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Short communication

Determination of (*R*)-(+)- and (*S*)-(−)-isomers of thiopentone in plasma by chiral high-performance liquid chromatography

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Abstract

A method for the determination of (*R*)-(+)- and (*S*)-(−)-isomers of thiopentone in plasma was developed. Following liquid–liquid extraction, the separation of enantiomers of thiopentone and the internal standard (racemic ketamine) was achieved by high-performance liquid chromatography on an α_1-acid glycoprotein (AGP) column with ultraviolet detection at 280 nm. The mobile phase consisted of 20 m*M* KH_2PO_4 buffer–2-propanol–methanol (93.5:5.0:1.5) at pH 5.0. The flow-rate was 0.9 ml/min. The limit of quantification for each isomer was approximately 10 ng/ml. The assay is suitable for pharmacokinetic studies of (*R*)-(+)- and (*S*)-(−)-isomers of thiopentone, following usual bolus intravenous clinical doses of the racemic drug.

Keywords: Enantiomer separation; Thiopentone

1. Introduction

Thiopentone [5-ethyl-5-(1-methylbutyl)-2-thiobarbituric acid, Fig. 1] is a commonly used intravenous anaesthetic agent, administered by bolus injection for induction of anaesthesia, or by continuous intravenous infusion for maintenance of anaesthesia [1]. Thiopentone is marketed as the racemate. There have been many studies of the pharmacokinetics of racemic thiopentone when given by intravenous bolus and continuous infusion [2]. There have also been studies which have attempted to define the relationship between racemic thiopentone plasma concentration and pharmacodynamic effects, such as encephalographic changes or duration of anaesthesia [3].

In 1976 Mark et al. [4] described preliminary findings of a study in which the two enantiomers of thiopentone had been administered separately to volunteers. It was found that the (−)-isomer was more potent than the (+)-isomer and that the (−)-isomer was eliminated more slowly than the (+)-isomer. These differences in the isomers have not been explored since. They suggest that the plasma concentration of racemic thiopentone following administration of the racemic drug will be an imprecise measure of the concentration of

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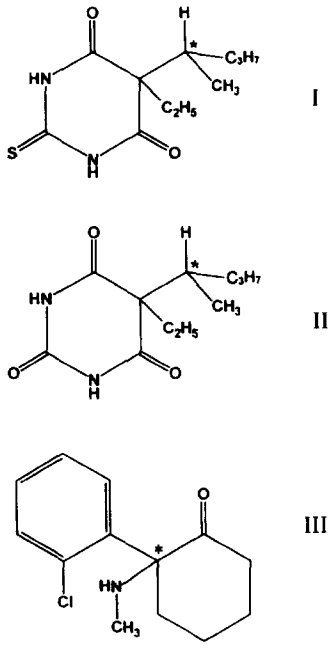

Fig. 1. Structures of thiopentone (I), pentobarbitone (II) and ketamine (III) with asymmetric centre shown.

pharmacologically "active" drug and that the concentrations of the separate enantiomers should be monitored.

This paper describes a relatively simple HPLC procedure which uses a chiral α_1-acid glycoprotein column for determining the plasma concentrations of (*R*)-(+)- and (*S*)-(−)-isomers of thiopentone, following dosing with the racemic thiopentone.

2. Experimental

2.1. Materials and reagents

Racemic thiopentone sodium was supplied by Abbott Australia (Sydney, Australia). Racemic ketamine hydrochloride, sodium hydroxide, hydrochloric acid and KH_2PO_4 were purchased from Sigma (St. Louis, MO, USA). Methanol, 2-propanol (Millipore, Bedford, MA, USA), pentane (Mallinckrodt, Paris, KY, USA) were all HPLC grade. Purified water was obtained using a MilliQ system (Millipore).

2.2. Equipment and chromatographic conditions

The HPLC system consisted of a Model 501 pump, a Model 450 variable wavelength UV absorbance detector and a Model 712B WISP autosampler (Waters, Milford, MA, USA), a chiral AGP column (10 cm × 4.0 mm, Chromtec, J.T. Baker, Phillipsburg, NJ, USA) and a guard column (10 mm × 3.0 mm, Chiral AGP, Chromtec, J.T. Baker). Detector output was recorded on a PC running Maxima 820 software (version 3.30, Millipore, Ventura, CA, USA).

The stereoselective separation was performed at room temperature with an isocratic mobile phase (20 m*M* phosphate buffer–2-propanol–methanol, 93.5:5.0:1.5, pH 5.0), at a flow-rate of 0.9 ml/min.

