Pharmacokinetics of thiopentone enantiomers following intravenous injection or prolonged infusion of *rac*-thiopentone

D. J. Cordato,¹ A. S. Gross,² G. K. Herkes,¹ & L. E. Mather³

¹Department of Neurology, Royal North Shore Hospital and University of Sydney

²Department of Clinical Pharmacology, Royal North Shore Hospital and

³Department of Anaesthesia and Pain Management, Royal North Shore Hospital and University of Sydney, Sydney, Australia

Aims Thiopentone is administered as a racemate (*rac*-thiopentone) for induction of anaesthesia as well as for neurological and neurosurgical emergencies. The pharmacokinetics and pharmacodynamics of *rac*-thiopentone have been extensively studied but the component R-(+)- and S-(−)- enantiomers, until very recently, have been largely ignored.

Methods The present study analyses the pharmacokinetics of R-(+)- and S-(−)-thiopentone in 12 patients given *rac*-thiopentone intravenously for induction of anaesthesia and five patients given a prolonged infusion of *rac*-thiopentone used for treatment of intracranial hypertension.

Results The mean total body clearance (CL_T) and apparent volume of distribution at steady-state (V_{ss}) showed trends towards higher values for R-(+)- than for S-(−)-thiopentone in both patient groups; CL_T and V_{ss} of unbound fractions of R-(+)- and S-(−)-thiopentone, however, did not show these trends. The time courses of R-(+)- and S-(−)- thiopentone serum concentrations were so similar that EEG effect could not be attributed to one or other enantiomer. Serum protein binding for S-(−)-thiopentone was greater than for R-(+)-thiopentone ($P=0.02$) and 24 h urinary excretion of R-(+)-thiopentone was greater than for S-(−)-thiopentone ($P=0.03$). In one patient, concomitant measurement of CSF and serum thiopentone concentrations found that serum: CSF equilibration of unbound fractions of both enantiomers was essentially complete.

Conclusions The study was unable to determine any pharmacokinetic difference of clinical significance between the R-(+)- and S-(−)-thiopentone enantiomers and concludes that minor differences in CL_T and V_{ss} could be explained by enantioselective difference found in serum protein binding.

Keywords: thiopentone, enantiomers, pharmacokinetics

Introduction

Thiopentone is most commonly used for the induction of anaesthesia. It is also used to a lesser extent in the treatment of acute neurological and neurosurgical emergencies including closed head injury and refractory status epilepticus due to its ability to reduce intracranial pressure, cerebral blood flow and oxidative metabolism.

Thiopentone is a racemic drug (*rac*-thiopentone) used as an equimolar mixture of R-(+)- and S-(−) enantiomers. It is well established that the enantiomers of a racemic drug may possess different pharmacokinetic and pharmacodynamic properties [1, 2]. The pharmacokinetics and pharmacodynamics of *rac*-thiopentone have been extensively investigated over many decades but most studies until very recently have ignored its enantiomeric duality, reporting only on the sum of enantiomer concentrations [3, 4]. This is despite Christensen & Lee [5] and Haley & Gidley [6] having demonstrated that S-(−)-thiopentone was a more potent

hypnotic than R-(+)-thiopentone in mice and Mark *et al.* [7] having shown that S-(−)-thiopentone was more potent in terms of duration of sleep and apnoea in human subjects.

There is no definitive published literature on the differential pharmacokinetic and pharmacodynamic effects of R-(+)- and S-(−)-thiopentone. Although pharmacodynamic studies are difficult to interpret when a drug is given as a racemate, recent studies, based upon separation of R-(+)- and S-(−)-thiopentone enantiomers using high-performance liquid chromatography (h.p.l.c.) [8, 9] have provided evidence of differential pharmacokinetics after administration of *rac*-thiopentone [10, 11].

The aim of the present study was to determine whether there is a pharmacokinetic difference between R-(+) and S-(−)-thiopentone which could result in a difference in the time course of effect following a single intravenous bolus of *rac*-thiopentone in human subjects. Blood and urine levels of R-(+)- and S-(−)-thiopentone were measured by chiral h.p.l.c. [8] in concert with an evaluation of pharmacological effect by spectral frequency electroencephalographic (EEG) analysis. The study also investigated whether there were pharmacokinetic differences between R-(+)- and

S-(–)-thiopentone under conditions of prolonged intravenous infusion of *rac*-thiopentone when used for treatment of intracranial hypertension.

