Enantioselective and Nonlinear Intestinal Absorption of Eflornithine in the Rat

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This study aimed to investigate if the absorption of the human African trypanosomiasis agent eflornithine was stereospecific and dose dependent after oral administration. Male Sprague-Dawley rats were administered single doses of racemic eflornithine hydrochloride as an oral solution (750, 1,500, 2,000, or 3,000 mg/kg of body weight) or intravenously (375 or 1,000 mg/kg of body weight). Sparse blood samples were obtained for determination of eflornithine enantiomers by liquid chromatography with evaporative light-scattering detection (lower limit of quantification [LLOQ], 83 μM for 300 μl plasma). The full plasma concentration-time profile of racemic eflornithine following frequent sampling was determined for another group of rats, using a high-performance liquid chromatography–UV method (LLOQ, 5 μM for 50 μl plasma). Pharmacokinetic data were analyzed in NONMEM for the combined racemic and enantiomeric concentrations. Upon intravenous administration, the plasma concentration-time profile of eflornithine was biphasic, with marginal differences in enantiomer kinetics (mean clearances of 14.5 and 12.6 ml/min/kg for L- and D-enantiomeric, respectively). The complex absorption kinetics were modeled with a number of transit compartments to account for delayed absorption, transferring the drug into an absorption compartment from which the rate of influx was saturable. The mean bioavailabilities for L- and D-enantiomeric eflornithine were 41% and 62%, respectively, in the dose range of 750 to 2,000 mg/kg of body weight, with suggested increases to 47% and 83%, respectively, after a dose of 3,000 mg/kg of body weight. Eflornithine exhibited enantioselective absorption, with the more potent L-isomer being less favored, a finding which may help to explain why clinical attempts to develop an oral treatment have hitherto failed. The mechanistic explanation for the stereoselective absorption remains unclear.

Human African trypanosomiasis (HAT), also known as sleeping sickness, is fatal if left untreated. The disease is spread in 36 countries throughout sub-Saharan Africa (13). Sixty million people are exposed to Trypanosoma-infected tsetse flies and are at risk of developing the disease. HAT was largely under control in the mid-20th century but has since reemerged (5). By 1997, an estimated 450,000 people were infected (13). Although this number has decreased in recent years, thanks to coordinated campaigns (1), it remains a neglected disease, with numerous patients not receiving treatment (2).

The human disease is caused by one of the subspecies of the kinetoplastid protozoan Trypanosoma brucei. T. b. rhodesiense is responsible for an acute form of the disease, while T. b. gambiense (causing 97% of reported cases) causes a chronic form in West and Central Africa (5, 6). There are two stages of HAT, namely, an early or hemolymphatic stage and a late or encephalitic stage. The early stage causes a variety of nonspecific symptoms, such as fever, headache, malaise, and weakness. As the disease progresses, specific organ dysfunction, e.g., tachycardia, heart failure, endocrine disturbances, and liver, spleen, and eye involvement, occurs (13). In the late stage, there is a wide spectrum of possible features that may occur, such as psychiatric disturbances, sleep disorders, motor system disorders, sensory syndromes, and abnormal reflexes. In the terminal stage, the patient develops seizures, incontinence, cerebral edema, progressive mental deterioration, and finally, death.

Only two drugs, melarsoprol and eflornithine, are registered for the treatment of late-stage HAT. There is an increasing resistance to melarsoprol, a toxic arsenical compound requiring intravenous injections (4, 8, 14, 21). Eflornithine (D,L-α-difluoromethylornithine [DFMO]) was initially synthesized as an antitumor agent, although it was never registered for that use. It is mainly effective against T. b. gambiense-infected patients. Eflornithine is efficacious compared to melarsoprol but requires four (100 mg/kg of body weight) daily infusions for a duration of 7 to 14 days. This complex mode of administration requires hospital-like settings, with the consequence that many patients living in rural areas are left untreated. It can be expected that a less complex mode of administration would enable more patients to be treated.

Results of oral eflornithine treatment in late-stage infected patients have been discouraging. Oral regimens with the same or similar doses to those for intravenous use (100 to 125 mg/kg of body weight, given four times daily) resulted in eflornithine racemic plasma concentrations of approximately half those observed after intravenous administration (16). Moreover, it was suggested that plasma eflornithine concentrations did not increase proportionally to the dose when the dose was increased from 100 to 125 mg/kg of body weight (60 to 70% of the expected increase), although this was not statistically significant. Relapse was observed in 6 of the 25 patients on oral...
treatment. When the drug is administered intravenously, race- 
cemic cerebrospinal fluid concentrations above 50 μM have been 
associated with a high cure rate, but this concentration ap- 
ppeared to be insufficient during oral treatment. Increasing the 
oral dose may improve the cure rate, but gastrointestinal side 
effects are likely to occur and possibly be dose-limiting (10, 16).

