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Mechanism for increased bioavailability of tacrine in fasted rats

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Abstract

The mechanism associated with the increased bioavailability of tacrine as a result of a 24-h period of fasting was examined in rats. The AUC value for tacrine after the fasting was 52% higher compared with normal feeding when 4 mg kg^{-1} of tacrine was orally administered, but the value for velnacrine, a hydroxylated metabolite of tacrine, was reduced by 10%. The relative metabolic ratio of tacrine in urinary excretion ($Au_{tacrine}$ divided by $Au_{velnacrine}$) was lower in fasted rats compared with normally fed rats. This clearly shows that metabolism of tacrine is reduced with 24-h fasting after oral administration. Altered intestinal permeation in the fasting state was hypothesized, and the transport of tacrine across the rat intestine was studied. When a fasted intestine was mounted in an Ussing chamber, the mucosal-to-serosal permeability of tacrine was increased to double that for a fed rat intestine. To examine the effect of absorption rate on the hepatic metabolism of tacrine, a direct pyloric vein infusion study was carried out. Compared with an infusion of tacrine for 5 min, a slow infusion of tacrine over a period of 30 or 60 min increased the hepatic metabolism of tacrine and decreased its systemic clearance in rats. Collectively, these results suggest that rapid transport across the intestine aids tacrine in avoiding hepatic first-pass metabolism and enhances its bioavailability in fasted rats. From these findings, we conclude that both oral administration before a meal and a reduction in the dose might be recommended in tacrine therapy considering the serious hepatotoxicity of tacrine in clinical use.

Introduction

Tacrine (Figure 1A) and its active metabolite velnacrine (Figure 1B) are clinically useful in the treatment of Alzheimer's disease (Puri et al 1989a; Siegfried 1993; Jann et al 2002). Although these drugs are believed to be pharmacologically active as a result of their ability to stabilize neurotransmitters in the brain (Ogura et al 2000; Jann et al 2002), adverse reactions, such as predictable cholinergic effects and non-predictable elevation in serum transaminase levels, are negative factors associated with tacrine therapy in dementia (Crismon 1994; Monteith et al 1996). Rectal and transdermal delivery systems (Ahlin et al 1994; Kim et al 2000) and the chemical modification of tacrine (Gong et al 2004) have been tried, in an attempt to increase the bioavailability and reduce the hepatotoxicity of tacrine.

Following its oral administration, tacrine is rapidly and well absorbed, with peak plasma concentration within 1 h (Madden et al 1995a), and has a low bioavailability which is thought to result from extensive first-pass metabolism (Hartvig et al 1990; Lou et al 1996). Tacrine appears to have a wide tissue distribution, reflected by its large volume of distribution. High concentrations of tacrine can be found in the kidney, liver, adrenal gland and brain following an intravenous or oral dose (McNally et al 1989). The distribution of tacrine in the brain is saturable and mediated by organic cation transport systems (Sung et al 2005). In-vitro metabolism studies have demonstrated the importance of CYP1A in the biotransformation of tacrine to 1-, 2-, 4- and 7-hydroxylated metabolites (Madden et al 1995b), and the 1-hydroxylated metabolite of tacrine was identified as a major stable metabolite (Truman et al 1991). When species differences in the formation of NADPH-dependent metabolites were studied, high levels of velnacrine were found in the urine of the rat compared with the dog and

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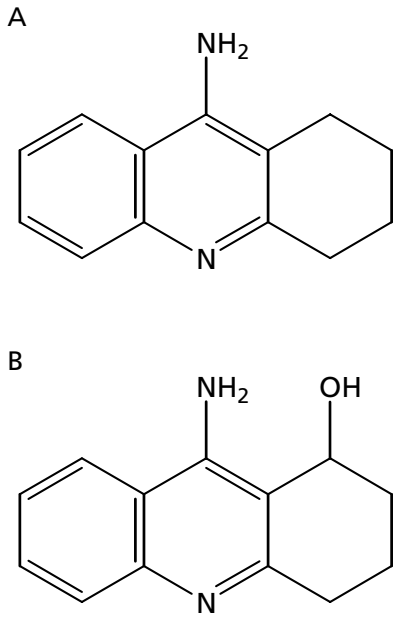