Methods

Two separate studies were performed to investigate whether there were differential pharmacokinetics of the thiopentone enantiomers in humans. Study 1 was based upon intravenous bolus doses of *rac*-thiopentone used in induction and maintenance of anaesthesia in surgical patients. Study 2 was based upon prolonged infusion of *rac*-thiopentone in patients with neurological or neurosurgical emergencies including severe head injury. All patients or relatives gave informed written consent. Approval for the studies was granted by the Medical Research Ethics Committee of Royal North Shore Hospital.

Study 1

Twelve patients (seven male, five female) undergoing back surgery (six lumbar and six cervical laminectomies and/or fusions) by the same surgical and anaesthetic team at Royal North Shore Hospital (RNSH) were enrolled in this study. The mean age of subjects was 62 years (age range 38–82 years). All patients had no prior history of cardiac failure, renal or hepatic dysfunction. Five patients were otherwise healthy and on no regular medications. Intercurrent problems in the remaining seven subjects included well controlled hypertension in five, non-insulin dependent diabetes mellitus in three, previous peptic ulcer disease in one and rheumatoid arthritis in one. Individual patient details are given in Table 1.

Approximately 30 min prior to induction of anaesthesia, morphine sulphate (10 mg) and atropine sulphate (0.6 mg) were given as premedication in six patients, pethidine hydrochloride (100 mg) in one patient and temazepam (10 to 20 mg) and metoclopramide (10 mg) in the remaining five patients. *Rac*-thiopentone sodium (Abbott Australasia, 50 mg mL⁻¹) was given as an intravenous bolus injection over 10 s. The mean dose of *rac*-thiopentone used was

277 mg (range 125–500 mg), as decided by the treating anaesthetist on clinical grounds. Pancuronium or vecuronium (8–14 mg) were also given by the anaesthetist at induction of anaesthesia in all 12 patients and a further 10–20 mg morphine sulphate was given in nine patients and 50–300 µg fentanyl citrate in two patients at induction. Following induction of anaesthesia all 12 patients also received 1 g cephazolin. General anaesthesia was maintained during the operation using nitrous oxide: oxygen 2:1 and isoflurane 0.5–1%.

Arterial blood samples were collected from an indwelling cannula inserted prior to surgery for the purpose of intraoperative haemodynamic monitoring. Blood samples (1–2 ml) were collected at 1, 3, 5, 7, 10, 15, 30 and 60 min after *rac*-thiopentone administration into 5 ml tubes (SST Becton Dickinson and Co.). Further samples were taken every 1–2 h following administration of the thiopentone until removal of the arterial cannula after surgery, with a total post-dose sampling time of 180–480 min (average: 280 min). The maximal sampling time was limited by the Ethics Committee approval which required cessation of blood sampling after removal of the arterial cannula. Twenty-four hour urine samples were also collected in eight patients.

Electroencephalographic analysis

Eight channel bipolar EEG (electroencephalographic) recordings (Fp1–F3, Fp2–F4, F3–C3, F4–C4, C3–P3, C4–P4, F4–F3, P4–P3) were performed successfully in eight patients. Leads were placed in accordance with the international 10/20 system [12]. After gentle scrubbing of the scalp with Omniprep (D.O. Weaver and Co) abrasive solution and attachment of silver/silver chloride disc electrodes to the relevant scalp site using B5 collodion (Specialised Laboratory Equipment Ltd), electrodes were connected to a Vickers Medical Medelec DG 32 Compact EEG recorder. Baseline EEG recording was performed in six of the eight patients for a minimum of 5 min prior to induction of anaesthesia and in all eight patients for at least 60 min after intravenous injection of *rac*-thiopentone. Baseline EEG monitoring was

Table 1 Demographic data and R-(+)- to S-(–)- thiopentone ratios of CL_T, V_{ss}, 24 h urinary excretion and serum protein binding for individual patients in study 1. N/A: not available.

