Journal of Pharmaceutical & Biomedical Analysis
Vol. 5, No. 7, pp. 695–700, 1987
Printed in Great Britain

0731–7085/87 $3.00 + 0.00
Pergamon Journals Ltd.

Gas–liquid chromatographic method for the determination of tolperisone in human plasma: pharmacokinetic and comparative bioavailability studies

P. MISKOLCZI,[1]* L. VERECZKEY[1] and R. FRENKL[2]

[1] *Chemical Works of Gedeon Richter Ltd., Department of Pharmacokinetics and Drug Metabolism, Budapest*
[2] *University of Physical Education, Budapest, Hungary*

Abstract: A new capillary GLC method for the determination of tolperisone in human plasma was developed. Pharmacokinetic and comparative bioavailability studies were carried out after i.v. administration and after oral administration of two different preparations of tolperisone. After i.v. administration of the drug the elimination half-life was found to be 1.55 ± 0.7 h (mean ± S.D.), the apparent volume of distribution to be 5.1 ± 1.0 l/kg (mean ± S.D.) and total body clearance to be 140.8 ± 33.8 l/h (mean ± S.D.). The oral bioavailability was found to be 22.3 ± 6.3% for Mydeton tablets and 16.7 ± 8.9% for Mydocalm tablets. There was no significant difference between the bioavailability of two oral tablets.

Keywords: *Capillary GLC analysis; tolperisone; pharmacokinetics; bioavailability.*

Introduction

The term "spasticity" is commonly applied to abnormalities of regulation of skeletal muscle tension that result from lesions of various levels in the CNS. Tolperisone is an antispastic drug acting predominantly within the CNS, which has been found to be an effective agent for controlling spasticity. There is little information about the pharmacokinetic behaviour of tolperisone in humans. The drug concentrations in plasma were studied in the 1970s using gas chromatography equipped with electron capture detection (ECD) [1] and by mass fragmentographic and mass chromatographic techniques in combination with an internal standard labelled with a stable isotope [2]. The aims of this work were to develop a new sensitive method for the determination of tolperisone in human plasma, to investigate its pharmacokinetics and to compare the oral bioavailability of two different brands of tolperisone tablets.

* To whom correspondence should be addressed.

Materials and Methods

Reagents and formulations

Mydeton injections (production batch 30016-0584) and tablets (production batch 51114-0384) were supplied by Gëdeon Richter Ltd. (Budapest, Hungary) and Mydocalm tablets (production batch NK 1053-3840) were supplied by Nippon Kayaku Co. Ltd. (Tokyo, Japan). Hydrochloric acid and benzene (distilled twice in glass) were obtained from Reanal (Budapest, Hungary) and Biogal (Debrecen, Hungary). Ethanol and hexane were obtained from Janssen Chimica (Belgium).

Apparatus

The apparatus was a Hewlett–Packard 5730A gas chromatograph equipped with a nitrogen–phosphorus flame ionisation (NP-Fid) detector. The separation was carried out on a cross-linked methyl silicone glass capillary column (OV-1; 25 nm × 0.2 mm i.d.). Nitrogen was used as carrier gas (flow 1.6 ml min $^{-1}$) and auxilliary gas (flow 30 ml min $^{-1}$). The column pressure was adjusted to 137 kPa; the split ratio was 1:30. The temperature of the oven was 210°C; the injection port, 250°C; and detector, 300°C.

Extraction

To 1.0 ml of plasma in a centrifuge tube the internal standard solution (methadone, 50 ng) and 0.1 ml of ammonia (specific gravity 0.88) were added and mixed. Fifteen millilitres of ethanol were then added and shaken well. The tube was allowed to stand for 1 h then centrifuged at 2000 rpm for 10 min. The supernatant solution was separated and evaporated to dryness. Five millilitres of benzene and 5 ml of saturated potassium carbonate solution were added to the residue and shaken well for 5 min. After centrifugation, the benzene layer was separated and shaken with 5 ml of 0.01 M hydrochloric acid. The mixture was centrifuged and the benzene layer was discarded. 0.5 ml of saturated potassium carbonate solution and 1 ml of *n*-hexane were added to the acid layer and shaken for 5 min. After centrifugation, the hexane layer was separated and evaporated to dryness and the residue was dissolved in 20 μl of ethanol. 1 μl of this ethanolic solution was injected onto the gas chromatograph.