Figure 1 Chemical structures of tacrine (A) and velnacrine (B).

man (Pool et al 1997). In man, mono- and dihydroxylated tacrine and glucuronylated conjugates have been identified in the urine, which was the primary route of excretion (Truman et al 1991; Pool et al 1997). The elimination half life of tacrine is short, 1.5 to 2.5 h after a single oral or intravenous dose (Madden et al 1995a).

Welty et al (1994) studied the temporal effect of food on tacrine bioavailability in man. The mean AUC values after tacrine administration during breakfast (70.2 ng h mL^{-1}) and 2 h after breakfast (74.2 ng h mL^{-1}) were significantly lower than the values determined after the administration of tacrine to subjects that had fasted for 8 h overnight (91.8 ng h mL^{-1}). However, the administration of velnacrine with food resulted in slightly lower peak plasma levels of unconjugated velnacrine, and delayed time-to-peak plasma levels without affecting the AUC and half-life of the drug (Puri et al 1989b). Although the bioavailability of tacrine after overnight fasting was increased, the underlying mechanism for this was not investigated (Welty et al 1994).

The objective of this study was to investigate the effect of fasting on the bioavailability of tacrine after oral administration in rats and to elucidate the mechanism of its altered systemic pharmacokinetics. Of particular interest was the transport process across the intestine and hepatic first-pass metabolism before entering the systemic circulation.

Materials and Methods

Materials

Tacrine (1,2,3,4-tetrahydro-9-aminoacridine, Figure 1A) was purchased from Sigma Chemical Co. (St Louis, MO, USA), and velnacrine was synthesized according to a previous report (Shutske et al 1989). The condensation of anthranilonitrile with 1,3-cyclohexanedione for enamine formation (refluxing toluene, *p*-toluenesulfonic acid, H_2O separation) gave the enamino ketone, which was cyclized in refluxing THF in the presence of K_2CO_3 and CuCl to give 9-amino-3,4-dihydroacridin-1(2H)-one. Reduction with $LiAlH_4$ in THF afforded velnacrine, which was converted to its maleic acid salt. Ketamine (Ketalar; Yuhan Co., K.P. VII) and acepromazine (Sedaject; Samu Chemical Co., K.P. VII) were also used for anaesthesia. Solvents were of HPLC grade and all other chemicals were of analytical grade.

Animals

Male Sprague-Dawley rats (Dae-Han Biolink, Korea) were used in all of the in-vivo pharmacokinetic experiments. Experimental protocols involving the rats used in this study were reviewed by the Animal Care and Use Committee in College of Pharmacy, Seoul National University according to the NIH guidelines (NIH publication number 85-23, revised 1985) of Principles of Laboratory Animal Care.

Pharmacokinetic study

Rats fasted for 24 h and normally fed rats, 250–280 g, were anaesthetized by intramuscular administration of 20 mg kg^{-1} ketamine and 10 mg kg^{-1} acepromazine. The femoral artery and vein were catheterized with PE 50 tubing (Becton Dickinson and Company, Sparks, MD, USA), filled with a heparin solution (i.e., 40 U of heparin per mL of saline), and connected to the cannulae to prevent blood clotting. One hour after recovery from anaesthesia, tacrine (4 mg kg^{-1} dose) was orally administered to the rats. Blood samples (300 μL) were collected from the catheter connected to the artery at pre-determined time points, and centrifuged to collect plasma. Rat plasma (100 μL) was deproteinized by adding 2 volumes of acetonitrile, centrifuged and the supernatant (200 μL) was directly injected on HPLC for assay.