After each day's use, the column was washed overnight with 10% 2-propanol in water at a flow-rate of 0.1 ml/min.

2.3. Determination of the optical rotation of thiopentone isomers

The eluate from each peak (corresponding to individual enantiomers, Fig. 2) was collected for approximately 500 injections of racemic thiopentone. The combined eluates, containing approximately 1 mg of each isomer, were lyophilised and reconstituted with water (5 ml). UV and optical rotatory dispersion (ORD) spectra, recorded on a Hitachi 150-20 spectrophotometer and a Jasco 720 spectropolarimeter, respectively, were obtained for each enantiomer.

2.4. Standards

Stock aqueous solutions of racemic thiopentone sodium (0.1, 1.0, 10, 50 and 100 μg/ml, i.e. 0.05–50 μg/ml for each enantiomer) were prepared and stored at 4°C in the dark. Drug-free human plasma samples (200 μl) were spiked with stock solution (50 μl) to produce racemic thiopentone sodium concentrations of 0.02, 0.2, 2, 10 and 20 μg/ml (i.e. 0.01–10 μg/ml for each enantiomer) for use as standards.

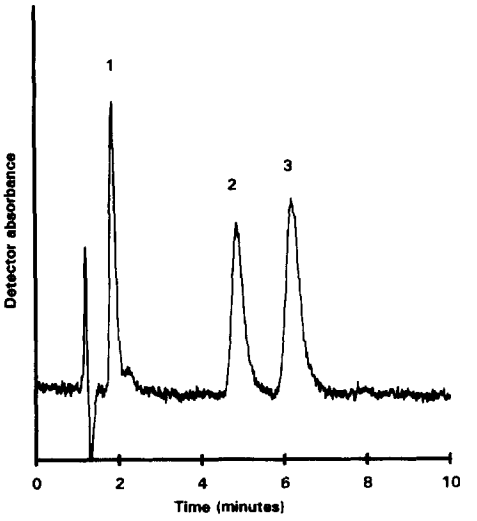

Fig. 2. Representative chromatogram of an extract of a plasma sample obtained after intravenous administration of racemic thiopentone. The concentrations of the (*R*)-(+)-thiopentone (2) and (*S*)-(+)-thiopentone (3) were 2.81 μg/ml and 4.02 μg/ml, respectively. Racemic ketamine (1) (internal standard) concentration was 40 μg/ml.

2.5. Collection of blood samples

A male patient (85 kg in body weight, 180 cm in height and 73 years of age) undergoing surgery, with no evidence of hepatic or renal impairment, received a single 300-mg intravenous dose of racemic thiopentone sodium (Pentothal, Abbott, Sydney, Australia). Blood samples (10 ml) were taken from a radial artery cannula placed for blood pressure monitoring and collected into heparinised tubes (Johns Professional Products, Melbourne, Australia) at 0.5, 1, 2, 3, 5, 7, 10, 15, 30 and 45 min and 1, 1.5, 2, 3, 4, 5, 6, 7, 8, 10, 12, 16, 24, 28 and 32 h. Plasma was separated by centrifugation at 4000 *g* for 10 min and stored frozen for later analysis. The study protocol was approved by the Hospital Ethics Committee on Research.

2.6. Assay procedure

For the extraction of plasma samples, 250 μl of plasma, 50 μl of racemic ketamine (2-*o*-chlorophenyl-2-methylaminocyclohexane, Fig. 1) in methanol (200 μg/ml), 1 ml of KH_2PO_4 buffer pH 6.0 (10 m*M*) and 3 ml of pentane were added to a 10-ml polypropylene tube. The mixture was vortex-mixed for 20 s and centrifuged at 4000 *g* for 10 min. After freezing the aqueous phase in dry ice, the organic (upper) phase was transferred to another 10-ml polypropylene tube and evaporated to dryness under a stream of nitrogen. The residue was reconstituted in 120 μl of mobile phase and vortex-mixed for 10 s before injecting 10–100 μl onto the column.

For the standards, 200 μl of plasma and 50 μl of racemic thiopentone stock solution were extracted as above.

