PHARMACOKINETICS OF BUDESONIDE AND ITS MAJOR ESTER METABOLITE AFTER INHALATION AND INTRAVENOUS ADMINISTRATION OF BUDESONIDE IN THE RAT

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ABSTRACT:
Fatty acid esterification of budesonide (BUD) has previously been documented in vitro as well as in large airway tissues after in vivo administration. This reversible esterification has the potential to prolong the anti-inflammatory effect of BUD and improve its airway selectivity. In the present study we characterized the plasma and tissue kinetics of BUD in the rat after inhalation and intravenous administration, and fitted a semiphysiological compartment model to the data. After inhalation, BUD half-life was longer (8.2 h) in trachea than in plasma (3.7 h), with similar data after intravenous dosing. BUD-oleate was formed in all tissues and had a longer half-life than BUD in trachea (18–20 h) but a similar half-life in plasma and muscle. Although the major fraction of BUD and BUD-oleate in the body was found in muscle, the airways, especially trachea, possessed a high capacity to form BUD-oleate. According to steady-state simulations, BUD-oleate accumulated in trachea, giving rise to persistent and higher concentrations of active BUD as compared with a situation wherein esters were not formed. BUD esters had no effect on plasma levels of BUD at steady state, however. BUD and BUD-oleate were shown to have a 2-fold and 10- to 50-fold selectivity, respectively, in airways as compared with muscle tissue after intravenous administration. After inhalation, the corresponding figures for selectivity were 10 and 50 to 1000, respectively.

Budesonide (BUD) is a relatively fast-dissolving glucocorticosteroid (Talton et al., 2000), with a rapid absorption from the lung in both humans and animals. It was therefore not expected to show a prolonged duration of action. Contrary to what could be expected from early pharmacokinetic documentation, however, BUD is effective during once-daily treatment in patients with asthma, rhinitis, and inflammatory bowel diseases (Brogden and McTavish, 1992; Campieri et al., 1997; Campbell, 1999).

BUD has been shown to be retained in airway tissue to a greater extent than more lipophilic glucocorticosteroids, due to the intracellular formation and retention of BUD fatty acid esters (Miller-Larsson et al., 1998). These esters, the primary one being BUD-21-oleate, are produced in a variety of tissues in both animal and humans, and they are pharmacologically inactive and rapidly formed intracellularly (Wieslander et al., 1998). BUD esterification requires ATP and CoA, and it is inhibited by the acyl-cholesterol CoA transerase inhibitor cyclandelate, which together suggest that the acyl-cholesterol CoA transerase group of enzymes is involved in the esterification (Tunek et al., 1997). The BUD esters are slowly hydrolyzed back to intact BUD by lipases, but not by carboxyl esterase (Tunek et al., 1997). Experimental evidence indicates that an intracellular depot of BUD esters is at least partly responsible for the unexpectedly long duration of effect of the drug (Wieslander et al., 2000).

The aim of the present work was to characterize the pharmacokinetics of BUD and BUD-oleate in rat, after both local and systemic administration. A basic understanding of the tissue pharmacokinetics of budesonide and its esters may contribute to the understanding of effect duration and effect selectivity in the clinical situation.

Experimental Procedures
Materials. Male Sprague-Dawley rats were supplied by Möllegaard Breeding Center (Skensved, Denmark). Unlabeled BUD and 1,2-3H-labeled BUD (specific activity, 38 mCi/mmol with a radiochemical purity > 98.3%) were supplied by AstraZeneca Research and Development (Lund, Sweden).

Study Design and Sampling. 3H-Labeled BUD, dissolved in a saline vehicle containing 3.8% ethanol, was administered to rats via a tail vein as a bolus dose of 16 nmol/kg. The rats were sacrificed by heart puncture after i.p. administration of 1 ml of sodium pentobarbital at the following time points: 5, 15, 30, 45, and 60 min and 1.5, 2, 4, 8, 12, and 24 h after BUD administration (three animals at each time point). Plasma was separated after centrifugation at 70°C before analyses.

A micronized dry powder of BUD was administered by inhalation to rats using an aerosol delivery system (Nerbrink et al., 1997) adapted for small animals. The inhaled dose was 210 nmol/kg. The rats were put in restraining tubes connected to the inhalation chamber and exposed to the BUD for approximately 10 min. The rats were sacrificed 15, 30, 45, 60, and 90 min and 2, 4, 8, 12, and 24 h after start of the inhalation (three animals at each time point). Plasma, lungs, and trachea were collected at all time points.