Urine collection

Rats fasted for 24 h and normally fed rats, 250–280 g, were orally administered a 4 mg kg^{-1} dose of tacrine. The rats were housed in individual metabolite cages for the collection of urine at 2, 4, 6, 8 and 24 h after dosing. Fifty microlitres of Tris buffer (pH 10) was added to 50 μL of the urine sample. Tacrine and velnacrine were extracted with a mixed solvent (1 mL) containing ethylacetate and cyclohexane (1:1 by volume). The mixture was centrifuged and the organic layer was transferred and evaporated to dryness. The residue was reconstituted with 200 μL mobile phase (see below), and a 50-μL volume was injected to the HPLC system.

Determination of tacrine by HPLC

Tacrine and velnacrine concentrations in the plasma and urine were determined by HPLC with a fluorescence

detector (Shimadzu, Kyoto, Japan). The same mobile phase and sample pretreatment was used as in our previous study (Sung et al 2005). Separation was performed on a reversed-phase HPLC column (4.6 × 150 mm C-18 column; GL Science Inc., Tokyo, Japan) and analytes were eluted with a mixture of triethylamine, filtered double-distilled water and methanol (5:650:350 by volume; pH adjusted to 4.5). The separation was carried out at a flow rate of 1.2 mL min^{-1}. Fluorescence in the eluent was monitored at an excitation wavelength of 330 nm and an emission wavelength of 365 nm. Calibration curves for the tacrine and velnacrine were constructed in the concentration range of 10–5000 nM. The retention time for velnacrine and tacrine was 2.8 and 4.9 min, respectively. Detector response was linear in the concentration range examined, and inter- and intra-day variations in the assay were less than 15%, indicating that the assay was valid in the concentration range of interest.

Ussing chamber study with the rat intestinal epithelium

Male Sprague-Dawley rats, 320–350 g, after 24-h fasting and normal feeding, were used in this study. Experiments were performed as described in a previous publication with minor modifications (Lee et al 2000). Segments of the jejunum were removed from the rats, immediately rinsed twice with ice-cold saline and placed in ice-cold Hank's Balanced Sodium Salts (pH 7.4) under continuous oxygenation with O_2–CO_2 (95%/5%) bubbling. The specimen was cut open along the mesenteric border with blunt-end scissors and divided into 2.5-cm segments and the intestinal sheet mounted in an Ussing diffusion chamber (Navicyte Inc., San Diego, CA, USA) with 0.64 cm^2 of tissue area exposed.

After mounting, each half-cell was filled with 1 mL of the medium in both the mucosal and serosal sides. After a 30-min preincubation, 1 mL buffer in the mucosal side was replaced by an equal volume of Hank's Balanced Sodium Salts buffer containing various concentrations of tacrine. Samples (500 μL) were taken from the serosal side of chamber at 30-min intervals and replaced with fresh Hank's Balanced Sodium Salts. Experiments were run for 120 min and the tacrine concentration in the medium of the serosal side of the chamber was determined by an HPLC assay. When it was necessary to characterize paracellular leakage, the transport of Lucifer yellow (50 μM initial concentration) was measured in fasted and normal fed intestines.

Pyloric vein administration study

Tacrine was administered via the pyloric vein to study the effect of absorption rate on the first-pass metabolism for tacrine in rats. After rats, 250–280 g, were anaesthetized with an intramuscular injection of ketamine (50 mg kg^{-1}) and acepromazine (10 mg kg^{-1}), the pyloric vein and femoral artery was catheterized with polyethylene tubing, filled with heparinized saline (25 U mL^{-1}). Tacrine was administered at a dose of 4 mg kg^{-1} via a 5-, 30- or 60-min infusion interval to the catheter connected to the pyloric vein. Blood samples (300 μL) were collected from the femoral artery at pre-determined time points, and centrifuged to collect plasma. The plasma samples (100 μL) were deproteinized by adding 2 volumes of acetonitrile, centrifuged and the supernatant (200 μL) was directly used in the HPLC assay.

Data analysis

The area under the tacrine concentration in the plasma versus time curve from time zero to infinity ($AUC_{0\rightarrow\infty}$) was calculated using linear trapezoidal and area extrapolation methods (Gibaldi & Perrier 1982).

