inconsistent effect on cyclosporin absorption. In this investigation, standard rat chow with 2% corn oil did not appear to alter the oral absorption of cyclosporin from the gastrointestinal tract. For ethical reasons, in laboratory investigations, one would not wish to subject animals to any greater degree of stress than is necessary. Our findings suggest that if one does not wish to fast animals before cyclosporin oral administration, standard rat chow is a suitable feed.

We thank Sandoz Ltd for their generous gifts of cyclosporin solutions and for Sandimmun-Kits. S. Hedayati wishes to thank the SERC for financial support.

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J. Pharm. Pharmacol. 1992, 44: 124–126
Communicated May 25, 1991

Toloxatone pharmacokinetics in the plasma and cerebrospinal fluid of the rabbit

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Abstract—The pharmacokinetics of toloxatone (5 and 10 mg kg^{-1}, i.v.) was studied in anaesthetized rabbits. There was a biexponential decline in plasma concentration with time. No differences were observed in the pharmacokinetic parameters with the increase of the dose. The terminal half-life was short ($47{\cdot}4 \pm 2{\cdot}8$ and $41{\cdot}5 \pm 4{\cdot}2$ min for 5 and 10 mg kg^{-1}, respectively). The total clearance was 79 ± 18 mL min^{-1} after a dose of 5 mg kg^{-1} and 106 ± 40 mL min^{-1} after a dose of 10 mg kg^{-1}. The volume of distribution was $5{\cdot}8 \pm 2{\cdot}8$ (5 mg kg^{-1}) and $5{\cdot}4 \pm 1{\cdot}8$ L (10 mg kg^{-1}). The average percentage of toloxatone bound to plasma protein was 30% and was not affected by concentrations within the investigated range. In cerebrospinal fluid (CSF), the highest concentrations of toloxatone were obtained within 15 min after the end of the injection. The CSF level of toloxatone was equal to that of plasma unbound toloxatone and declined at a rate similar to toloxatone in plasma. These results suggest that the toloxatone passage through the blood–brain barrier may be completed by passive diffusion. In addition, the unbound plasma concentration would accurately reflect the toloxatone concentration in CSF and could be a useful tool for drug monitoring.

A new generation of reversible, selective MAO-A inhibitors has recently been developed with fewer potentially dangerous side effects. One of these, toloxatone (5-(hydroxymethyl)-3-(3-methylphenyl)-2-oxazolidinone), has been found to be an effective antidepressant and is now used for this indication. However, although metabolic studies with this drug (Malnoë & Strolin Benedetti 1975, 1979; Strolin Benedetti et al 1982) are amply documented, no data on the penetration of toloxatone into the cerebrospinal fluid (CSF) has been published.

The present study was carried out to determine the level of toloxatone in the plasma and CSF after an i.v. injection in anaesthetized rabbits, a species in which we are studying the effects of toloxatone and other monoamine oxidase type A inhibitors on the central monoamine systems.

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Materials and methods

Animals. Adult male Fauve de Bourgogne rabbits, 2·5–3·0 kg (Elevage Scientifique des Dombes, Châtillon sur Chalaronne, France), were housed individually in stainless steel cages and were maintained on a 14 h light/dark cycle with lights on from 0600 to 1900 h in a controlled environment (temperature 20–22 °C and relative humidity 40–65%). The animals were acclimatized for one week before the start of the experiments and had free access to a commercial diet (Alimentation UAR, Epinay sur Orge, France) and water.

Study design. Rabbits were randomly assigned to the treatment groups. All experiments began between 0900 and 1000 h. After animals were anaesthetized with urethane (1·5 mg kg^{-1}) the femoral artery was cannulated for blood sampling. CSF was collected from the third ventricle according to the method described by Vistelle et al (1989a). Baseline blood and CSF samples were collected before drug administration. Toloxatone (Delalande, France) was dissolved in physiological saline. Drug (5 and 10 mg kg^{-1}) was injected i.v. in 1 mL kg^{-1} volume into the ear marginal vein at a rate of 1 mL min^{-1}. Blood samples (0·5 mL) were collected into polyethylene tubes containing lithium heparin at 5, 10, 15, 20, 30, 45, 60, 90, 120 and 180 min post injection. Plasma was separated by centrifugation (1000 *g*, 10 min, 20°C). Sequential 15 min samples of CSF (about 100 μL) were continuously withdrawn with a peristaltic pump into polyethylene microsample tubes placed on ice (+4°C). Plasma and CSF were stored at −20°C until assayed.