Eflornithine elicits its pharmacological effect by irreversible 
hindrance of ornithine decarboxylase, blocking polyamine bio-
synthesis in trypanosomes (3, 18). It has also been shown that 
compared to D-eflornithine, the L-enantiomer exhibits >20 
times higher affinity for the target enzyme ornithine decarbox-
ylase in mammalian cells (18). The L-isomer also appears to be 
more potent in cultured T. brucei parasites (R. Brun, Swiss 
Tropical Institute, personal communication). Taken together, 
these data suggest that the L-form is the principal trypanostatic 
moiety clinically.

Eflornithine human racemic pharmacokinetics are charac- 
terized by an oral bioavailability of approximately 50%, 
mainly renal elimination (>80%) of low extraction, no reported met- 
tabolites, negligible plasma protein binding, and a multiphasic 
plasma concentration-time profile, probably contributing to 
highly varying estimates of half-life ranging from 2 to 30 h (7, 
10, 12, 16). There are no published data on the pharmacoki- 
etics of eflornithine based on a stereoselective method of 
biomonitoring. The presence of enantioselective pharmacokin- 
etics should be considered in investigating the possibility of de-
veloping an efficacious oral treatment.

The aim of this work was to investigate if the oral absorption 
of racemic eflornithine was stereospecific and dose dependent 
in the rat. The secondary aim was to characterize the pharma-
cokinetic profile of eflornithine enantiomers and to investigate 
the absorption kinetics of eflornithine.

MATERIALS AND METHODS

Animals. Male Sprague-Dawley rats (B&K Universal AB, Sollentuna, Swe- 
den) weighing 260 to 320 g were acclimatized for at least 5 days after arrival at 
a certified animal facility (Experimental Biomedicine at Göteborg University, 
Göteborg, Sweden). The animals were housed under controlled environmental 
conditions (12-h light-dark cycle at 25 to 27°C and 60 to 65% humidity). Four rats 
were kept in each cage prior to surgery and thereafter were kept separately. Food 
(B&K Feed) and tap water were available ad libitum prior to and after surgery 
until 6 hours prior to drug administration. All experiments were performed 
during the light phase of the cycle. The study was approved by the Ethics 

Chemicals for in vivo experiments. Eflornithine hydrochloride was obtained 
from WHO/TDR (Geneva, Switzerland). Isofurane (Forene; Abbot Scandinavia 
AB, Go¨teborg, Sweden). The animals were housed under controlled environmental 
conditions (12-h light-dark cycle at 25 to 27°C and 60 to 65% humidity). Four rats 
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Drug formulation. Solutions for oral administration were prepared by dissolv-
ing an appropriate amount of eflornithine hydrochloride powder in saline solu-
tion. Eflornithine solutions for intravenous administration were prepared like-
wise but were pH adjusted to 7.2 with sodium hydroxide.

Experimental design. A total of 69 rats were administered eflornithine hydro-
chloride either orally (n = 52) or intravenously (n = 17). Selections of dose levels 
were based on allometric scaling and the lower limit of quantification (LLOQ) 
for the enantioselective assay. Single oral doses of 750, 1,500, 2,000 or 3,000 
mg/kg of body weight of racemic eflornithine hydrochloride were administered 
by gavage (10 ml/kg). Single intravenous eflornithine doses of 375 or 1,000 mg/kg 
of body weight were administered as a short-term infusion (3 min) via the jugular 
vein catheter (3.3 ml/kg) (Table 1).

Blood samples were drawn from the jugular vein catheter or carotid artery 
catheter after oral or intravenous doses, respectively. Sample volumes were 
replaced with an equal volume of saline solution, and catheters were flushed with 
heparinized saline solution (20 μl/ml) after each sampling occasion.

One to three blood samples (700 μl) per rat were obtained up to 13 h after 
dosing and analyzed with chiral quantification. In another group of rats, frequent 
(8 to 16 samples per rat) blood samples (130 μl per sample) were drawn up to 
25 h after dosing and quantified with the racemic method (Table 1). Plasma was 
separated by centrifugation for 8 min at 12,000 g within 30 min after blood 
collection and kept at −22°C until analysis.