3. Results

3.1. Determination of the optical rotation of thiopentone isomers

The UV spectra were similar to that of racemic thiopentone [5]. The eluates from the first and second peak of racemic thiopentone produced qualitatively similar ORD spectra (Fig. 3) as previously reported for the enantiomers of the

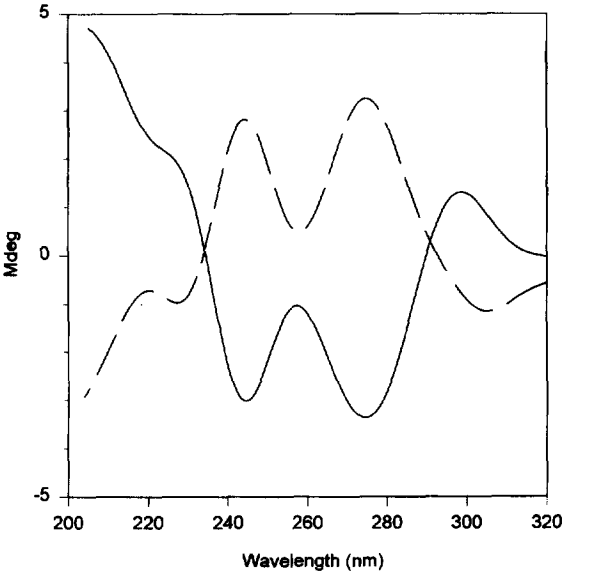

Fig. 3. ORD spectra of the concentrated eluates from the first (solid line) and second (dashed line) peak of racemic thiopentone.

Table 1
Intra-day accuracy and precision ($n = 5$)

Racemic thiopentone spiked (μg/ml)	(*R*)-(+)-Thiopentone		(*S*)-(−)-Thiopentone	
	Measured (mean ± S.D.) (μg/ml)	C.V.[a] (%)	Measured (mean ± S.D.) (μg/ml)	C.V.[a] (%)
0.02	0.0099 ± 0.0011	11.1	0.0120 ± 0.0013	11.2
0.2	0.0990 ± 0.0086	8.8	0.121 ± 0.0094	7.9
2.0	1.03 ± 0.044	4.3	1.03 ± 0.040	3.9
20.0	10.0 ± 0.14	1.4	10.1 ± 0.16	1.6

[a] Coefficient of variation.

desulphurated analogue of thiopentone (*R*)-(+)- and (*S*)-(−)-pentobarbitone [5-ethyl-5-(1-methylbutyl)-2-barbituric acid, Fig. 1], respectively [6]. Thus, by analogy, the first thiopentone isomer to elute was designated as (*R*)-(+)-isomer and the second as (*S*)-(−)-isomer. This was considered reasonable since the only difference between thiopentone and pentobarbitone is the replacement of a carbonyl oxygen with sulphur.

3.2. Assay procedure

A representative chromatogram of the extract of plasma obtained after intravenous administration of racemic thiopentone is shown in Fig. 2. Retention times were 4.84 and 6.18 min for (*R*)-(+)-thiopentone and (*S*)-(−)-thiopentone, respectively, and 1.84 min for racemic ketamine (internal standard). The enantiomers of thiopentone were well resolved from each other, the resolution factor being 2.1. The capacity factor (k') was 2.7 and 3.7 for (*R*)-(+)-thiopentone and (*S*)-(−)-thiopentone, respectively, and the enantioselectivity factor (α) was 1.37.

3.3. Standard curves

The standard curves were linear from 0.01 to 10 μg/ml with r^2 coefficients greater than 0.99 for both (*R*)-(+)-thiopentone and (*S*)-(−)-thiopentone (data not shown). When 250 μl of plasma was used, the minimum detectable level of (*R*)-(+) and (*S*)-(−)-thiopentone, at a signal-to-noise ratio of 4, was 10 ng/ml.

3.4. Accuracy and precision

The intra- and inter-day coefficients of variation, at concentrations of 0.02, 0.2, 2 and 20 μg/ml racemic thiopentone, are shown in Tables 1 and 2, respectively. The recovery, determined in drug-free plasma samples spiked with racemic

Table 2
Inter-assay reproducibility of (*R*)-(+)- and (*S*)-(−)-thiopentone ($n = 3$)

Racemic thiopentone spiked (μg/ml)	(*R*)-(+)-Thiopentone			(*S*)-(−)-Thiopentone		
	Measured (mean ± S.D.) (μg/ml)	C.V.[a] (%)	Mean recovery (%)	Measured (mean ± S.D.) (μg/ml)	C.V.[a] (%)	Mean recovery (%)
0.02	0.0117 ± 0.0018	15.2	78.5	0.0109 ± 0.0011	10.1	79.2
0.2	0.102 ± 0.0022	2.17	70.1	0.108 ± 0.011	9.92	72.3
2.0	1.02 ± 0.02	1.92	65.9	1.03 ± 0.0092	0.89	65.3
20	10.1 ± 0.037	0.37	64.3	10.1 ± 0.027	0.26	61.4

[a] Coefficient of variation.