Analytical method. Plasma and CSF samples were analysed by HPLC as previously described (Vistelle et al 1989b). The calibration curves were linear up to 10 μg mL^{-1} in plasma and 5 μg mL^{-1} in CSF. The limit of sensitivity (signal-to-noise ratio 3:1) of this method was 70 ng mL^{-1}, the recovery of toloxatone from plasma and CSF was about 75%. Inter-assay coefficients of variation (CV) for replicate analysis of 10 and 0·25 μg mL^{-1} plasma samples were 4·64% (n = 5) and 7·66% (n = 5), respectively. Similarly, the CV values for replicate analyses of 5 and 0·125 μg mL^{-1} CSF samples were 3·21 (n = 5) and 4·38% (n = 5), respectively. The intra-assay CV values for the same controls ranged between 3·72 and 5·74%, respectively, for plasma, and between 2·59% (n = 5) and 3·40% (n = 5), respectively, for CSF.

Protein binding. The extent of binding of toloxatone to plasma proteins was investigated, in-vivo, by ultrafiltration dialysis using the disposable Centrifree micropartition system (Amicon, Danvers, MA, USA) which separates free from protein-bound microsolute. Plasma samples were taken from each animal at different times after i.v. drug injection. Percent binding was calculated as [(Cp − Cu)/Cp] × 100, where Cu is the unbound drug concentration determined in the ultrafiltrate solution.

Pharmacokinetic analysis and statistical analysis. The plasma terminal half-life ($t^{1}_{\frac{1}{2}\lambda 2}$) was estimated from the terminal phase by linear regression using a computer program (Shumaker 1986) based on the ESTRIP procedure (Brown & Manno 1978). The area under the plasma concentration time curve (AUC) and the first moment of the curve (AUMC) were calculated by the linear trapezoidal rule with the addition of the extrapolated part: $AUC_t + (C_t/\lambda_2)$ where AUC_t is the area from t = 0 to the last concentration C_t estimated. The apparent volume of distribution (Vd_{ss}) was determined using the ratio: (dose × AUMC)/AUC^2 and the total clearance (CL) was calculated according to: dose/AUC. The mean residence time (MRT) was AUMC/AUC, the terminal half-life ($t^{1}_{\frac{1}{2}\lambda}$) was obtained by 0·693/$\lambda$2 and $t^{1}_{\frac{1}{2}\lambda 1}$ by 0·693/λ_1. The non-parametric Mann–Whitney U test was used to assess significance at a level of $P < 0.05$. Results were expressed as the mean ± s.d.

Results

Plasma pharmacokinetics. A semilogarithmic plot of the concentrations of toloxatone is shown in Fig. 1. Five min after the injection of the drug, total concentrations were 6·5 ± 1·6 μg mL^{-1} (mean ± s.d.) and 15·0 ± 2·2 μg mL^{-1} for 5 and 10 mg kg^{-1} of toloxatone, respectively and the free concentrations were 3·1 ± 0·6 μg mL^{-1} for 5 mg kg^{-1} and 6·8 ± 1·0 μg mL^{-1} for 10 mg kg^{-1}. Elimination of drug was rapid; the total concentrations measured at 180 min after administration (0·24 ± 0·05 and 0·31 ± 0·15 μg mL^{-1} for 5 and 10 mg kg^{-1}, respectively) were less than one tenth of those obtained 5 min after the end of the injection.

Table 1. Pharmacokinetic parameters (mean ± s.d.) of total and free toloxatone in male rabbits (n = 5) after 5 and 10 mg kg^{-1} i.v.

