Journal of Chromatography, 434 (1988) 278–282
Biomedical Applications
Elsevier Science Publishers B.V., Amsterdam — Printed in The Netherlands

CHROMBIO. 4456

Note

High-performance liquid chromatographic determination of mianserin in plasma and brain and its application to pharmacokinetic studies in the rat

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(First received July 5th, 1988; revised manuscript received August 22nd, 1988)

Mianserin (MIS) is a tetracyclic antidepressant that has a pharmacological effect on presynaptic α-receptors. The concentrations of MIS in various biological tissues have previously been determined by mass fragmentography [1, 2] or gas chromatography (GC) with nitrogen detection [3, 4]. To our knowledge there has been only one report concerning the high-performance liquid chromatographic (HPLC) assay of MIS [5], even though two reports have referred to the HPLC separation of MIS [6, 7]. There are few pharmacokinetic data on MIS in experimental animals [8, 9]. In the present study, a simple HPLC method for the determination of MIS in plasma and brain was developed, and single-dose kinetics were investigated after the intravenous administration of MIS using this HPLC method.

EXPERIMENTAL

Chemicals

Mianserin hydrochloride, desmethylmianserin (DMMIS) and 8-hydroxymianserin (8-OH-MIS) maleate were kindly supplied by Organon (Oss, The Netherlands) and imipramine (IMP) hydrochloride was supplied by Ciga-Geigy (Tokyo, Japan). The standard solutions of MIS, DMMIS, 8-OH-MIS and IMP (100 mg/l) were prepared in methanol and stored at 4 °C, conditions under which the solutions were found to be stable for at least one month. Working solutions of MIS (250 μg/l) were prepared by diluting the standard solutions in 0.02 *M* hydrochloric acid. All other reagents were of analytical grade. For the pharma-

0378-4347/88/$03.50

cokinetic study, MIS (22–24 g/l) was prepared in 30% dimethyl sulphoxide aqueous solution on every day of the experiment.

High-performance liquid chromatography

The chromatographic system consisted of an LC-6A (Shimadzu, Kyoto, Japan) solvent-delivery system, a Model 7125 injector (Rheodyne, Cotati, CA, U.S.A.) and an SPD-6A variable-wavelength detector (Shimadzu) operated at 215 nm. The data were recorded with an integrating recorder (C-R3A, Shimadzu). The instrument was fitted with a 25 cm×4.6 mm I.D. reversed-phase Zorbax-ODS column (5-μm particles; DuPont, Wilmington, DE, U.S.A.), operated at 40°C. The mobile phase was prepared by mixing 680 ml of 1% triethylamine aqueous solution with 320 ml of acetonitrile, with the pH adjusted to 3.5 by the addition of phosphoric acid. The flow-rate was 1.6 ml/min.

Animal experiments

Male Wistar rats weighing 260–290 g were used for all the experiments. Two days before the drug administration, a cannula was inserted surgically into the right jugular vein of each rat to facilitate systematic blood sampling. At zero time, a 20 mg/kg dose of MIS was administered intravenously through another jugular vein with an infusion pump over a 4-min period. Serial blood samples (0.2–2.0 ml) were collected into heparinized tubes at 2, 5, 7, 10, 20, 30 min and 1, 1.5, 2, 4, 6, 8, 12, 16, 20 and 24 h following the dosage. The rats were decapitated, and the blood samples and brains were collected at 2, 12, 20 and 24 h after the injections. The plasma and brain samples were stored at −80°C until assay.

Extraction procedures

The drugs in the brain and plasma samples were extracted according to the previously described method for a tricyclic antidepressant drug, with slight modification [10]. Silanized glassware was used during all extraction processes. Each brain sample was weighed and homogenized in 19 volumes of distilled water by a Polytron homogenizer (Kinematica, Luzern, Switzerland). Then 0.02–1.5 ml of plasma or brain homogenate was transferred to a test-tube containing 0.5 ml of 2 *M* sodium hydroxide and 5.0 ml of internal standard solution (IMP, 10 μg/l in hexane–isoamyl alcohol, 99:1), shaken for 90 min and centrifuged at 3000 *g* for 10 min. The organic phase was collected after the aqueous phase was frozen in a dry ice–acetone bath. The drugs in the organic layer were then back-extracted into an aqueous phase by the addition of 0.1 ml of 0.05 *M* hydrochloric acid and shaking for 30 min. The organic phase was discarded. A sample of the aqueous solution (50–80 μl) was injected into the chromatograph.

Pharmacokinetic analysis

The plasma concentration–time data were estimated using a three-compartment open model in accordance with the following equation:

$$C_p = D[A \exp(-\alpha t) + B \exp(-\beta t) + C \exp(-\gamma t)] \quad (1)$$

where C_p and D are the plasma concentration and administered dose (mg/kg

body weight), respectively. A, B, C, α, β and γ correspond to the pharmacokinetic parameters representing the plasma concentration of MIS. An integrative non-linear least-squares analysis of all the data to be fitted to eqn. 1 was made using the NONLIN computer program.

RESULTS AND DISCUSSION

Selectivity

Fig. 1 shows chromatograms obtained from the plasma extracts with and without drugs. The retention times of DMMIS, MIS and IMP were 4.6, 6.2 and 10.2 min, respectively. These three compounds were well separated, and no substance interfering with the analysis of MIS was detected in the plasma and/or brain homogenate. The other metabolite of MIS, 8-OH-MIS, whose retention time was 1.8 min, was hidden in the large front peak and did not prevent the assay. It seems that the reversed-phase column is better than the adsorption column for HPLC assay of MIS [5, 6]. DMMIS was scarcely present in the plasma of the rats in the single-dose experiment (Fig. 1).