Extraction of Intact BUD and Fatty Acid Esters. The lung and muscle were partially thawed and cut into pieces. Ethanol was added to the tissue to give an ethanol content exceeding 74%. For homogenization, the tissue was...
shaken for a short period with a steel marble at 2000 rpm using a Mikro-Dismembrator U (Lab Vision, Göteborg, Sweden). Trachea was cut into pieces and ethanol was added (same content as described above). The samples were extracted in a microwave oven MES 1000 (CEM Corp., Matthews, NC) for 30 min at 90°C. Samples were kept frozen (–20°C) until analysis with high-pressure liquid chromatography (HPLC).

**Tissues.** Extracts were purified according to a method previously described (Miller-Larsson et al., 1998) with the following modifications. The HPLC system consisted of a 2150 solvent delivery system (Pharmacia LKB, Stockholm, Sweden) and an AS3000 AutoSampler (Spectra Physics, San Jose, CA). An ethanol gradient was used. Phase A contained 5% ethanol and 0.1% acetic acid; phase B contained 95% ethanol and 0.1% acetic acid; and phase C contained 0.1% acetic acid. The gradient was as follows: 0 to 7.6 min, 35% solvent B and 65% solvent A; 7.6 to 15.2 min, 85% solvent B and 15% solvent A; and 15.2 to 20 min, 100% solvent B. Phase C was added to the HPLC flow, through a T-connection between the injector and column, during the injection phase and the first minute of the gradient. In this system, approximate retention times were 7.5 min for intact BUD and 15.5 min for BUD fatty acid esters. The esters were collected together as one fraction.

**Plasma.** Plasma was added to solid phase extraction columns where unesterified BUD bound to the packing material. The eluting plasma was collected into tubes with saturated sodium trichloroacetate and methanol to dissociate the BUD esters from lipoproteins in the plasma. The obtained solution was added to another solid phase extraction column, where the esters bound to the packing material. After these steps, the BUD and BUD ester samples were run separately. After washing and elution (with acetonitrile), the eluate was injected into an enrichment system, where the sample was further purified on an NH2 column, enriched on a C18 column, and fractions collected.

**Radioactivity Measurements.** Samples from plasma and tissue extracts were measured in a scintillation cocktail (Packard, Groningen, The Netherlands). The level of quantification (LOQ) was set at twice the background dpm count. Values below the LOQ were only obtained for muscle tissue, at the 12- and 24-h measurements.

**Liquid Chromatography-Mass Spectrometry Analyses.** For unlabeled samples collected after inhalation, tissue and plasma extracts were prepared similarly as described above and processed by HPLC connected to a Finnigan TSQ mass spectrometer via an atmospheric pressure chemical ionization interface (ThermoFinnigan, San Jose, CA). Selected reaction monitoring tandem mass spectrometry was used for quantifying BUD and BUD-oleate. A minor fraction of another fatty acid conjugate of BUD, BUD-palmiate, was also detected; however, since concentrations were less than a tenth as high as those of BUD-oleate at all timepoints, results on BUD-esters are reported as BUD-oleate only.

LOQ was 0.025 nM at a sample volume of 1 ml of plasma. The LOQ was set to 0.4 pmol/g of tissue, and samples of 0.09 to 0.15 g, 0.9 to 1.2 g, and 0.25 to 0.27 g were used for analyses of trachea, lung, and muscle, respectively.

**Pharmacokinetics.** Data are shown as mean concentration in the different tissues, including plasma, for the three animals per group. Concentrations are expressed in nanomolar, assuming a tissue density of 1 g/ml.

Noncompartmental analyses were performed using WinNonlin version 3.0. (Pharsight Corp., Mountain View, CA) with standard pharmacokinetic nomenclature (Benet and Galeazzi, 1979). Area under the concentration-time curve (AUC) was estimated using the log-linear trapezoidal method.