	5 mg kg^{-1}		10 mg kg^{-1}	
	Total	Free	Total	Free
$t^{1}_{\frac{1}{2}\lambda 1}$ (min)	4·9 ± 1·2	7·7 ± 2·8	3·8 ± 1·7	10·3 ± 6·0
$t^{1}_{\frac{1}{2}\lambda 2}$ (min)	47·4 ± 2·8	42·5 ± 3·8	41·5 ± 4·2	36·1 ± 3·8
MRT (min)	72 ± 22	79 ± 25	52 ± 4	60 ± 20
AUC (mg $min\ L^{-1}$)	172 ± 35	139 ± 15	322 ± 83	268 ± 48
CL (mL min^{-1})	79 ± 18	112 ± 16	106 ± 40	145 ± 52
Vd_{ss} (L)	5·8 ± 2·8	10·1 ± 4·8	5·4 ± 1·8	9·7 ± 2·9

The pharmacokinetic parameters of toloxatone are reported in Table 1. For the two doses, plasma concentrations were fitted to a biexponential equation. The AUC and the extrapolated value C_0 increased proportionally to the dose for the total and unbound drug as demonstrated by the lack of statistical differences after normalization to dose. The other parameters were not statistically different. The average percentage of toloxatone bound to plasma protein was about 30% (32·2 ± 12·4%, 5 mg kg^{-1} and 28·1 ± 14·8%, 10 mg kg^{-1}). It was not concentration dependent as shown by the linear regression observed between free (x) vs total (y) concentrations over the whole concentration range studied (y = 1·709x − 0·210; n = 116; r = 0·9632; $P < 0.05$).

CSF pharmacokinetics. Fig. 2 shows the CSF concentrations of toloxatone vs time profiles. The penetration of toloxatone into CSF was very fast; the highest concentrations (1·77 ± 0·23 and 4·66 ± 0·54 μg mL^{-1} for 5 and 10 mg kg^{-1}, respectively) were obtained within 15 min after the end of infusion. The CSF level

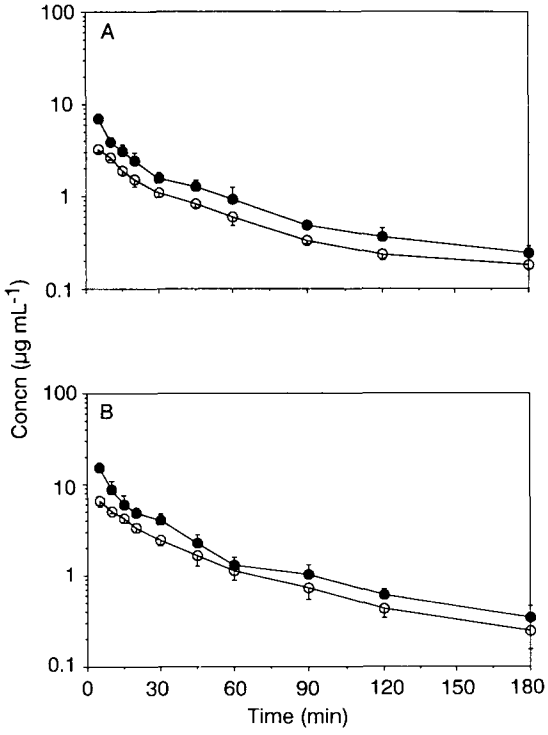

FIG. 1. Log-averaged plasma concentrations (±s.e.m.) of toloxatone after 5 (A) and 10 (B) mg kg^{-1} i.v. doses to 5 male rabbits. ● Total plasma concentration; ○ unbound plasma concentration.

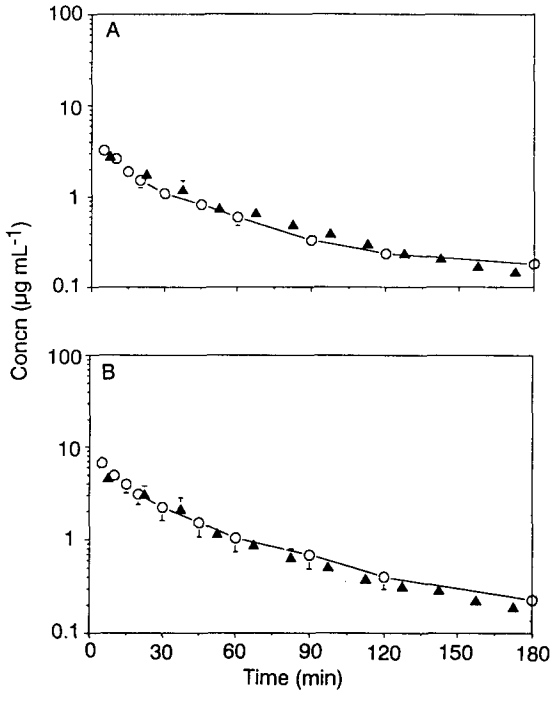

FIG. 2. Log-averaged CSF and unbound plasma concentrations (±s.e.m.) of toloxatone after 5 (A) and 10 (B) mg kg^{-1} i.v. doses to 5 male rabbits. ▲ CSF concentration; ○ unbound plasma concentration.

