The pharmacokinetics of teniloxazine in healthy subjects and patients with hepatic cirrhosis

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The single-dose and steady-state pharmacokinetics of teniloxazine, an investigational drug with antidepressant and anti-anoxic properties, were compared in 12 healthy volunteers and 12 cirrhotic patients, following oral administration of 80 mg teniloxazine maleate every 12 h for 7 days. In healthy volunteers, an increase in oral clearance, CL_o (from a mean (s.d.) value of 14.6 (3.9) to 18.0 (6.6) ml min⁻¹ kg⁻¹; mean % ratio between the two values (95% CI), 123 (94–151)) and a significant shortening of $t_{1/2}$ (from 6.2 (2.7) to 4.8 (1.4) h; mean % ratio (95% CI), 78 (58–98)) were observed upon repeated administration, suggesting autoinduction of teniloxazine metabolism. In cirrhotic patients, the pharmacokinetic parameters of teniloxazine remained essentially invariant with time. Compared with normal subjects, CL_o was about halved in cirrhotic patients, whereas $t_{1/2}$ was more than doubled. As a consequence of these modifications, the multiple-dose regimen resulted in a two-fold mean drug accumulation in cirrhotic patients, compared with virtually no accumulation in healthy volunteers. Although no adverse events were noted in either study group, it is suggested that maintenance doses for patients with liver dysfunction should initially be at the lower end of the therapeutic range.

Keywords teniloxazine pharmacokinetics liver cirrhosis

Introduction

Teniloxazine is an investigational drug currently undergoing clinical evaluation for the treatment of affective disorders and cerebrovascular insufficiency. The basic pharmacodynamic properties of teniloxazine have been summarized by Ogura *et al.* [1]. This drug is a potent and fairly selective inhibitor of the neuronal reuptake of noradrenaline, with minor inhibitory activity against the uptake of serotonin and dopamine. At daily doses of 60–120 mg, it exerts clinically significant antidepressant actions, but, compared with tricyclic antidepressants, it is virtually devoid of anticholinergic, sedative and cardiovascular adverse effects. Teniloxazine has also been shown to exert anti-amnesic and anti-anoxic actions in various animal models of amnesia and cerebral anoxia.

Current knowledge of teniloxazine pharmacokinetics derives essentially from animal studies [2, 3]. This drug is well absorbed from the gastrointestinal tract, undergoes extensive enterohepatic recycling, and is cleared from plasma with first-order elimination

kinetics, primarily by hepatic oxidative biotransformation. Little is known of teniloxazine pharmacokinetics in man, since only a single-dose study conducted in healthy Oriental subjects is currently available [1]. The present investigation had two main aims:

1. To characterize both the single-dose and steady-state pharmacokinetics of teniloxazine in Caucasian subjects.
2. To evaluate the effect of liver function impairment on the drug disposition kinetics, since preliminary pharmacokinetic data have shown that biotransformation (presumably in the liver) is the main determinant of teniloxazine elimination also in humans, only 6 to 14% of an oral dose being excreted unchanged by the kidney (Yoshitomi Pharmaceutical Ind., data on file).

The safety and tolerance of this multiple-dose administration were also evaluated.

Methods

Protocol

Twelve healthy volunteers, nine males and three females, mean (s.d.) age 38 (5) years, weight 68 (14) kg, and 12 male patients with biopsy-proven liver cirrhosis gave their informed written consent to participate in this study, which was approved by the local Ethics Committee. The criteria for the selection of volunteers (all Italian), who were recruited from the attendant and nursing staff of the ward, were that they should not be heavy consumers of alcohol or tobacco and did not require any regular medication. They were diagnosed as healthy subjects by means of a thorough clinical examination, including medical history, physical examination, electrocardiogram, complete blood count and laboratory test values indicating normal functions of kidney and liver. Mean (s.d.) age and weight of cirrhotics were 54 (5) years and 72 (12) kg, respectively. Serum albumin, total serum bilirubin and % prothrombin levels, expressed as means (s.d.), were 38 (4) g l⁻¹, 23 (13) µmol l⁻¹ and 64 (17), respectively. Pugh's score [4] ranged from 5 to 9 with a mean (s.d.) of 6.5 (1.3), indicating that all cirrhotic patients were in a compensated state. No patient was a carrier of any other disease. During the preceding week and the period of investigation all participants abstained from alcohol and took no drugs.