The elimination clearance of tacrine after pyloric vein administration was also calculated as follows:

$$CL_{elimination} = Dose/AUC_{0\rightarrow\infty} \quad (1)$$

The transport rate across the intestine was evaluated from the slopes obtained from the linear regression of the amount of tacrine transported versus the time plot for each initial concentration of tacrine. The rate was plotted against initial concentration of tacrine in the apical side. When it was necessary to determine the apparent permeability (P_{app}), the following equation was used:

$$P_{app} = dQ/dt \times 1/C_0A \quad (2)$$

where dQ/dt represents the rate of transport across the intestine, C_0 the initial tacrine concentration and A the surface area of the monolayer.

When it was necessary to compare the means in the pyloric vein infusion, one-way analysis of variance was used. To compare fasted with normally fed rats, Student's *t*-test was used to compare the means. $P < 0.05$ was accepted as denoting statistical significance. Data were expressed as the mean ± standard deviation (s.d.).

Results

Pharmacokinetics of tacrine after 24-h fasted and normally fed rats

Figure 2 shows the temporal profiles for tacrine and velnacrine after fasting and normal feeding. Although 6-h sampling was attempted, it was not possible to detect tacrine at the last time point due to the sensitivity of the assay (Figure 2A). Tacrine concentration increased in the absorption phase and decreased exponentially after reaching a maximum, and the C_{max} was higher in the fasting state compared with the feeding state. Based on a moment analysis, the $AUC_{tacrine}$ value of the fasted rats was 52% greater than that for normally fed rats (Table 1), indicating that bioavailability was increased by a 24-h fasting. On the other

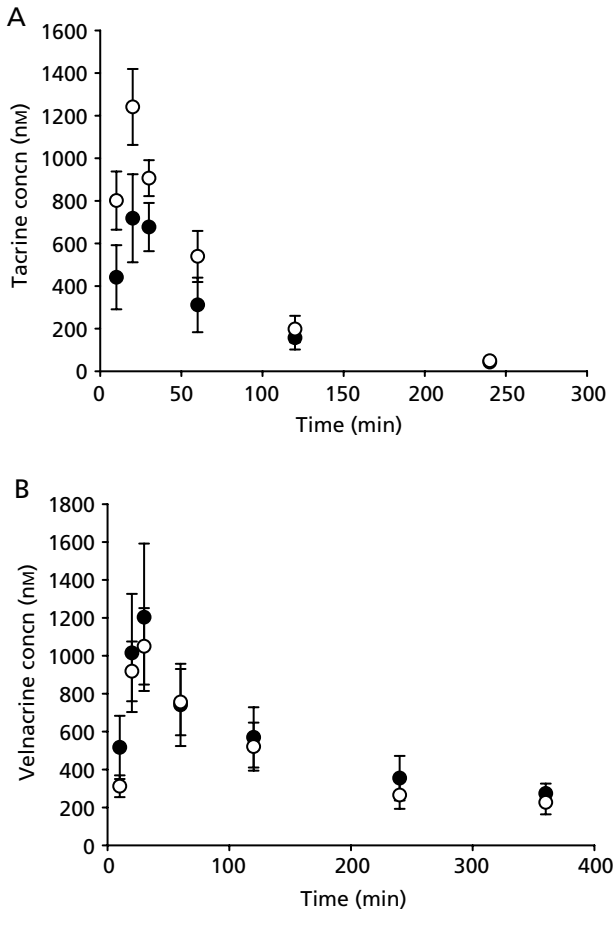

Figure 2 Temporal profiles for tacrine (A) and velnacrine (B) concentrations in plasma after an oral administration of tacrine in fasted (open circles) and fed (closed circle) rats. Data are expressed as the mean ± s.d. of quadruplicate runs.