For the compartmental analyses, data were pooled regardless of individual identity when fitting a model to data from both experiments simultaneously. The concentrations at each timepoint, as analyzed from three different rats, showed only a small variation, which made it possible to use only the group mean values and thereby limit run time. The model includes the two different analytes (BUD and BUD-oleates), and compartments were numbered according to Fig. 1. The rate of mass transfer from compartment i to compartment j is denoted \( k_{ij} \). The volume of the ith compartment is denoted \( V_i \) and its concentration is denoted \( C_i = q_i/V_i \), where \( q_i \) is the mass content at time \( t \). The volumes of the excised lung and trachea tissues (compartments 6, 7, 8, 9, and 9') were estimated based on their weights, assuming a density of 1 g/ml. Concentrations of analytes in the soleus muscle (compartments 4 and 5) were set to represent all muscle tissues, the latter with a volume of 200 ml/rat. The volume of the plasma compartment (compartments 1 and 2), representing all extracellular water, was set to 50 ml (Bernareggi and Rowland 1991; Davies and Morris, 1993).

The model fitting was done with the computer program NONMEM (University of California, San Francisco, CA), using the ADVAN 8 routine. A proportional error model with a separate error for the trachea BUD oleate was used. As a summary of our final model, the lung was described by three compartments representing solid BUD (deposition compartment), dissolved BUD, and BUD-oleate; the trachea was described by four compartments representing solid BUD (deposition compartment), dissolved BUD, and BUD-oleate (two compartments were needed to describe the nonlinear kinetics of BUD-oleate in trachea); the soleus muscle was described by two compartments representing BUD and BUD-oleate; plasma was described by two compartments representing BUD and BUD-oleate; the gut was described by one compartment; and one extra peripheral compartment accounted for BUD outside the characterized organs (Fig. 1). The model was implemented as a set of 13 differential equations with Michaelis-Menten functions, to describe the rate of formation and rate of hydrolysis of BUD-oleate, and first order rate constants to describe the rates of all remaining processes.

The fraction of inhaled dose deposited in lung and trachea was fixed at 30% during the fitting procedure. The distribution between lung (94%) and trachea (6%), the oral bioavailability (20%), and the half-life for gut absorption (8.3

![Fig. 1. Compartmental model describing the kinetics of BUD and BUD-oleate after inhalation and intravenous administration.](image-url)

Dotted lines, Michaelis-Menten kinetics; solid lines, first order processes. Dark boxes represent BUD-oleate compartments. \( k_{diss} \) and \( k_{diss2} \), dissolution rate constants of BUD in lung and trachea, respectively; \( k_{p} \), oral absorption rate constant.
of BUD-oleate was unique for each tissue. The parameters were the Michaelis-Menten processes describing the formation and hydrolysis of BUD-oleate. We tried to use one set of Michaelis-Menten model for the formation and hydrolysis of BUD-oleate. After some exploration, we chose a model using two different linear compartments, or alternatively, modeling using a nonlinear process. After some exploration, we chose a Michaelis-Menten model for the formation and hydrolysis of BUD-oleate. We tried to use one set of $K_m$ values for each of these processes, throughout all tissues/compartments. The $V_{\text{max}}$ for the Michaelis-Menten processes describing the formation and hydrolysis of BUD-oleate was unique for each tissue. The parameters were the same after both intravenous administration and inhalation, except in trachea and lung, where different $V_{\text{max}}$ values for the ester formation were used. To get a satisfactory fit of the concentrations of BUD in plasma, a further compartment was needed; this compartment (“other tissues” in Fig. 1) lumped together all tissues not otherwise accounted for.

We noted that our basic model predicted concentrations of both analytes in all tissues but the trachea. Thus, there was a need for an additional BUD-oleate compartment for this tissue. We included a second Michaelis-Menten process with unique $V_{\text{max}}$ and $K_m$ to resolve this misfit of trachea concentrations.

The compartmental model described the experimental concentrations of BUD and BUD-oleate well in all tissues (Fig. 4, A and B) after both i.v. administration and inhalation. Parameters and coefficients of variation, obtained by fitting the model to the experimental data, are given in Table 2, A and B.

Absorption and reabsorption between the tissues and plasma were generally rapid processes. The transports from plasma to trachea and from the “other tissue” compartment to plasma were slow processes.

### Results

**Noncompartmental Analysis.** Results from the noncompartmental analysis of data obtained in the intravenous and inhalation experiments are shown in Table 1; concentrations after i.v. administration are plotted in Fig. 2, A and B, and after inhalation in Fig. 3.