Chiral quantification of eflornithine. D- and L-difluoromethylornithine was 
extracted from plasma by solid-phase extraction and separated on a Chirobiotic 
TAG (250 mm × 4.6 mm) column with isocratic LC and evaporative light-
scattering detection, as previously described, using identical instrumentation and 
chromatographic conditions (15). The chiral method was developed for deter-
mination of eflornithine enantiomers in 1,000-μl human plasma samples but was 
used in this study for 300-μl rat plasma samples. Rat plasma samples (300 μl) 
were mixed with 110 μl water (700 μl) and run with calibrators prepared in 
human plasma (1,000 μl), ranging from 25 μM to 1,000 μM for each enantiomer.

The dilution of plasma samples did not significantly affect the accuracy or pre-
cision of the method. No significant differences were observed in slopes or 
intercepts in regressing nondiluted and diluted calibration curves (r test; P > 0.99).

No matrix differences were observed in analyzing quality control (QC) samples 
(n = 5) at two levels (250 and 2,500 μM) prepared in rat and human plasma. The 
LLOQ was set to 83 μM (300 μl). Duplicates of two QC levels (750 and 7,500 
μM) were analyzed without using runs of rat plasma samples to ensure that 
accuracy and precision were within acceptable limits.

Racemic quantification of eflornithine. Racemic eflornithine was quantified 
using precolumn derivatization, followed by LC and UV detection according to 
a published method (20), modified as described below. The LC system consisted of 
a 48-well-plate Propekt 2 autoinjector (SparkHolland, Emmen, Holland), two 
interconnected Schimadzu LC10AD pumps (Schimadzu, Kyoto, Japan), and a 
Schimadzu SPD 10-A UV-Vis detector, set at 330 nm. Data acquisition was 
performed using Chromatographic Station for Windows, version 1.2.3 (Data-
ax, Prague, The Czech Republic).

Plasma samples (50 μl) were precipitated with ice-cold methanol (270 μl) 
containing an internal standard (L-α,α,α-3-hydroxybutyric acid) at a concen-
tration of 0.155 μM. The samples were placed on a vortex mixer for approxi-
ately 10 s, centrifuged for 10 min at 12,000 × g, and thereafter kept at −37°C 
for 10 minutes. The supernatants were transferred to new tubes and evaporated 
to dryness at 65°C under a gentle stream of air. The dried samples were redis-
solved in 50 μl phosphate buffer (0.1 M; pH 7.5). The derivatization mixture was 
prepared daily by mixing o-phthalaldehyde (20 mg), ethanol (1 ml), nitrolicthic 
acid (4 mg), mercaptoethanol (100 μl), and 10 ml phosphate buffer (0.1 M; pH

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TABLE 1. Experimental design for the study

<table>
<thead>
<tr>
<th>Route of administration</th>
<th>Racemic dose (mg/kg of body wt)</th>
<th>Chiral analysis (1 to 3 plasma samples/rat)</th>
<th>Racemic analysis (8 to 16 plasma samples/rat)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oral</td>
<td>750</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1,500</td>
<td>9</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>2,000</td>
<td>7</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>3,000</td>
<td>10</td>
<td>4</td>
</tr>
<tr>
<td>Intra venous</td>
<td>375</td>
<td>5</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td>1,000</td>
<td>5</td>
<td>3</td>
</tr>
</tbody>
</table>

Vol. 52, 2008 2843  ENANTIOSELECTIVE INTESTINAL ABSORPTION OF EFLORNITINE
The data were modeled simultaneously to obtain pharmacokinetic parameters for L- and D-enantiomers, based on a two-compartment disposition model with linear elimination.

The temperature for the autoinjector was kept constant at 20°C.

The plots illustrating the concentration-time profiles for L-eflornithine (A), D-eflornithine (B), and racemic eflornithine (C) after intravenous administration of 375 mg/kg of body weight of racemic eflornithine to laboratory rats (n = 17). The data were modeled simultaneously to obtain pharmacokinetic parameters for L- and D-enantiomers, based on a two-compartment disposition model with linear elimination.
sums of the enantiomer concentrations were in agreement with racemic concentrations. The plasma concentration profile was described with a two-compartment model with first-order elimination. Clearance (CL) and central volume were estimated for each enantiomer, whereas intercompartment clearance ($Q$) and peripheral volume were set to be identical for both isomers (Fig. 2; Table 2). Interindividual variability was estimated for CLD-eflornithine and CL L-eflornithine. Although the OFV did not decrease significantly in estimating CL and central volume of distribution individually for each enantiomer compared with their estimation as shared parameters, they were nevertheless estimated individually in consideration of diagnostic plots as well as the further employment of the estimates as fixed parameters for fitting of the oral plasma concentration-time data. The estimated population CLD-eflornithine was 15% higher than the estimated population CL L-eflornithine, with dose having no effect on CL. The diagnostic plots and parameter estimates for the intravenous data are shown in Fig. 3 and Table 3.