Recovery, linearity and reproducibility

The extraction efficiency was determined using 0.5 ml of rat plasma and brain homogenate spiked with 50 ng of MIS and IMP. We performed six extractions each. The recovery rates from the plasma were 80.0 ± 4.4 and $92.3 \pm 4.8\%$ for MIS and IMP, respectively. Those from the brain homogenate were 80.2 ± 4.2 and $93.2 \pm 4.2\%$.

Plasma standards were prepared from 0.5 ml of rat plasma spiked with MIS in six concentration ranges from 6.25 to 100 ng. Linear responses were obtained by measuring the ratios of the heights (drug peak to internal standard). The regres-

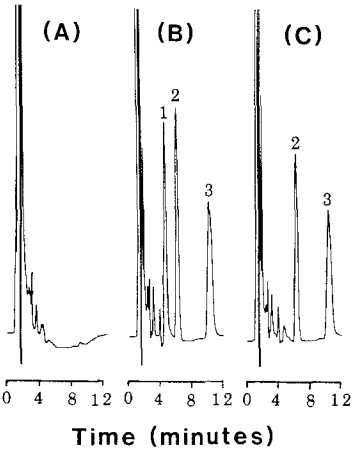

Fig. 1. Chromatograms obtained from 0.5 ml of rat plasma samples. (A) Plasma blank; (B) plasma spiked with 50 ng of MIS, DMMIS and IMP; (C) plasma from rats receiving MIS (the MIS content was calculated to be 84.8 ng/ml). Peaks: 1 = DMMIS; 2 = MIS; 3 = IMP.

sion line was $y=0.0332x-0.022$ ($n=12$, $r=0.999$, $P<0.001$). The minimum detectable level (determined at a signal-to-noise ratio of 2) was 3 ng.

The within-run reproducibility was determined by analysing 0.5 ml of six replicate standard serum samples containing 50 ng of MIS and 0.2 ml of pooled serum (MIS, 216 ng/ml). The coefficient of variation (C.V.) of the standard sample was 1.7% and that of the pooled serum was 1.0%. The between-run reproducibility was also assessed by analysing six standard samples and pooled serum. The C.V. of the former was 1.5% and that of the latter was 3.7%.

The recovery rate, reproducibility and assay sensitivity in the present study corresponded to those of previously reported methods [1–5]. The HPLC method is very simple in comparison with mass fragmentometry and GC.

Pharmacokinetic applications

The time course of the plasma concentration of MIS fitted well to a three-compartment open model by non-linear least-squares regression (Fig. 2). The pharmacokinetic parameters are presented in Table I. The brain/plasma concentration ratio was 14.3 ± 4.5 ($n=12$), similar to that reported by Altamura et al. [9]. The pharmacokinetic profiles in the rats were analogous to those of clomipramine, a basic antidepressant drug, as well as MIS [11]. The values of the Cl_{total} and V_{dss} in the present study correspond closely to those of the previous study, even though the blood samplings were performed within 100 min and the data were analysed according to a two-compartment open model in the previous study

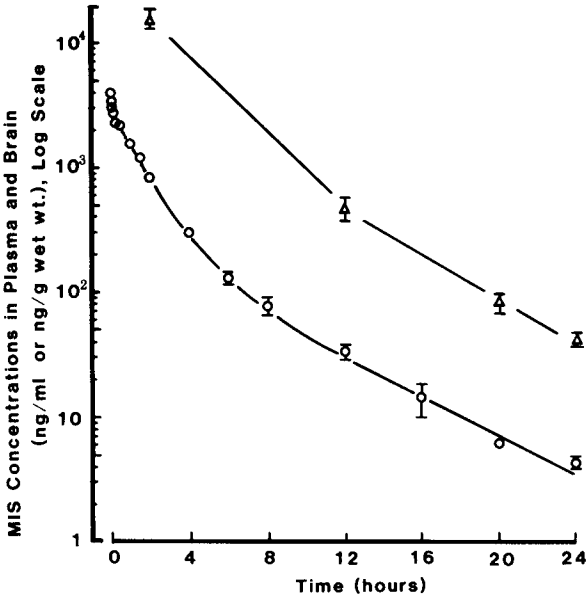

Fig. 2. MIS brain and plasma concentrations as a function of time after an intravenous administration of 20 mg/kg MIS. MIS levels in the plasma and brain at each time point are shown as the means and standard errors in three rats. (○) Plasma MIS; (△) brain MIS.

TABLE I

PHARMACOKINETIC PARAMETERS OF MIS IN RATS

MIS was infused in rats through the jugular vein over 4 min. Plasma concentration–time data following administration were fitted to a three-compartment open model.

A	4838 ng/ml	B	2791 ng/ml	C	254.7 ng/ml
α	0.373 min^{-1}	β	0.0115 min^{-1}	γ	0.00294 min^{-1}
k_{12}	0.206 min^{-1}	k_{21}	0.151 min^{-1}	k_{13}	0.00378 min^{-1}
k_{31}	0.00363 min^{-1}	k_{10}	0.0231 min^{-1}	V_p	2.54 l/kg
Cl_{total}	58.4 ml/min/kg			V_{dss}	8.64 l/kg
$t_{1/2(\alpha)}$	1.86 min	$t_{1/2(\beta)}$	60.3 min	$t_{1/2(\gamma)}$	3.93 h

[8]. The large values of the Cl_{total} and V_{dss} indicate extensive tissue uptake and large hepatic extraction ratios of MIS. The value of Cl in the rat is much higher than that in humans, but the value of the V_{dss} is close to that in humans [12, 13]. The method presented here may permit basic studies of MIS pharmacokinetics in small animals and clinical applications to routine therapeutic monitoring.

ACKNOWLEDGEMENT

The authors thank K. Yokogawa, Ph.D., Hospital Pharmacy, for helpful discussions.

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