Patient	Sex	Age (years)	Operation (site)	<i>rac</i> -thiopentone dosage (mg)	EEG study	CL _T R/S ratio	V _{ss} R/S ratio	24 h urine excretion R/S ratio	Serum protein binding R/S ratio
1	M	69	Cervical	400	No	1.08	0.89	N/A	N/A
2	F	79	Lumbar	125	Yes	1.20	1.16	1.15	N/A
3	M	76	Lumbar	250	Yes	1.04	1.24	N/A	N/A
4	M	82	Lumbar	250	Yes	1.07	0.98	0.96	N/A
5	F	76	Lumbar	200	No	1.17	0.97	1.45	0.99
6	F	44	Cervical	300	Yes	1.07	1.24	1.42	N/A
7	M	60	Lumbar	350	Yes	0.78	0.78	0.97	1.01
8	M	68	Lumbar	250	No	0.99	1.04	1.31	0.99
9	F	40	Cervical	250	Yes	1.06	1.12	N/A	N/A
10	M	38	Cervical	500	Yes	1.04	1.08	1.52	0.98
11	F	54	Cervical	225	Yes	0.99	1.34	N/A	0.95
12	M	60	Cervical	225	No	1.34	1.28	1.05	0.98

of short duration in two patients and not included in the data analysis.

Power spectrum analysis, a measurement of power or vertical peak of a sampled EEG region against frequency values for the same region of EEG, was performed on the recordings of all eight patients. The spectral edge was defined as the upper boundary of that frequency containing 95% of the total power in the channel's power spectrum. The median frequency was the frequency at which half the total power was below and half the total power above that frequency. Spectral EEG data were analysed from the time of onset of induction of anaesthesia with *rac*-thiopentone to 10 min following induction. Results of mean values of spectral edge and median frequencies were documented for all eight patients before, then every 30 s following the intravenous injection of thiopentone for 5 min and every 60 s for a further 5 min for each of the eight bipolar channels used. It is acknowledged that the EEG could have been influenced by the other medications administered but it is believed that thiopentone produced the dominant drug effect during this time frame.

Study 2

Five patients receiving prolonged intravenous infusions of *rac*-thiopentone sodium (Abbott Australasia) and undergoing arterial blood sampling during and for a maximum of 5 days post-infusion were studied. There were four male patients and one female patient, mean age 32 years (range 20–47 years). The mean total dose of *rac*-thiopentone was 37.6 g (range 26.1 to 54.5 g) given over a mean total duration of 62.6 h (range 31 to 92 h). Details of individual patients are given in Table 2.

Rac-thiopentone infusion was commenced to reduce intracranial pressure and/or cerebral oxidative metabolism in all five patients: three had suffered severe head injury following motor vehicle accidents, one a subarachnoid haemorrhage due to aneurysmal rupture and one cerebral venous sinus thrombosis (patient 17). Two of the patients with severe head injury died during hospitalisation, patient 15 within 12 h of cessation of *rac*-thiopentone infusion from the effects of a closed head injury and patient 14 5 days following completion of *rac*-thiopentone infusion as a result of sepsis and post-traumatic pancreatitis.

CSF was collected at regular intervals during and following infusion of *rac*-thiopentone in patient 17.

Sample preparation and analytical techniques

Within 30–60 min of collection all blood samples were centrifuged at 3000 rev min⁻¹ for 10 min and the serum was stored at -20°C until analysis. Measurement of thiopentone enantiomer concentrations in serum, urine, buffer and CSF was performed within 3 weeks of collection by the chiral stationary phase h.p.l.c. method described by Huang *et al.* [8]. The limit of quantitation for R-(+)- and S-(-)- thiopentone in serum, buffer and urine were 5 ng ml⁻¹ and 6 ng ml⁻¹ respectively (signal to noise ratio >4:1). At concentrations ranging from 1–100 µg ml⁻¹ in serum, buffer and urine, the recovery of R-(+)- and S-(-)-thiopentone enantiomers was greater than 70% and there was less than 5% loss of recovery of R-(+)- or S-(-)-thiopentone when serum and buffer samples were stored at -20°C for 3 months. The intra-day and inter-day variabilities for the measurement of R-(+)- and S-(-)-thiopentone in serum, buffer and urine were less than 8%. The calibration curves were linear with *r*² coefficients greater than 0.99.