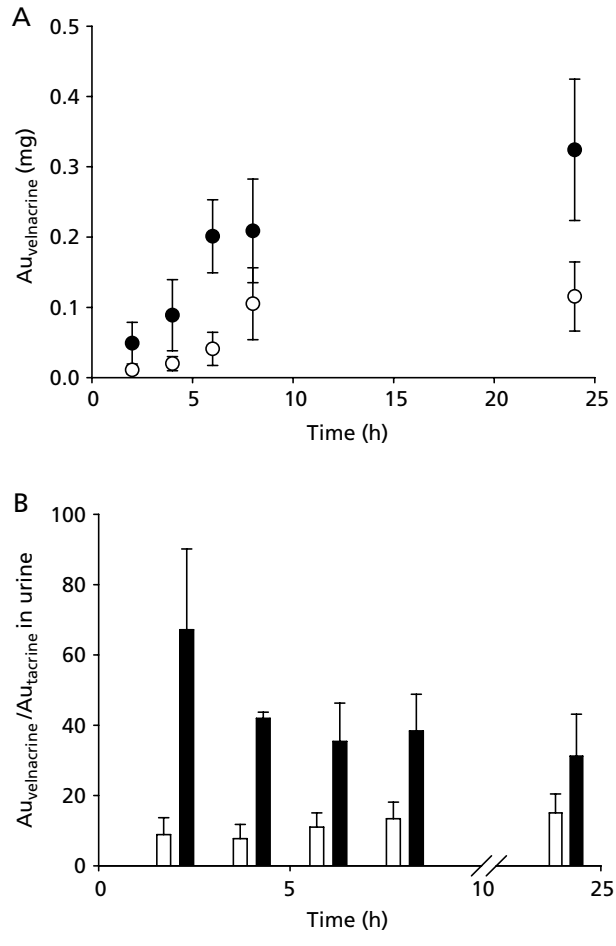

Figure 3 Temporal profiles of cumulative amount of velnacrine (A) and relative metabolic ratio ($Au_{velnacrine}/Au_{tacrine}$) (B) in urinary excretion after an oral administration of tacrine in fasted (open circles and bars) and fed (closed circles and bars) rats. Data are expressed as the mean ± s.d. of quadruplicate runs.

Table 1 Pharmacokinetic parameters for tacrine (4 mg kg^{-1}) after oral administration to rats

	Fed rat	Fasted rat
$AUC_{tacrine}$ (μg min mL^{-1})	11163 ± 3776	16931 ± 2538
$AUC_{velnacrine}$ (μg min mL^{-1})	29046 ± 8085	26015 ± 5385
$AUC_{velnacrine}/AUC_{tacrine}$	2.76 ± 0.68	1.52 ± 0.38*
Bioavailability (%)[a]	27.6 ± 9.3	41.8 ± 6.1*

[a]$AUC_{tacrine,oral}$ divided by $AUC_{tacrine,iv}$. *$P < 0.05$ compared with fed rats.

hand, the $AUC_{velnacrine}$ value after fasting was 10% lower than that for normal feeding. Therefore, the metabolic ratio of tacrine ($AUC_{velnacrine}$ divided by $AUC_{tacrine}$) was decreased by approximately 45% in the fasted rats.

The temporal profiles of velnacrine levels in urine samples are shown in Figure 3A. Compared with tacrine (data not shown), the cumulative amount of velnacrine excreted in fasted rats was significantly lower than in fed rats after oral administration of tacrine. The relative metabolic ratio of tacrine in urinary excretion ($Au_{velnacrine}$ divided by $Au_{tacrine}$) was highest at early time points in normally fed rats, while the ratio appeared to increase with time in the case of fasted rats (Figure 3B). The relative metabolic ratios for the normally fed rats were high at all time points compared with the fasted rats, indicating that the formation of velnacrine was significantly diminished in fasted rats.

