) was shown to be slower than elimination of the inactive (S;S)-formoterol (Paper IV). A similar trend, albeit not statistically significant, has been reported after administration of racemic formoterol via the dry powder inhaler Aerolizer® (57). More rapid glucuronidation of (S;S)-formoterol could contribute to this enantioselectivity in the pharmacokinetics of formoterol (63). The capacity for glucuronidation is normally high and independent of age (64). Thus, if the capacity for phase-1 reactions, e.g. O-demethylation, were reduced, this would probably be metabolically compensated for by conjugation of formoterol.

Formoterol and O-demethylated formoterol, but no conjugates were shown to be excreted in faeces (Paper III). Deconjugation in the intestine of systemically formed and excreted conjugates (65) might explain this difference in proportions of formoterol and metabolites in faeces compared with urine and plasma.

Despite a long duration of bronchodilation, the systemic duration of action of inhaled formoterol, represented by serum potassium suppression, was not prolonged compared with an inhaled systemically equieffective salbutamol dose. Thus, the systemic exposure to inhaled therapeutically relevant doses of formoterol, and to some extent to pharmacologically active metabolites, seems to be too low to prolong the systemic action compared with a short-acting analogue.
The mean relative therapeutic index between formoterol (Oxis) 4.5–54 µg given via Turbuhaler and salbutamol 200–1800 µg given via pMDI was estimated at 2.5 (Paper V). Thus, systemically mediated side-effects of inhaled formoterol may be less pronounced than with an equieffective bronchodilating dose of inhaled salbutamol. The octanol/water partition coefficient of formoterol is approximately 200 times that of salbutamol (66). Although not being the only factor behind the observation, this physicochemical difference is likely to contribute to the observed difference in duration of bronchodilation between these two drugs after administration via the lung (2). However, because the measured ratio did not statistically significantly differ from unity, further studies of this issue are desirable.

Concluding remarks

Regularity is a key element of maintenance drug treatment; either the goal is to exert continuous or intermittent effects. Compliance is crucial for treatment success, particularly for lengthy treatment of chronic diseases. Education plays a prominent role in the patient’s disease awareness, comprehension and ultimately compliance with a prescribed drug regimen. The importance of simplicity should therefore not be underestimated. Once- or twice-daily intake of a drug is always easier to comply with than regimens requiring more frequent dosing. Regular treatments of asthma and chronic obstructive pulmonary disease with long-acting β₂-agonists are examples where continuously exerted effect can be a desirable outcome (67, 68). This thesis illustrates how that goal can be achieved by two different approaches: oral administration of the prodrug bambuterol and inhalation of formoterol. The pharmacological challenge was to prolong duration of bronchodilation without getting troublesome side-effects. To achieve this, the systemic plasma concentration of terbutaline, generated from bambuterol, and local lung concentration of formoterol are to be kept within therapeutically relevant ranges for extended periods of time. The systemic plasma concentration of generated terbutaline was used as a marker of the sustained bronchodilating potential of bambuterol, whereas systemic and local effect variables were used to illustrate the selective long-acting potential of inhaled formoterol.

Recommended doses of bambuterol, 10-20 mg once daily in adults, produced plasma concentrations of the active moiety terbutaline, normally being within therapeutically relevant limits. Achieving this by once-daily dosing is a unique property of bambuterol among prodrugs of β₂-agonists. Dose proportionality with respect to generated terbutaline makes dosing of bambuterol predictable. Children should be given higher doses than indicated by their lower body weight compared with adults. Parametric and non-parametric pharmacokinetic analysis of plasma concentrations showed that absorption of bambuterol is slow and multi-phasic and indicated that biotransformation to terbutaline, which is also slow, occurs both presystemically and systemically. The steady-state level of terbutaline was higher than after a single-dose, indicating that the full effect of bambuterol cannot be expected until after a few days.
Although bronchodilation is sustained by inhaled formoterol, Paper IV in this investigation suggested that the duration of systemically-mediated cardiovascular or metabolic side-effects of formoterol do not differ from inhaled salbutamol. There was a trend in Paper V that the magnitude of systemic side-effects may even be less pronounced after inhalation of formoterol compared with a locally equieffective dose of inhaled salbutamol. The local action of inhaled formoterol is probably maintained by substance retained in the lungs. Once absorbed into the systemic circulation, formoterol is rapidly eliminated or transformed into inactive metabolites - primarily glucuronide conjugates. This could explain why systemically-mediated side-effects were less pronounced and wore off more rapidly than the bronchodilating effect of inhaled formoterol.