Urine was kept in sterile 4 l glass containers at room temperature until completion of each 24 h collection. A preliminary study, performed and repeated under similar conditions with known concentrations of R-(+)- and S-(-)-thiopentone enantiomers and known urine volumes, excluded any significant loss of recovery of thiopentone in urine by this method. The total volume of urine was quantitated and 10 ml of urine was stored at -20°C until analysis.

Serum protein binding analysis was performed using pooled serial serum samples of six patients from study 1 and four patients from study 2. Equilibrium dialysis was performed in glass cells containing 2 ml serum adjusted to pH 7.4 by the addition of 10–20 µl 0.5 M H₃PO₄ and separated by a cellulose membrane (Selbys Scientific Ltd) from 2 ml pH 7.4 0.067 M phosphate buffer solution. The glass cells were equilibrated at 35°C for 8 h. At the completion of equilibration, pH measurement confirmed that no change occurred during dialysis and protein dipstick testing (Bayer Diagnostic Multistix 8 SG) of phosphate buffer confirmed there was no leakage of protein across the cellulose membrane. The mean post-dialysis serum concentrations of R-(+)- and S-(-)-thiopentone were 0.57 µg ml⁻¹ (range 0.17–1.5 µg ml⁻¹) and 0.58 µg ml⁻¹ (range 0.32–1.4 µg ml⁻¹), respectively for patients in

Table 2 Demographic data and R-(+)- to S-(-)-thiopentone (CL_T values and serum protein binding for individual patients in study 2. N/A: not available.

Patient	Sex	Weight (kg)	Age (years)	Clinical diagnosis	Total <i>rac</i> -thiopentone dosage (g)	Duration of infusion (h)	CL _T		Serum protein binding R/S ratio
							R-(+)- (L min ⁻¹)	S-(-)- (L min ⁻¹)	
13	M	90	20	Closed head injury	26.9	75	0.022	0.021	N/A
14	M	75	20	Closed head injury	37.5	31	0.160	0.156	0.99
15	F	56	47	Closed head injury	26.1	53	0.103	0.100	0.97
16	M	100	47	Subarachnoid haemorrhage	54.5	62	0.153	0.145	0.98
17	M	95	36	Cerebral venous thrombosis	43.0	92	0.060	0.058	0.95

study 1. The mean post-dialysis serum concentrations of R-(+)- and S-(−)-thiopentone were $21.9 \mu\text{g ml}^{-1}$ (range $11.4\text{--}39.4 \mu\text{g ml}^{-1}$) and $25.5 \mu\text{g ml}^{-1}$ (range $13.3\text{--}43.6 \mu\text{g ml}^{-1}$), respectively for patients in study 2. Pilot studies confirmed that there was no significant or differential binding of thiopentone enantiomers to the cellulose membrane used and that equilibration of thiopentone enantiomers across the membrane was achieved within 8 h.

Pooled cerebrospinal fluid (CSF) from patient 17 of study 2, stored after collection at -20°C , was also used to determine the degree of protein binding of R-(+)- and S-(−)-thiopentone enantiomers using the same technique.

Pharmacokinetic and pharmacodynamic data analysis

A two compartment modelling approach using a non-linear least squares algorithm of Marquardt with the reciprocal squared of the concentration as a weighting factor was applied to analyse serum concentration–time data for study 1 [13]. This approach gave the simplest model that fit the data without skew to the residuals. The initial dilution volume (V_c), total apparent volume of distribution at steady-state (V_{ss}) of total and unbound fractions, the mean total body clearance (CL_T) of total and unbound fractions and mean residence time (MRT) of the separate thiopentone enantiomers were calculated using standard equations [13]. For study 2, CL_T was calculated from the ratio of the mean infusion rate for the last 12 h to the mean serum thiopentone enantiomer concentration measured during this period. The doses of R-(+)- and S-(−)-thiopentone enantiomers for studies 1 and 2 were deemed to be half the dose of *rac*-thiopentone.