Intestinal permeability of tacrine in Ussing chamber experiment

The amount of tacrine (10 μM initial concentration) transported across the intestinal epithelium increased linearly with time (Figure 4A), and increased by approximately twice in the fasting state. The permeability of tacrine (10 μM initial concentration) across the rat intestinal epithelium in fasted rats was found to be $9.23 \pm 1.01 \times 10^{-6}$ cm s^{-1}, while that for the fed was $4.26 \pm 1.48 \times 10^{-6}$ cm s^{-1}. To assess paracellular leakage

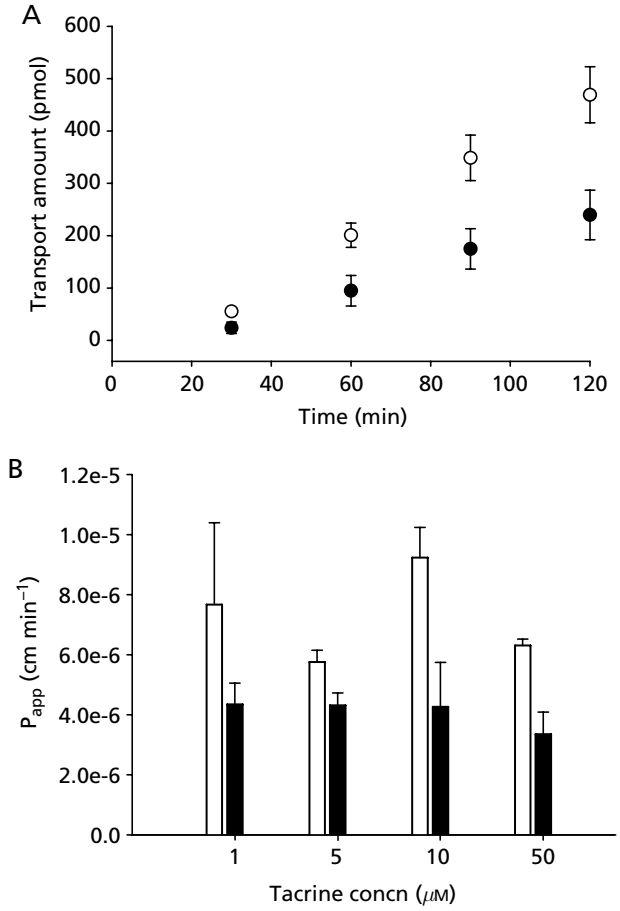

Figure 4 Temporal profiles for the mucosal to serosal transport of tacrine (10 μM initial concentration) in fasted (open circles) and fed (closed circles) rat intestine (A), and the increased transport of tacrine across a fasted (open bars) intestine compared with a fed intestine (closed bars) regardless of initial tacrine concentration (B).

in the intestine, the transport of Lucifer yellow across the intestine was determined simultaneously. The permeability of Lucifer yellow in the fasted rats was $1.72 \pm 0.44 \times 10^{-5}$ cm s^{-1}, approximately 70% higher than that in the fed rats ($1.06 \pm 0.26 \times 10^{-5}$ cm s^{-1}), implying that the increased permeability of tacrine across the rat intestine was mainly via the paracellular route.

The 1-hydroxylated metabolite of tacrine (velnacrine) was not detected in the serosal side of the chamber when tacrine was loaded in the mucosal compartment (data not shown), and as a result the possibility of intestinal metabolism can be ignored during tacrine absorption.

When the concentration dependency of tacrine across the intestine from the apical to basal side was examined, the transport rates were proportional to the initial tacrine concentration in the range of 1–50 μM and the P_{app} values of tacrine were not statistically different (i.e., concentration independent) within fasted and fed groups. However, there were significant differences between fasted and fed groups. The P_{app} values of tacrine in the fasted rats were approximately twice those in the fed rats in the concentration range of 1–50 μM (Figure 4B), and the increased permeability might contribute to the enhanced bioavailability of tacrine after a 24-h period of fasting.