An 80 mg tablet of teniloxazine maleate, equivalent to 57 mg teniloxazine base, was administered to each subject with 200 ml water every 12 h for 7 days. Venous blood samples were collected into heparinized plastic tubes at 0 (predose), 0.5, 1, 2, 3, 4, 6, 8 and 12 h after the 1st and 13th doses. Additional blood samples were collected before each morning dose (trough concentrations). Blood was centrifuged immediately after collection and plasma samples were stored at -20°C until analysis. Subjects were fasted overnight only before the 1st and 13th doses. After dosing, all subjects remained sitting for 2 h. A light standard meal was provided after 4 h. Electrocardiogram and clinical laboratory tests (haematology, urine analysis and serum chemistry) of each participant were repeated the days before (baseline) and after the dosing period. Pulse rate and blood pressure were monitored before and 1, 2 and 4 h after each administration. Tolerance was assessed by means of general questioning. Adverse events were recorded throughout the dosing period and the following 4 days to detect possible withdrawal reactions.

Drug analysis

A reverse-phase h.p.l.c. method with u.v. detection was developed for the determination of teniloxazine in human plasma. Plasma (1 ml), containing 300 ng imipramine as internal standard, was alkalinized with 0.2 ml of NaOH 1 N and extracted with 6 ml of a mixture of *n*-hexane/isoamylalcohol 98.5/1.5. After centrifugation, the organic phase was transferred into

a glass tube and dried under a gentle stream of nitrogen at 40°C. The residue was dissolved with 250 µl of a mixture of methanol/0.025 N hydrochloric acid 1/1 and injected into a Perkin Elmer h.p.l.c. apparatus equipped with a C-8 reverse-phase column (5 µm particle size; 4.6 mm by 15 cm). The mobile phase was a mixture of 27% v/v acetonitrile and 73% v/v phosphate buffer 0.02 M, pH 3, containing 0.3% v/v triethylamine. The assay was linear ($r^2 > 0.99$) between 5 and 1000 ng ml⁻¹. The intra- and inter-assay coefficients of variation ($n = 8$), determined at 5 and 300 ng ml⁻¹, were below 10 and 5%, respectively. Detection limit was 2 ng ml⁻¹.

Pharmacokinetic parameters were estimated by standard non-compartmental methods [5]. The peak concentration ($C_{\max}$) and the time of its occurrence ($t_{\max}$) were the observed values. The elimination rate constant (λ_z) was determined by log-linear regression analysis of the last 3–4 data points. Elimination half-life ($t_{1/2}$) was calculated as $\ln 2/\lambda_z$. The area under the curve (AUC) was calculated using the linear trapezoidal rule up to 12 h, i.e. within one dosing interval (τ). For first-dose studies, these values were extrapolated to infinity by adding the quotient of the last-measured concentration to the elimination rate constant. Oral clearance (CL_o) was calculated as dose/AUC. The accumulation ratio was calculated from the following equations [6]:

$$R_1 = \text{AUC}(0-12) \text{ after 13th dose} / \text{AUC}(0-12) \text{ after 1st dose}$$

$$R_2 = C_{\min} \text{ after 13th dose} / C_{\min} \text{ after 1st dose}$$

$$R_3 = 1/(1 - e^{-\lambda_z \tau})$$

We performed a power analysis [7] based on the coefficients of variation obtained by Ogura *et al.* [1] for $t_{1/2}$ and AUC. This analysis indicated that 12 subjects should be sufficient to detect differences of 25 and 30%, for $t_{1/2}$ and AUC respectively, with a significance level (α) of 0.05 and a power ($1 - \beta$) of 0.85.