Quantification

The concentrations of tolperisone were calculated from the peak height ratios and appropriate calibration curves. Different calibration curves were prepared for both the i.v. and oral studies. The curves were obtained by extracting plasma samples spiked with increasing amounts of tolperisone (3–200 ng/ml^{-1} for the oral study and 5–1000 ng ml^{-1} for the i.v. study). Calibration curves were prepared every day.

Pharmacokinetic parameters

The pharmacokinetics of the drug was analysed according to a two-compartment open model based on the biphasic decay of the plasma concentration–time curves [3].

The areas under the plasma concentration–time curve (AUC) were estimated by the trapezoidal rule up to the last measured point and thereafter to infinity by integration. The following pharmacokinetic parameters were derived: apparent volume of distribution V_d = $Dose_{i.v.}/\beta AUC$; total plasma clearance Cl_p = $Dose_{i.v.}/AUC$; elimination half-life $t_{½}$ = $ln2/\beta$; bioavailability F = $(AUC_o/DOSE_o)/(AUC_{i.v.}/DOSE_{i.v.})$, where the subscripts o and i.v. represent oral and intravenous administration, respectively.

Tolperisone was administered in a single dose of 100 mg intravenously and in Mydocalm and Mydeton tablets in a cross-over study, with an interval of 3 weeks between doses. The volunteers (Table 1) received the drug at 8 a.m. after an overnight fast. No food was allowed for the following 4 h, but the water intake was not restricted. All the subjects had undergone physical and routine clinical chemistry examinations and none of them had any hepatic, cardiac, gastrointestinal or renal dysfunction. They were non-smokers, had no previous history of alcohol abuse and had taken no other drugs in the previous five weeks.

Blood samples were drawn by venepuncture immediately before drug administration and at 2, 5, 10, 15, 20, 30, 45, 60, 90 min and 2, 3, 4, 6, 7 h after i.v. administration and at 15, 30, 45, 60, 90 min and 2, 3, 4, 6, 7 h after oral administration. 0.5 ml of sodium citrate (3.8% w/v) was added to 4.5 ml of blood as anticoagulant. (The dilution of the plasma was taken into account in the calculations.) After separation from the red cells, the plasma was stored at −20°C until analysed.

Table 1
Details of the volunteers

Subject code	Sex	Weight (kg)	Age	Order of administration
VI	m	75	22	Mydeton; Mydocalm; i.v.
ST	m	70	22	Mydeton; Mydocalm; i.v.
LS	m	80	22	Mydocalm; i.v.; Mydeton
KA	m	67	25	Mydocalm; i.v.; Mydeton
KJ	m	76	23	i.v.; Mydeton; Mydocalm

Results and Discussion

Sensitivity, recovery and precision

The gas–liquid chromatograms for a blank plasma and two samples of plasma spiked with 3 and 20 ng of tolperisone are shown in Fig. 1. The GLC method has adequate sensitivity for the pharmacokinetic studies on the drug; 2 ng of tolperisone can be measured in 1 ml of human plasma. As labelled tolperisone was not available, the recovery from plasma was determined in the following way: a methanolic solution of the drug and the internal standard in known amounts was injected three times and the average peak height ratio was determined. Known amounts of tolperisone were added to 5-5 plasma (1 ml) and the samples were extracted. After extraction, known amounts of internal standard were added to the hexane layer and the peak height ratios were determined. These determinations were carried out at the concentrations shown in Table 2. From these peak height ratios the recovery was calculated to be 65.1 ± 4.5%. The within-day reproducibility was checked on 5-5 identical plasma samples, each spiked with tolperisone in various concentrations. The results shown in Table 2 indicate that within-day precision is satisfactory. Day-to-day reproducibility was less satisfactory confirming the need for daily calibration of the peak height ratios.