Pyloric vein administration study

Temporal profiles of tacrine and velnacrine after pyloric vein administration are shown in Figure 5. The concentration of tacrine for a 5-min infusion was significantly higher (in the case of early sampling time points) than those for an infusion of 30 and 60 min (Figure 5A insert). However, there was no significant difference in the concentration of velnacrine. The AUC of tacrine was reduced with an increase in infusion time, so elimination clearance (CL) of tacrine (Equation 1) was increased with a slow infusion (Table 2). The metabolic ratios ($AUC_{velnacrine}$ divided by $AUC_{tacrine}$) for 5, 30 and 60 min infusions were 1.34 ± 0.06, 1.76 ± 0.12 and 1.86 ± 0.13, respectively, which tended to increase with infusion time. The hepatic first-pass metabolism of tacrine might decrease if the absorption rate of the drug was increased. Collectively, these data suggest that enhanced intestinal permeability

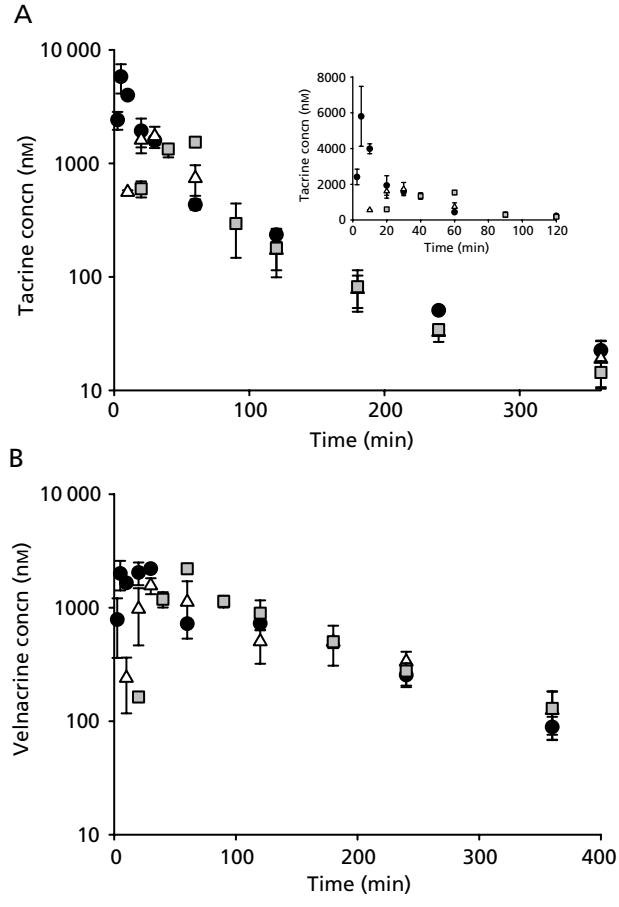

Figure 5 Temporal profiles for tacrine (A) and velnacrine (B) concentrations in plasma after pyloric vein administration of tacrine for 5 (circles), 30 (triangles) or 60 min (squares) in rats. Insert shows the time profiles in detail for periods of up to 120 min. Data are expressed as the mean ± s.d. of triplicate runs.

Table 2 Pharmacokinetic parameters for tacrine (4 mg kg^{-1}) after pyloric vein administration in rats

Parameter	Infusion time		
	5 min	30 min	60 min
$AUC_{tacrine}$ (μg min mL^{-1})	31.78 ± 1.52	21.74 ± 3.86*	17.18 ± 7.46*
$CL_{tacrine}$ (mL min^{-1} kg^{-1})	0.13 ± 0.004	0.18 ± 0.01*	0.23 ± 0.02*
$AUC_{velnacrine}$ (μg min mL^{-1})	42.64 ± 15.27	38.32 ± 3.83	37.69 ± 7.36
$AUC_{velnacrine}/AUC_{tacrine}$	1.34 ± 0.06	1.76 ± 0.12*	1.86 ± 0.13*

*$P < 0.05$ compared with infusion time for 5 min.

followed by avoiding hepatic first-pass metabolism contribute to the increased bioavailability of tacrine in the fasting state.