The AUC values calculated up to infinity were similar to values for the AUC calculated up to the last sampling time. The extrapolated areas were small (<10%), the exception being AUC for the trachea. After intravenous dosing, a plasma clearance of 4.4 l/h/kg was calculated. There were persistent levels of the BUD-oleate and BUD in trachea. The terminal half-life of BUD-oleate in trachea was 3- to 5-fold longer than in plasma and other tissues. Thus, BUD-oleate exposure in trachea exceeded that in other tissues, as shown in Figs. 2 and 3 and Table 1.

Exposure to BUD was higher in the tissues than in plasma: the AUC-values obtained after intravenous administration were 34, 24, 14, and 3.7 nM · h in tissues from lung, trachea, muscle, and plasma, respectively. Regarding the AUC values for BUD-oleate, again after intravenous administration of BUD, there was a large difference in AUC-values obtained after intravenous administration were 34, 24, 2 and 3 and Table 1.

**Compartmental Analysis.** BUD-oleate was rapidly formed in all tissues but plasma, and the BUD-oleate showed biphasic decay over time. Thus, kinetics of the BUD-oleate could only be successfully modeled using two different linear compartments, or alternatively, using a nonlinear process. After some exploration, we chose a Michaelis-Menten model for the formation and hydrolysis of BUD-oleate. We tried to use one set of $K_m$ values for each of these processes, throughout all tissues/compartments. The $V_{\text{max}}$ for the Michaelis-Menten processes describing the formation and hydrolysis of BUD-oleate was unique for each tissue. The parameters were the same after both intravenous administration and inhalation, except in trachea and lung, where different $V_{\text{max}}$ values for the ester formation were used. To get a satisfactory fit of the concentrations of BUD in plasma, a further compartment was needed; this compartment (“other tissues” in Fig. 1) lumped together all tissues not otherwise accounted for.

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The compartmental model described the experimental concentrations of BUD and BUD-oleate well in all tissues (Fig. 4, A and B) after both i.v. administration and inhalation. Parameters and coefficients of variation, obtained by fitting the model to the experimental data, are given in Table 2, A and B.

Absorption and reabsorption between the tissues and plasma were generally rapid processes. The transports from plasma to trachea and from the “other tissue” compartment to plasma were slow processes. The model fitting led us to conclude that all tissues except plasma and the second trachea compartment had a high capacity for forming BUD-oleate, as judged from the estimated $V_{\text{max}}$ values. Hydrolysis

### Table 1

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Analyte</th>
<th>Route of Administration</th>
<th>Intravenous</th>
<th>Inhalation</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\text{AUC}_{\text{inf}}$</td>
<td>$t_{1/2}$</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>$\text{nM} \cdot \text{h}$</td>
<td>h</td>
</tr>
<tr>
<td>Lung</td>
<td>BUD</td>
<td></td>
<td>34.2</td>
<td>4.9</td>
</tr>
<tr>
<td></td>
<td>BUD-oleate</td>
<td></td>
<td>21.8</td>
<td>5.6</td>
</tr>
<tr>
<td>Trachea</td>
<td>BUD</td>
<td></td>
<td>23.5</td>
<td>6.9</td>
</tr>
<tr>
<td></td>
<td>BUD-oleate</td>
<td></td>
<td>104</td>
<td>18</td>
</tr>
<tr>
<td>Muscle</td>
<td>BUD</td>
<td></td>
<td>14.1</td>
<td>2.3</td>
</tr>
<tr>
<td></td>
<td>BUD-oleate</td>
<td></td>
<td>1.6</td>
<td>2.5</td>
</tr>
<tr>
<td>Plasma</td>
<td>BUD</td>
<td></td>
<td>3.7</td>
<td>4.7</td>
</tr>
<tr>
<td></td>
<td>BUD-oleate</td>
<td></td>
<td>1.3</td>
<td>5.4</td>
</tr>
</tbody>
</table>

* $\text{AUC}_{\text{inf}}$ denotes AUC measured from time zero to infinity.
capacity showed large variation and was high in muscle and lowest in the second trachea compartment.

Simulations Based on Compartmental Analysis. Steady-state infusion. A 48-h simulation covering a continuous 24-h infusion shows that all tissues except BUD-oleate in trachea reached steady state at 24 h (Fig. 5). Interestingly, on comparing the simulated concentrations with experimental rat tissue concentrations (individual data) obtained after an intravenous infusion of 144 nmol/kg over 24 h (data on file), good agreement is seen in all tissues except in muscle, where the model predicts too high a concentration at 24 h (Fig. 5). The level of BUD-oleate in plasma was not investigated during this experiment.