Both approaches to sustaining stimulation of β₂-adrenoceptors have their pros and cons. Bambuterol can be dosed orally once daily, but full effect is reached slowly. The effect of formoterol is reached within a few minutes, but administration must occur via the lungs, often twice daily. Both treatments, however, give 24-h symptom relief during regular treatment.
ACKNOWLEDGEMENTS

This dissertation has been made possible by the kind help and generous support from my colleagues at AstraZeneca R&D Lund. Particularly, I wish to express my gratitude to

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AstraZeneca, for its benevolent attitude to my dissertational work.

I also wish to thank my parents.

And finally, I thank my wife Agnes.
REFERENCES


APPENDIX

Program code used to model the pharmacokinetics of bambuterol and terbutaline:

```plaintext
$PROBLEM Bambuterol (iv+po) & terbutaline (iv): C-B, C-T, C-T-B
$INPUT ID AMT TIME RATE EVID CMT ADDL II DV FLAG VAR TYPE CP MIN
$DATA data_all.prn IGNORE=#
$SUBROUTINES ADVAN5
$MODEL
  COMP=(DEFOBS1)
  COMP=(PERIPH1)
  COMP=(PERIPH2)
  COMP=(DEFOBS2)
  COMP=(PERIPH3)
  COMP=(PERIPH4)
  COMP=(PERIPH5)
  COMP=(DEPOT1)
  COMP=(DEPOT2)
  COMP=(DEPOT3)
$PK
  X3=0
  X4=0
  IF(VAR.EQ.1)X3=1
  IF(VAR.EQ.2)X4=1
  ;----------------------------------------------------------------------------
  ;BASIC PK OF BAMIBUTEROL
  ;----------------------------------------------------------------------------
  K10=THETA(1)*(1+ETA(1))
  K12=THETA(2)*(1+ETA(2))
  K21=THETA(3)*(1+ETA(3))
  K13=THETA(4)*(1+ETA(4))
  K31=THETA(5)*(1+ETA(5))
  ;----------------------------------------------------------------------------
  ;BASIC PK OF TERBUTALINE
  ;----------------------------------------------------------------------------
  K40=THETA(6)*(1+ETA(6))
  K45=THETA(7)*(1+ETA(7))
  K54=THETA(8)*(1+ETA(8))
  K46=THETA(9)*(1+ETA(9))
  K64=THETA(10)*(1+ETA(10))
  ;----------------------------------------------------------------------------
  ;VOLUMES OF DISTRIBUTION
  ;----------------------------------------------------------------------------
  S1 =THETA(11)*(1+ETA(11))
  S4 =THETA(11)*(1+ETA(11))
  ;----------------------------------------------------------------------------
  ;SYSTEMIC GENERATION OF TERBUTALINE FROM BAMIBUTEROL
  ;----------------------------------------------------------------------------
  K74=THETA(12)*(1+ETA(12))
  K17=THETA(13)*(1+ETA(13))
  ;----------------------------------------------------------------------------
  ;GI ABSORPTION OF BAMIBUTEROL (K81, K91) AND
  ;PRESYSTEMIC RATE OF TERBUTALINE GENERATION (K104)
  ;----------------------------------------------------------------------------
  K81 =THETA(14)*(1+ETA(14))
  K91 =THETA(15)*(1+ETA(15))
  K104=THETA(15)*(1+ETA(15))
  F8 =THETA(16)*(1+ETA(16))
  F9 =THETA(17)*(1+ETA(17))
  F10 =THETA(18)*(1+ETA(18))
$ERROR
  IF (EVID.EQ.0.AND.F.GT.0) THEN
    IPRED=LOG(F)
  ELSE
    IPRED=LOG(F+1)
```

40
Figure 17. Pharmacokinetic modelling of plasma bambuterol and terbutaline, basic goodness of fit plots. DV=measured plasma concentration; PRED=predicted concentration based on population parameter mean estimates; IPRE=predicted concentration based on individual parameter mean estimates; WRES=weighted residual; IWRE=individual weighted residual