Due to the relatively brief duration of sampling time, the emphasis of data analysis in study 1 was a comparison of the two enantiomers. This method of analysis was held to be valid given that the enantiomer concentrations were determined in the same serum samples and thus under concurrent and identical circumstances. The limitations of the approach, however, are acknowledged with a drug having a long apparent terminal half-life as does thiopentone [14].

Values for CL_T , V_{ss} , MRT, 24 h urinary excretion, and fractional serum protein binding of R-(+)- and S-(−)-thiopentone were not found to deviate significantly from normality (Wilks-Shapiro test) and were compared using Student's *t*-test for paired data as well as the Wilcoxon Signed Rank test.

Mean values of R-(+)- and S-(−)-thiopentone serum concentrations for the eight patients who underwent EEG analysis at 1,3,5,7 and 10 min following intravenous bolus injection were also plotted against mean spectral edge and median frequency EEG results for all channels used in each patient.

Results

The mean time course for R-(+)- and S-(−)-thiopentone enantiomers of all 12 patients in study 1 is shown in Figure 1. The pharmacokinetic parameters for R-(+)- and S-(−)-thiopentone including CL_T , V_{ss} , 24 h urinary

Figure 1 The time course for R-(+)- and S-(−)-thiopentone enantiomer mean serum concentrations following intravenous injection of *rac*-thiopentone for the 12 patients in study 1. S-(−)-thiopentone = O; R-(+)-thiopentone = ●. Symbols are superimposed when only one is visibly present.

excretion and serum protein binding in study 1 and statistical analysis of results are given in Table 3. R-(+)- to S-(−)-thiopentone ratios of the above parameters for individual patients in study 1 are given in Table 1.

For the 12 patients in study 1, CL_T and V_{ss} showed trends to higher values for R-(+)- than S-(−)-thiopentone and the 24 h urinary excretion of R-(+)-thiopentone exceeded that of S-(−)-thiopentone. A concentration-dependent difference in the ratio of R-(+)-:S-(−)-thiopentone enantiomer serum protein binding was not observed when studies 1 and 2 were compared ($P=0.2$), although the patient numbers were small. Analysis of data for 10 patients (6 patients from study 1 and 4 patients from study 2) showed that the fractional serum protein binding of S-(−)-thiopentone was higher than that of R-(+)-thiopentone ($P=0.02$). When normalised by the appropriate free fractions in the six patients from study 1 the trends in CL_T and V_{ss} were no longer apparent suggesting that enantioselective protein binding was responsible for the trends observed in pharmacokinetic parameters.

In only one patient (7 of study 1), was the protein binding of R-(+)-thiopentone greater than S-(−)-thiopentone. However the absolute difference was small. It is of interest that patient 7 was the only patient from studies 1 and 2 to demonstrate considerably higher CL_T and V_{ss} of S-(−)- compared to R-(+)-thiopentone.

Median frequency spectral EEG analysis of the eight patients in study 1 who had EEG monitoring during surgery demonstrated initial slowing to delta frequency range ($<4\text{ Hz}$) within 30 s of commencement of a single intravenous bolus of *rac*-thiopentone and reversion to beta frequency ($>12\text{ Hz}$) after 180 s. At approximately 8 min post-administration of *rac*-thiopentone there was a transient increase in median frequency most probably due to concomitant stimulation from intubation which was performed at approximately this time in the majority of patients. Initial EEG changes were simultaneous, symmetric and generalised in that similar intraindividual spectral edge and median frequency findings were evident at all eight channels of EEG recording. The time course of the median and spectral edge frequency EEG data for F4–F3 (average results

Table 3 Comparative pharmacokinetic data and statistical analysis of the difference between R-(+)- and S-(-)-thiopentone enantiomers when administered as *rac*-thiopentone in study 1.