Ester inhibition. The simulated effect of total ester inhibition revealed that during once-daily inhalation of 210 nmol/kg BUD, the concentration of BUD decreased only in trachea, within the 24-h dosing interval (Fig. 6). In other tissues, a difference is only seen at later timepoints, as is evident from data after the last dose simulated in Fig. 6. Thus, under the conditions used for this simulation, the esterification process only influences the BUD concentration in trachea. For BUD in trachea, terminal half-life increased 2-fold during repeated dosing from a value of 8 h at first dosing to about 16 h (Fig. 6).

Dose and time dependence of esterification. The fraction of BUD-oleate, calculated as the ratio between the amount of BUD-oleate and the sum of BUD-oleate and BUD, was simulated in lung and trachea after inhalation of three different doses. Figure 7 shows that the ratios at early timepoints changed with dose. The lowest ratio was found with highest dose, and the ratio approached asymptotically the same value at later timepoints, regardless of the administered dose.

Discussion

Reversible fatty acid esterification is a common mechanism for intracellular storing of steroid hormones and their precursors (Hochberg, 1998). Although oleates are the predominant steroid esters formed, palmitate, palmitoleate, and arachidonate esters have also been identified. Among the corticosteroids, both cortisol and corticosterone are esterified, but BUD appears to be unique in this respect among the corticosteroid xenobiotics used in medical therapy (Tunek et al., 1997). In man, the presence of BUD-oleate has hitherto been confirmed in lung and nasal tissue in vivo (Thorsson et al., 1998; Petersen et al., 2001).

The presented semiphysiological compartment model effectively describes experimental data from two different studies with two different administration routes. The model offers an explanation and parameterization of both the distribution of BUD and the tissue-specific formation and hydrolysis of BUD-oleate after intravenous and inhalation administration in rat. Whereas the Michaelis-Menten kinetics for BUD-oleate were defined by \( K_m \) values not dependent on tissue, the \( V_{max} \) for the Michaelis-Menten processes describing the formation and hydrolysis of BUD-oleate was unique for each tissue.

The differences in \( V_{max} \) among the different tissues are probably an indication of different amounts or activity of the enzymes responsible for the formation and hydrolysis of BUD-oleate. A possible reason for the need to have a different \( V_{max} \) for the formation of BUD-oleate in lung and trachea with the i.v. versus the inhalation routes of administration is a nonuniform cellular distribution of the enzymes responsible for the esterification of BUD, such that a higher enzyme concentration is found on the luminal than on the endothelial side of the airways. This could explain why more BUD-oleates are formed after inhalation than after intravenous administration.

BUD-oleate in trachea seems to behave in a less linear fashion than BUD-oleate in the other tissues. Two compartments with different Michaelis-Menten kinetics (both \( K_m \) and \( V_{max} \)) were needed here to describe the kinetics of BUD-oleate. Again, one can only speculate about the reason for this finding, but it might be due to differences regarding distribution, capacity, or specificity of the enzymes involved in the synthesis and hydrolysis of BUD-oleate. Further animal studies are needed to measure tissue-specific \( K_m \) and \( V_{max} \) values.

Our estimated \( V_{max} \) values showed all tissues but plasma and the second trachea compartment to have a high esterification capacity. The second trachea compartment could actually have been modeled by a first order process, but not understanding the detailed mechanisms for intratracheal BUD-oleate disposition, e.g., redistribution within tissue subcompartments, we chose a Michaelis-Menten process.

The finding of low BUD-oleate amounts also in plasma raises the question of its origin. Our model does not contain any steps for distribution of BUD-oleate. Literature data on similar esters of endogenous steroids indicate only intracellular localization (Sviridov, 1999), which is in accordance with in vitro experimental data on BUD-oleate (Wieslander et al., 1998). It could also be speculated that a small amount of BUD-oleate might be actively transported out of the cell. Preliminary in vitro data indicate that BUD-oleate is not formed in human plasma per se, but to some extent in human whole blood (data on file, AstraZeneca Research and Development).