Apart from $t_{\max}$, intragroup differences in pharmacokinetic parameters after the 1st and 13th doses were evaluated by means of Student's two-tailed paired *t*-test, whereas Student's non-paired *t*-test was used for comparisons between control subjects and cirrhotic patients. Wilcoxon's signed rank test and rank-sum test respectively were used as non-parametric tests for $t_{\max}$. Confidence intervals for ratios between parameters were calculated according to Armitage [8] and Hollander & Wolfe [9] for parametric and non-parametric tests, respectively. Intragroup comparisons between differently calculated accumulation ratios and between daily trough concentrations were made by two-way analysis of variance (ANOVA) using the general linear model (GLM) procedure of the statistical analysis system ((SAS (1988) Release 6.03. SAS Institute, Cary, NC, USA)). In the case of significant differences (α : 0.05), the ANOVA was followed by the Newman-Keuls multicomparison test for pairwise comparisons. Correlations were examined by linear regression analysis. Probability values < 0.05 were considered significant.

Results

In most of the subjects examined, oral teniloxazine administration produced irregular plasma concentration-time profiles, with either a convexity in the decay phase or a distinct secondary peak. Nine subjects in each group exhibited such curve irregularities between 4 and 6 h, either after both the 1st and 13th doses or on at least one occasion. The mean values of the pharmacokinetic parameters of teniloxazine obtained after the 1st and 13th doses are listed in Table 1 for both healthy volunteers and cirrhotic patients. In normal subjects, the absorption parameters (C_{max} and t_{max}) after the 13th dose were similar to those observed after the 1st dose. On the contrary, differences were noted in the parameters describing teniloxazine disposition: a nearly significant increase in CL_o ($P = 0.059$) and a proportional decrease in $t_{1/2}$, which became significantly shorter on multiple dosing ($P = 0.040$). Consistent with the shortening of $t_{1/2}$, the degree of teniloxazine accumulation actually observed at steady-state (R_1 and R_2 values) was significantly lower than that predicted on the basis of first-dose half-life (R_3 value). The effect of this time-dependent increase in elimination rate can be further appreciated from Figure 1 which shows that, after an initial significant increase, plasma trough concentrations decreased to a level not significantly different from that measured after the 1st dose.

No significant time-dependent modification of teniloxazine disposition parameters was observed in the cirrhotic group. Consistently, Figure 1 shows that, once steady-state had been attained, trough concentrations remained invariant with time.

Apart from t_{max} and the first-dose value of C_{max} , all

Figure 1 Trough plasma teniloxazine concentrations in a) healthy volunteers and b) patients with liver cirrhosis, given 80 mg teniloxazine maleate every 12 h for 7 days. Bars represent means $\pm$ s.e. mean. Data points having no common letters are significantly different ($P < 0.05$, Newman-Keuls multicomparison test; see **Methods**). Trough concentrations in cirrhotics differed significantly from corresponding concentrations in controls ($P < 0.05$ after doses 1–10; $P < 0.01$ after doses 12–14).

pharmacokinetic parameters in cirrhotic patients differed significantly from those observed in normal volunteers. The terminal half-life and the area under the plasma level-time curve were more than doubled, whereas apparent oral clearance was about halved. Thus, in contrast to control subjects, a two-fold mean

Table 1 Mean (s.d.) pharmacokinetic parameters of teniloxazine following oral administration of 80 mg teniloxazine maleate every 12 h to 12 healthy volunteers and 12 cirrhotic patients. Data obtained after the 1st and 13th doses (left and right columns, respectively)