of toloxatone was equal to those of plasma unbound toloxatone and declined at a rate similar to toloxatone in plasma. Toloxatone had no apparent effect on the CSF flow rate.

Discussion

After an i.v. injection of 5 and 10 mg kg^{-1} toloxatone in the rabbit, the biexponential decrease of concentrations is close to that found by Strolin Benedetti et al (1982) in man; the concentration at 180 min being less than one-tenth of that at 20 min. Because this drug is known to be extensively metabolized in rats (Malnoë & Strolin Benedetti 1979) and man (Strolin Benedetti et al 1982), the lack of a statistical variation of the total body clearance of toloxatone, following the injection of a dose of 10 mg kg^{-1}, indicates that a saturation of the metabolism of the drug does not appear over this dose range. The kinetics of toloxatone are not therefore modified in the range of the studied doses. The very short elimination half-life is in agreement with that (0·6 h) reported in the rat and close to those (1·1, 1·2 and 1·55 h) determined in man (Strolin Benedetti et al 1982), and as in the other species studied, the high total clearance of toloxatone justifies the short elimination half-life of this drug. The distribution volume is comparable with that (1·3 L kg^{-1}) determined in man (Strolin Benedetti et al 1982); however, the protein binding of toloxatone to plasma proteins is lower than that found in the rat (43%) and man (43–53%).

The highest toloxatone concentrations in CSF are observed after the first 15 min. These data reflect an important and fast passage of the drug through the blood–brain barrier (BBB). This result is not surprising considering the physico-chemical properties of toloxatone. This drug is lipid soluble with a partition coefficient n-octanol/buffer aqueous phase (pH 7·4) equal to 15·4 (log P 1·19) (unpublished work), weakly bound to plasma proteins and has a low mol. wt. After 30 min, the toloxatone concentrations in CSF and plasma are similar and decrease in parallel. These results suggest that the toloxatone passage through the BBB is by passive diffusion. In addition, the free

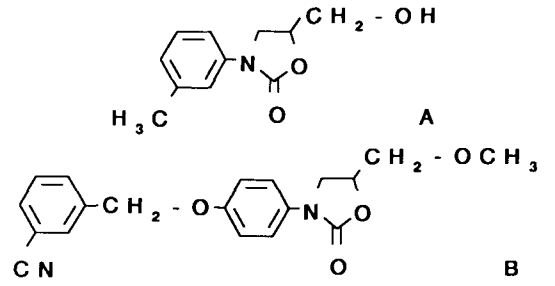

FIG. 3. Structures of toloxatone (A) and cimoxatone (B).

form concentration would accurately reflect the toloxatone concentration in CSF and could be an interesting tool for drug monitoring.

The comparison of toloxatone pharmacokinetics with those of another oxazolidinone is only possible with cimoxatone. The elimination half-life is about 3 h and its protein binding is 95% (Amrein et al 1989). The results obtained in the monkey (single oral dose of 2 mg kg^{-1}) have shown that concentrations of this drug in CSF are lower than those in plasma (Garrick et al 1985), which may reflect, in part, the known propensity of this drug to be highly bound to plasma proteins. Although the structural modifications: substitution of the hydroxymethyl group by a methoxymethyl in position 5 and a benzyloxy residue with a CN in position 3 on the phenyl ring (Fig. 3), increase the affinity and the specificity of this MAO-A inhibitor, they considerably reduce the passage of the drug to CSF.

In conclusion, the pharmacokinetic properties of toloxatone (short elimination half-life, moderate volume of distribution, high total clearance and a weak binding to plasma proteins) established in the rabbit are similar to those reported in rat and man, and the plasma free concentration of toloxatone is an accurate reflection of the drug concentration in CSF.

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