Discussion

The mechanism for the increased bioavailability of tacrine in rats after a 24-h period of fasting was examined. Compared with normally fed rats, the AUC value for tacrine was 52% higher, and the metabolic ratio ($AUC_{velacrine}$ divided by $AUC_{tacrine}$) was diminished in the fasting state. The relative metabolic ratio of tacrine in urinary excretion ($Au_{velnacrine}$ divided by $Au_{tacrine}$) also increased with time lapse after fasting, while the ratio was decreased with time in the case of normal feeding. For all time points, the relative metabolic ratio in urine samples was always low in fasted rats. This indicates that the bioavailability of tacrine is enhanced after fasting in the case of oral administration.

The possibility that metabolic enzymes are altered, thus increasing the bioavailability of tacrine, cannot be excluded because tacrine is extensively metabolized by CYP 1A and has a low bioavailability. In a previous study, Brown et al (1995) reported the induction of CYP 2E1 and 2B1/2 activity in the microsomes after fasting, while CYP 1A1 remained unchanged. In our preliminary study, the expression of CYP 1A1, together with CYP 1A2, was not changed by fasting (data not shown), which is inconsistent with our hypothesis.

Therefore, we investigated the involvement of active transport process in the intestine since tacrine is actively transported via organic cation transport systems in the brain and kidney (Sung et al 2005). Because the permeability of tacrine across the intestine, however, was concentration independent in the 1–50 μM concentration range, the involvement of active transport systems can be ruled out. However, when the permeability of fasted and normally fed intestine was compared, an increase in the transport of tacrine in the fasted intestine was found. We examined the underlying mechanism involving the increased permeability of tacrine in the fasted rat intestine and found that the paracellular transport of tacrine was largely increased (based on the results of Lucifer yellow, a paracellular marker, transport study). A similar phenomenon was previously reported in which the neural control of ion transport and paracellular permeability was altered by a 48-h period of fasting in the piglet intestine (Carey et al 1994; Carey & Hayden 2000). However, the altered permeability of tacrine across the intestine does not explain the overall mechanism of its increased bioavailability in the fasted rats. As reported previously (Puri et al 1989b), the administration of velnacrine with food resulted in delayed time-to-peak plasma levels without affecting the AUC values. To address this issue, the effect of absorption rate on the hepatic first-pass metabolism of tacrine was examined by means of a direct pyloric vein infusion method. Compared with an infusion of tacrine for 5 min, a slow infusion for 30 or 60 min increased hepatic metabolism and decreased clearance of tacrine. It can be concluded that the hepatic metabolism of tacrine decreases as the absorption rate of tacrine increases.These results suggest that rapid penetration via the paracellular route in the intestine helps tacrine to avoid hepatic metabolism and increases the bioavailability of tacrine in fasted rats.

The bioavailability of tacrine in the rat was enhanced by 24-h fasting when oral administration was used and the underlying mechanism of this increase was studied. The intestinal permeability of tacrine was determined in an Ussing chamber experiment and the mucosal-to-serosal transport of tacrine in the fasted intestine was double that in the fed state. In addition, the hepatic first-pass metabolism of tacrine was examined by a direct pyloric vein infusion method. Compared with an infusion of tacrine for 5 min, slow infusions for 30 or 60 min increased the hepatic metabolism of tacrine and decreased the AUC value. Collectively, those results suggest that the rapid absorption of tacrine across the intestine helps tacrine to avoid the hepatic first-pass metabolism and increases the bioavailability of tacrine in fasted rats. In clinical use, an oral administration before a meal and a reduction in the dose might be recommended in tacrine therapy, considering its serious hepatotoxicity.

Conclusions

Tacrine has mild to severe side effects, such as predictable cholinergic effects and nonpredictable elevations in serum transaminase levels, and these effects limit its use in the treatment of dementia. Tacrine administration after 24-h fasting increased its bioavailability from 28% to 42% by

enhancing the intestinal permeability and bypassing the first pass metabolism in rats. Based on these results, we conclude that tacrine administration before a meal, and reducing the dose, may help patients endure tacrine therapy by diminishing its hepatotoxicity and maintaining the effective therapeutic concentration of tacrine in clinical use.

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