Comparison of simulated and experimental concentrations after a 24-h infusion showed good agreement. Since this data set was not part of the modeling exercise, it could be regarded as a model validation. Interestingly, hydrolysis was most rapid in muscle tissues. The simulated 24-h infusion into rats showed rapidly declining BUD concentrations in muscle immediately after infusion ceased. A small deviation from nominal sampling time would thus result in a large deviation in observed concentrations, a phenomenon that must be taken into account when designing such studies. This might also explain the difference between predicted and experimental concentrations shown in Fig. 6. As opposed to muscle, hydrolysis is a slow process in the airways, and especially in the tracheal tissues.

Simulated repeated administrations to the rat showed a prolonged half-life (about 16 h) for BUD in airways. Thus, after repeated administrations, persistent high levels of BUD-oleate in trachea will
Fig. 4. Model-predicted and experimental concentrations of BUD and BUD-oleate in rats after intravenous administration of 16 nmol/kg BUD (A) and inhalation of 210 nmol/kg BUD (B).

Concentrations are expressed in nanomolar, assuming a tissue density of 1 g/ml.
give formation rate-limited elimination of BUD, resulting in persistent higher levels also of parent active steroid. The long terminal half-life for BUD in trachea could not be observed after a single i.v. dose administration, when its levels were influenced mainly by systemic influx to tissue.

In the present study we have characterized the plasma and tissue kinetics of BUD in the rat using different routes of administration. We have shown that BUD-oleate is formed in a variety of tissues. Since muscle is the largest tissue component in the body (as seen from a mass-balance point of view), the major amount of BUD and BUD-oleate in the body will reside in muscle. The differences between tissues in BUD-oleate kinetics are probably a result of the airways, the trachea especially, possessing a high capacity to form BUD-oleate. Furthermore, the BUD-oleate is retained here for a prolonged time. For the parent steroid, higher selectivity for lung and trachea over the muscle tissues was shown. One could argue that exposure of the muscle tissue to the drug could have been underestimated, due to the low muscle tissue concentrations and the relatively short calculated half-life. However, adding a 5-h half-life onto concentrations from the muscle tissue had minimal impact and still gives the same pattern of results. Thus, for BUD, selectivity for lung and trachea over the muscle tissue was about 2-fold, as calculated from AUC ratios after intravenous drug administration. Similar calculations performed on the esters after intravenous BUD showed a selectivity of BUD-oleate for lung and trachea over muscle tissue that was more than 10-fold. These figures, based on experimental data, are in good agreement with model-based calculations. Furthermore, based upon model predictions, the corresponding figures after inhalation for tissue selectivity for lung and trachea versus muscle were about 10-fold for BUD and 50- to 1000-fold for BUD-oleate, i.e., selectivity is even higher after inhalation.

BUD has a unique capability to reversibly build up an intracellular depot in the form of inactive esters. Due to nonlinear processes, the relative size of the ester depot is dose-dependent at early timepoints.

### Table 2a

First order rate constants with coefficients of variation (CV) and residual errors obtained by model fitting

<table>
<thead>
<tr>
<th>Rate Constants</th>
<th>CV (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$k_{10}$</td>
<td>23 10</td>
</tr>
<tr>
<td>$k_{13}$</td>
<td>16 45</td>
</tr>
<tr>
<td>$k_{1}$</td>
<td>0.28 32</td>
</tr>
<tr>
<td>$k_{34}$</td>
<td>76 19</td>
</tr>
<tr>
<td>$k_{41}$</td>
<td>4.1 36</td>
</tr>
<tr>
<td>$k_{61}$</td>
<td>16 18</td>
</tr>
<tr>
<td>$k_{16}$</td>
<td>3.0 12</td>
</tr>
<tr>
<td>$k_{11}$</td>
<td>3.2 4.1</td>
</tr>
<tr>
<td>$k_{13}$</td>
<td>0.19 14</td>
</tr>
</tbody>
</table>

Error_{trachea} (%): 3.9  
Error_{remaining compartments}: 14

*Residual error trachea compartment.