	C_{max} (ng ml ⁻¹)		t_{max} (h)		$t_{1/2}$ (h)		AUC^a (ng ml ⁻¹ h)		CL_{o1} (ml min ⁻¹ kg ⁻¹)		R_1^b	R_2	R_3
Controls													
Mean	281	282	1.5 ^c	1.5 ^c	6.2	4.8	1030	906	14.6	18.0	1.03	1.09	1.37
(s.d.)	(76)	(135)	(1.2)	(1.2)	(2.7)	(1.4)	(161)	(294)	(3.9)	(6.6)	(0.26)	(0.23)	(0.29)
<i>P</i> value ^e	NS		NS		< 0.05		NS		NS		< 0.05 ^d		< 0.05 ^d
Ratio % ^e	100		100		78		88		123		134 ^d		125 ^d
95% CI	71–130		100–133 ^f		58–98		71–106		94–151		110–157		103–147
Cirrhotics													
Mean	254	470	2 ^c	2 ^c	13.8	13.1	2104	2219	8.0	7.4	1.96	2.01	2.22
(s.d.)	(56)	(194)	(1–3)	(1–3)	(6.8)	(6.7)	(1348)	(1059)	(3.8)	(3.4)	(0.77)	(0.84)	(0.79)
<i>P</i> value (13th vs 1st dose)	< 0.01		NS		NS		NS		NS		NS ^d		NS ^d
(Cirrhotics vs controls)	NS	< 0.05	NS	NS	< 0.01	< 0.01	< 0.05	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01	< 0.01
Ratio % (cirrhotics to controls 13th dose)	166		133		271		244		41		191		184
95% CI	116–216		133–200		186–356		172–317		16–66		143–238		137–232
											125–199		

^aAUC values were calculated from 0 to infinity after 1st dose and from 0 to 12 h after 13th dose; ^baccumulation ratios are defined in **Methods**; ^cmedian value (range); ^dcompared with R_3 ; ^e13th vs 1st dose; ^f94.8% CI.

accumulation of teniloxazine was observed at steady-state.

None of the determined pharmacokinetic parameters was significantly correlated to any of the liver function tests or Pugh's scores.

Electrocardiogram, blood pressure and pulse rate showed no significant modifications with respect to pre-drug baseline levels. Neither were any clinically remarkable changes found in serum chemistry, haematology or urine analysis. No significant subjective adverse effects were reported during the dosing period and the following 4 days.

Discussion

The results of this study have shown that teniloxazine is absorbed and eliminated in our healthy volunteers at rates similar to those previously observed in a comparable group of Japanese subjects [1]. No statistically significant differences in $t_{\max}$ and $t_{1/2}$ ($P > 0.05$ and 0.1 , respectively) were in fact found between our control group and the healthy young volunteers investigated by Ogura *et al.* [1].

Various mechanisms have been proposed to explain the occurrence of secondary absorption maxima or convexities in the concentration-time profiles of orally administered drugs [reviewed in 10]. Although none of the theoretically possible mechanisms can in principle be discarded, the observation of extensive enterohepatic recirculation in animals [2] suggests that biliary recycling of teniloxazine is responsible

for the double-peak profiles observed in our subjects.

The results obtained in healthy volunteers indicate an increase in teniloxazine elimination rate over time and appear compatible with self-induction of teniloxazine metabolism. This conclusion is based on the following observations: 1) a time-dependent shortening of $t_{1/2}$ on multiple dosing, 2) a degree of accumulation less than that predicted on the basis of first-dose half-life, and 3) a biphasic time course of teniloxazine trough concentrations which, after an initial rise, dropped to a significantly lower level. The absence of time-dependent modifications of teniloxazine kinetics in the cirrhotic group is consistent with previous observations on autoinduction, indicating that the ability of liver enzymes to respond to inducing agents may be diminished or absent in liver cirrhosis [see 11 for a recent review]. Liver dysfunction, as assessed here histologically and biochemically, caused a halving of apparent oral clearance and a proportional lengthening of terminal half-life. As a consequence of these modifications, the multiple-dose regimen resulted in a two-fold drug accumulation in cirrhotic patients, compared with virtually no accumulation in the control group.

This study has shown that teniloxazine has a good tolerability in both controls and cirrhotic patients. However, in consideration of the significantly higher steady-state plasma concentrations observed in cirrhotic patients, it is suggested that initial maintenance doses for patients with liver dysfunction should be at the lower limit of the therapeutic range.

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