In contrast to intravenous administration, where L- and D-eflornithine concentrations were comparable, plasma concentrations after oral administration of L-eflornithine were lower than those after oral administration of D-eflornithine. The population mean bioavailability values for L- and D-eflornithine, in the dose range of 750 to 2,000 mg/kg of body weight, were 41% and 62%, respectively, with no apparent dose dependency. At the highest dose level, the population bioavailability values for L- and D-eflornithine were estimated to be higher, at 47% and 83%, respectively. Including the highest dose level as a categorical parameter for oral bioavailability for both D- and L-eflornithine significantly decreased the OFV ($P < 0.01; \Delta \text{OFV}, -11.4$).

The absorption delay and an increasing time to reach maximum concentration with dose were best described, among the models evaluated, by a transit model followed by a Michaelis-Menten function determining the absorption rate. Using a transit model instead of a lag time before absorption occurred reduced the OFV significantly ($P < 0.01; \Delta\text{OFV}, -148$). Parameters for the transit model could not be obtained individually for each enantiomer and were therefore assumed to be identical for both eflornithine enantiomers. Interindividual variability values for mean transit time and bioavailability could not be estimated separately for D- and L-eflornithine and were therefore assumed to be identical. Population parameters and population predicted concentration profiles are shown in Table 3 and Fig. 4, respectively. Estimates of maximum absorption rates ($T_{\text{max}}$) were similar for the two enantiomers, whereas the estimate for the amount of drug in the absorption compartment when the absorption rate was half of the maximum rate ($K_t$) was twofold higher for L-eflornithine than that for D-eflornithine. The selected structural model adequately described the plasma concentration profiles for D-, L-, and racemic eflornithine (Fig. 5).

**DISCUSSION**

The HAT drug eflornithine is efficacious following the complex mode of intravenous infusions in patients but has so far exhibited disappointing results when administered orally. The present study

![FIG. 3. Observed data versus population predictions and individual predictions for L-eflornithine (white squares), D-eflornithine (gray triangles), and racemic eflornithine (black circles) after intravenous administration. Concentrations are on a logarithmic scale.](image-url)
TABLE 3. Population pharmacokinetic parameter estimates for L- and D-eflornithine after oral administration of racemic eflornithinea

<table>
<thead>
<tr>
<th>Parameter</th>
<th>L-Eflornithine estimate (RSE)</th>
<th>Value for R-eflornithine</th>
<th>D-Eflornithine estimate (RSE)</th>
</tr>
</thead>
<tbody>
<tr>
<td>$T_{max}$ ($\mu$mol/min/kg of body wt)</td>
<td>11.1 (17)</td>
<td></td>
<td>14.5 (10)</td>
</tr>
<tr>
<td>$K_t$ ($\mu$mol/kg of body wt)</td>
<td>1.560 (25)</td>
<td></td>
<td>784 (28)</td>
</tr>
<tr>
<td>$n$</td>
<td>1.424 (21)</td>
<td>38 (29)</td>
<td></td>
</tr>
<tr>
<td>$F$ (%) at 750 to 2,000 mg/kg of body wt</td>
<td>41 (6.5)</td>
<td>10.0 (37)</td>
<td>62.3 (9.5)</td>
</tr>
<tr>
<td>Random residual variability (a)</td>
<td>27.7 (18)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a R-eflornithine represents parameters that were set to be identical for L- and D-eflornithine. CL, $Q$, and central and peripheral volumes of distribution were fixed to the values obtained in fitting the intravenous data. MTT, mean transit time to the absorption compartment; $n$, number of transit compartments before reaching the absorption compartment; $F$, absolute oral bioavailability; IIV, interindividual variability; a, additive residual error; RSE, relative standard error (standard error/mean) × 100.

is the first to describe the stereoselective pharmacokinetics of eflornithine and clearly demonstrates that, in the rat, intestinal absorption following oral administration of racemic eflornithine is stereoselective, with the more potent L-enantiomer being less well absorbed than D-eflornithine. Taken together with a 15% higher CL for L-eflornithine than that for the D-enantiomer, this results in systemic exposure of the L-isomer being only half that of the less active D-form after a single oral dose. Our results indicate that in order to achieve a comparable systemic exposure to the L-isomer after oral administration to that obtained with intravenous infusions, an approximately threefold higher dose is required. The present results, although containing single-dose data only, may therefore contribute to our understanding of why oral eflornithine has been ineffectual in the treatment of late-stage sleeping sickness.