**Residual error remaining compartments.

### Table 2b

Michaelis-Menten parameters for ester synthesis and hydrolysis obtained by model fitting

<table>
<thead>
<tr>
<th></th>
<th>Synthesis</th>
<th>Hydrolysis</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>$V_{max}$</td>
<td>$K_m$</td>
</tr>
<tr>
<td>Trachea</td>
<td>26 (108)</td>
<td>328 (19)</td>
</tr>
<tr>
<td>1st Compartment</td>
<td>220 (24)</td>
<td>30 (71)</td>
</tr>
<tr>
<td>2nd Compartment</td>
<td>0.26 (107)</td>
<td>2590 (109)</td>
</tr>
<tr>
<td>Lung</td>
<td>0.60 (69)</td>
<td>710 (408)</td>
</tr>
<tr>
<td>Muscle</td>
<td>0.201 (64)</td>
<td>30 (71)</td>
</tr>
<tr>
<td>Plasma</td>
<td>4.4 (32)</td>
<td>30 (71)</td>
</tr>
</tbody>
</table>

*Parameters obtained after intravenous administration.

**Parameters obtained after inhalation.

![Fig. 5](image_url)

**Fig. 5.** Concentrations of BUD and BUD-oleate after a 24-h infusion of 144 nmol/kg BUD in rats.

Solid lines, simulated concentrations; circles, experimental concentrations. Concentrations are expressed in nanomolar, assuming a tissue density of 1 g/ml.
At later timepoints, however, the relative size is identical, irrespective of dose. Our model suggests, however, that BUD-oleate at steady state should be proportional to dose over a wide dose span.

In the present study in rats, BUD esters accumulated in trachea, leading to higher and more persistent concentrations of active BUD in the airways. In contrast, by simulating complete block of ester formation, we found no evidence of any influence of BUD esters on the BUD pharmacokinetics in plasma. Budesonide has a longer duration of action than other inhaled steroids (Venables et al., 1996; Miller-Larsson et al., 2000) and is currently the only inhaled steroid approved for once-daily treatment of mild to moderate asthma. It has been speculated that esterification contributes to these findings (Lipworth and Jackson, 2000), which our findings support. Our simulations may also clarify why the pharmacokinetic properties of BUD in humans, as assessed by plasma concentrations of parent drug, do not deviate markedly from those of other inhaled steroids (Edsäter, 1999). In the clinic, the systemic effects of BUD are similar or fewer than for other inhaled steroids (Grahén et al., 1997). Our findings in the rat may, at least partly, explain why systemic effects are not affected by esterification to the same extent as is the local effect in the airways.

In conclusion, BUD-oleate accumulated in trachea, giving rise to high and persistent concentrations of active BUD, whereas BUD-
oleate appeared to have no effect upon plasma levels of BUD. Esterification contributes to the improved selectivity of BUD for lung and trachea over other tissues such as muscle.

**Appendix 1**

\[ \frac{dA_{\text{plasma}}}{dt} = k_{\text{gut dep}} \cdot A_{\text{gut}} - k_{\text{trachea}} \cdot A_{\text{trachea}} - k_{\text{muscle}} \cdot A_{\text{muscle}} - k_{\text{lung dep}} \cdot A_{\text{lung}} - k_{\text{other tissue}} \cdot A_{\text{other tissue}} + \frac{V_{\text{plasma}}}{V_{\text{hydr}}} \]

\[ \frac{dA_{\text{trachea}}}{dt} = k_{\text{diss}} \cdot A_{\text{trachea dep}} - k_{\text{form}} \cdot A_{\text{trachea form}} + \frac{V_{\text{trachea}}}{V_{\text{hydr}}} \]

\[ \frac{dA_{\text{muscle}}}{dt} = k_{\text{diss}} \cdot A_{\text{muscle dep}} - k_{\text{form}} \cdot A_{\text{muscle form}} + \frac{V_{\text{muscle}}}{V_{\text{hydr}}} \]

where \( k_p \) is the rate of mass transfer from compartment \( i \) to compartment \( j \) and \( A_i \) is the amount in compartment \( i \). For Michaelis-Menten processes the rates are denoted \( V_{\text{max}} \), using the Michaelis-Menten equation with the maximum rate, \( V_{\text{max}} \), and Michaelis-Menten constant, \( K_m \), according to

\[ V_i = \frac{V_{\text{max}} \cdot A_i}{K_m + A_i} \]

where \( A \) indicates compartment and \( b \) the enzymatic process (i.e., formation or hydrolysis). For trachea, two sets of Michaelis-Menten equations were used: see text for explanation. \( k_{\text{diss}} \) represents the dissolution rate constant and \( k_p \) represents the oral absorption rate constant of BUD.

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**References**