Pharmacokinetics and bioavailability

Figure 2 shows the average plasma concentration–time curves after i.v. and oral administration of 100 mg tolperisone. The time course of the plasma tolperisone

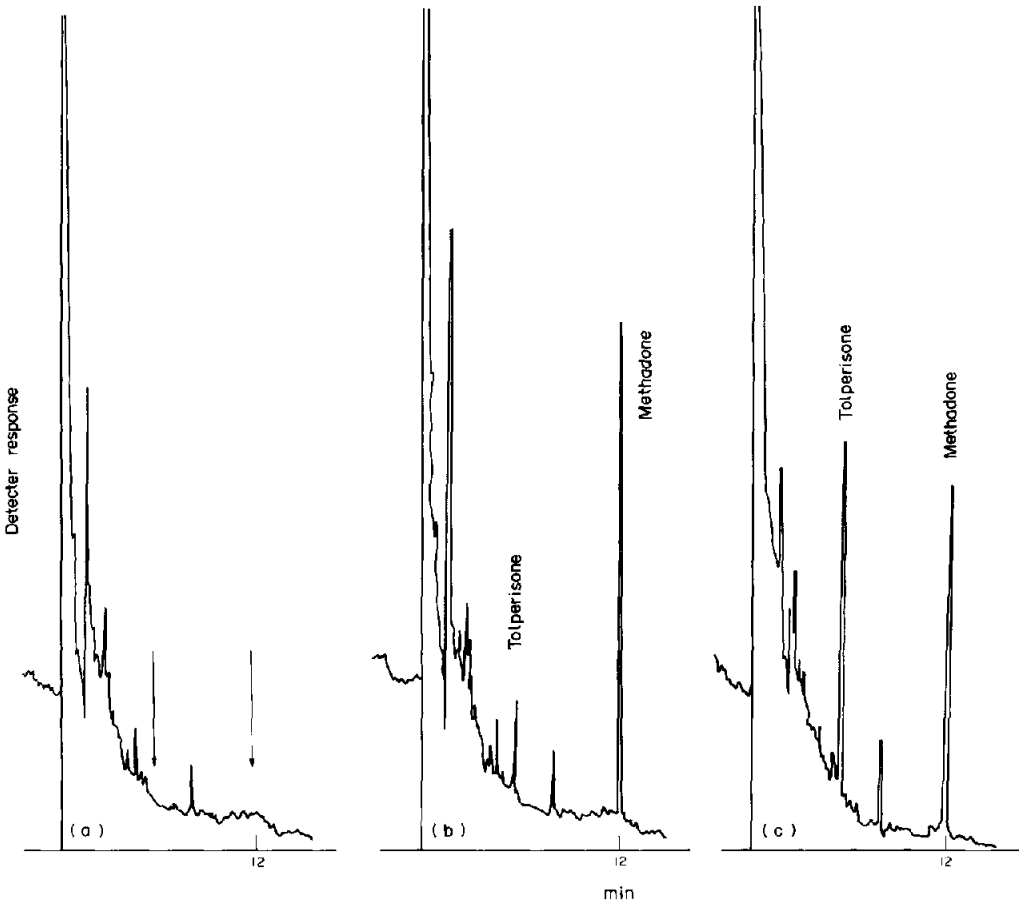

Figure 1
Chromatograms after extraction of human plasma samples: (a) blank plasma; (b) plasma spiked with 3 ng of tolperisone; (c) plasma spiked with 20 ng of tolperisone.

Table 2
Precision and recovery in the within-day determinations of tolperisone in spiked plasma

Amount added (ng ml^{-1})	No. of determinations	Mean amount found/±S.D. (ng ml^{-1})	Recovery (%)
3	4	2.8 ± 0.6	69.1
10	4	9.6 ± 1.8	64.3
50	4	50.2 ± 3.4	58.0
200	4	204.5 ± 8.0	67.4
600	4	597.1 ± 11.4	60.9

Table 3
Pharmacokinetic parameters after i.v. and oral administration of 100 mg of tolperisone