Differing bioavailabilities of the enantiomers are attributed to stereoselective absorption rather than first-pass metabolism. In both rats and humans, >80% of the drug is excreted renally as unchanged drug (7, 11). Together with a low total CL (approximately 90 ml/min and 4 ml/min in humans and rats, respectively), this suggests hepatic first-pass metabolism to be of minor importance for eflornithine bioavailability.

The mechanistic causes for the stereoselective intestinal absorption in the rat are unclear. Eflornithine has a molecular structure similar to those of natural amino acids and could be subject to active intestinal uptake. However, whereas one would generally expect the transport of L-isomers to be favored biologically, the bioavailability of this enantiomer is lower.

Since eflornithine is a relatively small and very polar molecule, paracellular intestinal absorption of eflornithine may occur, but whether such an absorption process would be stereoselective is unclear.

For humans, the available information on the oral absorption of eflornithine is contradictory. In one clinical study, the fraction absorbed was constant with the dose (12), whereas other studies, using higher doses, suggested that eflornithine may display saturable, dose-dependent absorption (10, 16). In rats, oral bioavailability of L-eflornithine was about 40 to 50% for all doses, with no evidence of dose-limiting bioavailability, whereas a slight increase in absolute oral bioavailability was observed following the highest dose. Efforts to model such a putative change in bioavailability with dose, using linear or power models over the four dose levels studied, did not improve fits. In the end, bioavailability was allowed to be estimated separately following the highest dose, although there is no immediate reason why a small but abrupt increase would occur at the highest dose. Our selected dose levels were high and may have resulted in higher intestinal drug concentrations than those in a clinical situation. Still, resulting plasma concentrations and the overall, racemic bioavailability (mean for L- and D-enantiomers) were not dissimilar to those seen clinically, suggesting that the rat is a suitable model for studying the
absorption of eflornithine. The laboratory rat has previously been found to be a good model for prediction of the oral fraction absorbed in humans (9). For further investigation of eflornithine absorption kinetics, lower dose levels given to rats would be of interest but would require a more sensitive stereoselective analytical method.

The atypical plasma concentration-time profile after oral dosing was adequately described with a transit model leading to an absorption rate described by a Michaelis-Menten function. The mechanistic explanation for this absorption profile cannot be explained by the dissolution rate because the drug was given as a solution. The successful characterization of the time profile by a Michaelis-Menten input function suggests that eflornithine is absorbed by active processes but is in conflict with the increased fraction absorbed at the highest dose level. Changes in the physiology of the gastrointestinal tract along its different segments, such as motility, luminal metabolism, epithelial expression of transporters, surface area, blood flow, etc., can result in variable permeability and absorption rates along the gastrointestinal tract. The time to reach maximum concentration increased with the dose from approximately 1.5 to 6 h, suggesting that the actual site of absorption varied with the dose due to the consequence of drug transfer along the gastrointestinal tract. This transfer of drug along the gastrointestinal tract may have given an absorption profile that could be described with a Michaelis-Menten-type function. Such a passage of the drug along the gastrointestinal tract with variable permeability and windows for active transport could contribute to the atypical absorption profile of eflornithine.

A sensitive stereoselective assay for eflornithine is hampered by the lack of chromophores and fluorophores. Realizing that even at the high doses employed we would have some difficulty in separately quantitating the enantiomers in an adequate number of samples for a sufficient duration of time with the analytical method at hand, we chose to support the enantiomeric data by including rich racemic concentration-time data determined with separate groups of rats. Only by combining racemic and chiral data could the pharmacokinetic parameters for the eflornithine enantiomers be estimated. There were insufficient stereospecific data after oral administration to allow estimation of separate interindividual variability values for L- and D-eflornithine, and therefore they were set to be identical. After intravenous administration, enantiospecific interindividual variability could be estimated for CL only.

In conclusion, the stereoselective eflornithine pharmacokinetics after oral and intravenous single dosing in the rat were determined by an approach based on the simultaneous modeling of sparse enantiomeric combined with rich racemic drug concentration-time data. Eflornithine was found to exhibit enantiospecific absorption after oral dosing, with the more potent L-isomer being less well absorbed. The stereospecific difference in absorption may explain why attempts to develop an oral treatment for eflornithine have so far failed.

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