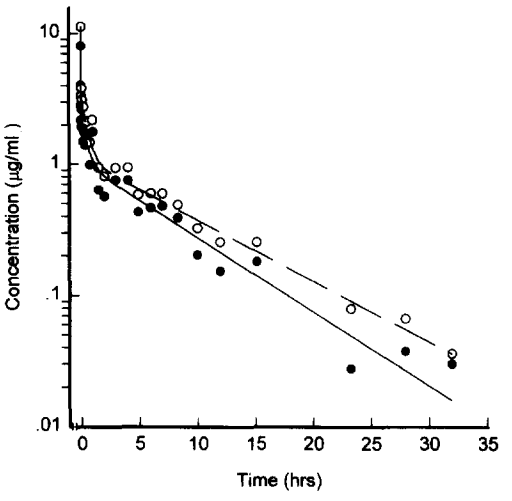

Fig. 4. Plasma concentrations of (*R*)-(+)-thiopentone (●, solid line) and (*S*)-(−)-thiopentone (○, dashed line) following bolus intravenous administration of 300 mg racemic thiopentone, plotted on a semilogarithmic scale.

thiopentone, is also shown in Table 2. The recovery of the internal standard, ketamine was 55.3% ($n = 5$, S.D. = 2.7).

3.5. Application of the assay

The plasma concentrations of the enantiomers of thiopentone, for a patient who received a 300 mg bolus dose of racemic thiopentone are shown in Fig. 4. Nonlinear least squares regression analysis (SigmaPlot, Jandel Scientific, San Rafael, CA, USA) showed that both plasma concentrations profiles were best characterised by a tri-exponential equation. The elimination half-life for the (*R*)-(+)-isomer was 5.34 h and that for the (*S*)-(−)-isomer was 6.49 h. Plasma clearance of each isomer was 282 and 197 ml/min, respectively.

4. Discussion

We have developed an assay, which uses a chiral AGP column, suitable for studying the pharmacokinetics of (*R*)-(+)- and (*S*)-(−)-thiopentone following an intravenous bolus injection. It is a direct method of assay which minimises the introduction of impurities and the possibility of incomplete derivatisation which can occur with derivatisation methods [7,8].

The degree of separation of (*R*)-(+)- and (*S*)-(−)-thiopentone was satisfactory, as indicated by the resolution factors and the chromatogram (Fig. 2). Various extraction solvents, such as dichloromethane, *n*-pentane, diethyl ether and ethyl acetate were tested. However pentane produced the highest extraction recovery in the assay (61%), and was relatively convenient to use. In the assay, there was no interference by pentobarbitone, a major metabolite of thiopentone, which has a UV maximum absorbance at 212 nm but no absorbance at 280 nm. We found that the assay cannot easily be adapted to analyse pentobarbitone however. Due to the greater polarity of pentobarbitone compared with thiopentone the extraction recovery of pentobarbitone was poor. Moreover, under the chromatographic conditions used the retention time of the pentobarbitone isomers was very short.

Racemic ketamine, the internal standard used in the thiopentone assay, is also an intravenous anaesthetic drug. We evaluated six achiral barbiturate compounds, including phenobarbitone and amylobarbitone, as possible internal standards, but all of these exhibited minimal chromatographic retention under the conditions of the assay. Ketamine is an organic base with a pK_a of 7.5 but thiopentone is an organic acid with a pK_a of 7.6. Although it is not possible to select a pH at which both drugs are completely unionised and completely extracted, there was sufficient of the highly lipophilic unionised moiety of each drug at pH 6.0 and a sufficient excess of organic compared with aqueous phase to enable a reliable recovery of each. At this pH, recovery of thiopentone and ketamine was 70 and 55%, respectively. While recovery of thiopentone was greater at pH 1.0, recovery of ketamine was only 10% at this pH. Under the present assay conditions, ketamine appeared as a single peak even though it was used as the racemic mixture. The absence of resolution of the enantiomers of ketamine is seen as an advantage for its use as an internal standard.

The assay is relatively simple and produces a

linear standard curve over the plasma concentration range studied. Intra-assay accuracy and precision are high with a sensitivity (10 ng/ml) making it suitable for measuring the plasma concentrations of thiopentone for up to 30 h after intravenous administration of usual induction doses of the racemic drug (Fig. 4). The results from this preliminary study indicate that the (+)-isomer was eliminated more rapidly than the (−)-isomer as reported by Mark et al. when the isomers were administered separately [4], and we are currently investigating this difference.

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