	$T_{1/2}$ (h)	V_d (l)	V_d (kg) (l/kg)	AUC (ng/ml/h)	Cl (l/h)	Cl (kg) (l/h/kg)	F (%)
Intravenous administration							
VI	2.32	364.3	4.9	920.1	108.7	1.5	
ST	2.47	486.4	6.9	733.3	136.4	1.9	
LS	1.52	391.9	4.9	558.0	179.2	2.3	
KA	2.01	286.9	4.3	1007.0	99.3	1.5	
KJ	1.46	338.9	4.5	622.5	160.7	2.1	
Mean ± s.d.	1.55 ± 0.7	373.7 ± 73.9	5.1 ± 1.0	768.2 ± 191.5	140.8 ± 33.8	1.9 ± 0.4	
Oral administration (Mydeton)							
VI	2.88	274.0	3.7	151.8			16.5
ST	2.67	279.2	4.0	137.9			18.8
LS	1.82	158.8	2.0	165.0			29.6
KA	2.37	185.7	2.8	182.5			18.1
KJ	2.60	210.6	2.8	178.4			28.7
Mean ± s.d.	2.46 ± 0.4	221.6 ± 53.4	3.1 ± 0.8	163.1 ± 18.6			22.3 ± 6.3
Oral administration (Mydocalm)							
VI	2.64	411.8	5.5	92.5			10.1
ST	2.26	398.7	5.7	82.0			11.2
LS	2.50	305.4	3.8	118.2			21.2
KA	2.57	345.7	5.2	107.1			10.6
KJ	2.84	217.4	2.9	188.6			30.3
Mean ± s.d.	2.56 ± 0.2	335.8 ± 78.9	4.6 ± 1.2	117.7 ± 41.9			16.7 ± 8.9

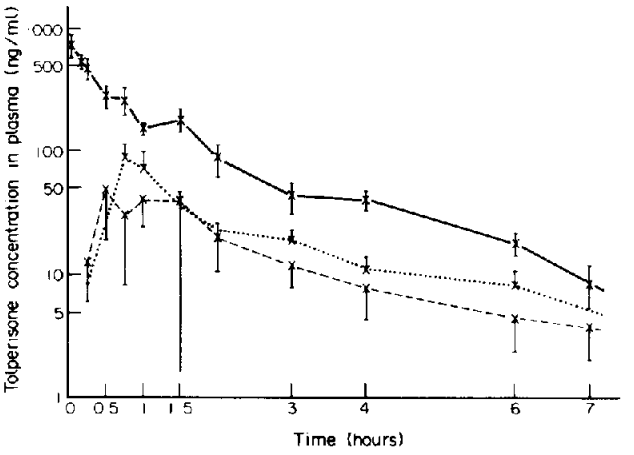

Figure 2
Average plasma concentration–time curves after administration of 100 mg tolperisone: ———, i.v.; · · · ·, Mydeton tablet; – – – –, Mydocalm tablet.

concentrations indicates that the pharmacokinetics of the parent drug can be described by a model containing at least two kinetically distinct compartments. The half-lives of elimination calculated from the slope of the terminal phases of the i.v. curves vary between 1.46 and 2.47 h.

The main pharmacokinetic behaviour (rate of elimination and distribution characteristics) did not alter after oral administration of the drug compared to that after i.v. administration. The absorption was rapid and peak plasma concentrations usually occurred 0.5–1.0 h after ingestion both of Mydeton and Mydocalm tablets. The AUC ratios for each individual showed 22.3 ± 6.3% (mean ± s.d.) bioavailability for Mydeton tablets and 16.7 ± 8.9% for Mydocalm tablets; there was no significant difference at a probability level of 95% ($P < 0.05$). The individual pharmacokinetic parameters and bioavailability data are presented in Table 3.

The low values for F are not necessarily the result of poor absorption; low bioavailability following oral administration can result from first-pass metabolism. Previous studies on the metabolism of tolperisone in humans [4] have shown that less than 0.1% of the dose is excreted unchanged in 24 h urine after i.v. administration. The relatively short half-life and extensive metabolism suggest that substantial first-pass metabolism of tolperisone occurs.

References

[1] J. A. Miller, *Cancer Res.* **30**, 559–562 (1970).
[2] H. Miyozaki, M. Ishibashi, T. Izawa, H. Takayama and G. Idzu, *Chem. Pharm. Bull.* **23**, 837–843 (1975).
[3] M. Gibaldi and D. Perrier, in *Pharmacokinetics* (J. Swarbrick, Ed.), pp. 49–86. Marcel Dekker, New York (1975).
[4] H. Miyazaki, M. Ishibashi, H. Takayama, H. Abaki, G. Idzu, N. Morishita and M. Ando, in *Proceedings of the 4th Symp. on Drug Metab. and Action*, Sendai, Japan, pp. 154–164. Sept. 21–22, 1972.

[*First received for review 3 October 1986; revised manuscript received 21 January 1987*]