Parameter	R(+) Mean (s.d.) Range (Median)	S(-) Mean (s.d.) Range (Median)	R(+)/S(-) Student's paired t-test	R(+)/S(-) Wilcoxon Signed Rank	R(+)-S(-) Difference Mean (s.d.) (95% CI)***	R(+)/S(-) Ratio Mean (s.d.) (95% CI)***
V_c (l)	20.2 (9.7) 6.8–38.6 (17.1)	18.8 (7.9) 7.4–32.9 (15.9)	$P=0.12$	$P=0.12$	1.4 (2.9) (-0.5–3.3)	1.1 (0.1) (0.97–1.14)
V_{ss} (l)	91 (53) 36–222 (90)	80 (37) 32–174 (84)	$P=0.06$	$P=0.09$	10.6 (17.5) (-0.4–21.8)	1.1 (0.2) (0.99–1.2)
Clearance ($l\ min^{-1}$)	0.59 (0.23) 0.31–1.0 (0.59)	0.56 (0.20) 0.26–0.87 (0.57)	$P=0.16$	$P=0.054$	0.04 (0.09) (-0.02–0.09)	1.1 (0.1) (0.98–1.16)
MRT (min)	165 (88) 62–322 (157)	168 (103) 60–384 (137)	$P=0.80$	$P=0.97$	-2 (29) (-20–16)	1.0 (0.2) (0.9–1.1)
Protein binding (%)*	83.5 (8.1) 68.3–91.9 (85.4)	84.4 (6.9) 71.8–90.9 (86.4)	$P=0.22$	$P=0.21$	-0.9 (1.6) (-2.6–0.8)	0.98 (0.02) (0.96–1.0)
24 h urinary excretion (% dose)**	0.30 (0.11) 0.15–0.47 (0.35)	0.26 (0.11) 0.11–0.38 (0.24)	$P=0.03$	$P=0.04$	0.05 (0.05) (0.006–0.09)	1.2 (0.2) (1.04–1.42)
Unbound clearance ($l\ min^{-1}$)*	4.8 (2.3) 2.1–8.5 (4.7)	5.3 (1.6) 2.6–6.8 (5.7)	$P=0.57$	$P=0.53$	-0.4 (1.7) (-2.3–1.4)	0.9 (0.3) (0.6–1.3)
Unbound V_{ss} (l)*	680 (585) 260–1848 (570)	684 (464) 253–1585 (507)	$P=0.95$	$P=0.53$	-3.7 (139) (-149–142)	0.9 (0.1) (0.8–1.1)

* $n=6$ patients from study 1 who underwent serum protein binding studies, ** $n=8$ patients from study 1 who underwent 24 h urinary collection,

***95% confidence interval.

for the eight patients) plotted against the R-(+)- and S-(-)-thiopentone concentrations for the same patients are shown in Figures 2a and b.

Maximal EEG slowing occurred between 30–60 s post-administration of *rac*-thiopentone and corresponded to mean R-(+)- and S-(-)-thiopentone enantiomer serum concentrations of 10.6 and 10.8 $\mu g\ ml^{-1}$ respectively, or a total *rac*-thiopentone serum concentration of 21.4 $\mu g\ ml^{-1}$ at 60 s post-induction (range 7.4 to 42.6 $\mu g\ ml^{-1}$). The change from EEG slowing to beta frequency activation corresponded to mean R-(+)- and S-(-)-thiopentone enantiomer serum concentrations of 5.6 and 5.7 $\mu g\ ml^{-1}$ and a total *rac*-thiopentone serum concentration of 11.3 $\mu g\ ml^{-1}$ (range 7.3 to 23.8 $\mu g\ ml^{-1}$). Similar findings were noted for changes in spectral edge frequency relative to concentrations over time. The time course of concentration of the two enantiomers was similar and therefore the EEG effect could not be attributed to either R-(+)- or S-(-)-thiopentone.

Serum concentrations of R-(+)- and S-(-)-thiopentone enantiomers in the five patients who required prolonged intravenous infusions of *rac*-thiopentone as part of their intensive care management were similar both during and after infusion. Comparison was also unable to distinguish any differences of clinical significance. Values of CL_T for R-(+)- and S-(-)-thiopentone in study 2 are given in Table 2. Figures 3 and 4 show R-(+)- and S-(-)-thiopentone enantiomer serum concentrations plotted

against time following completion of *rac*-thiopentone infusion for patients 13 and 17 of study 2.

Cerebrospinal fluid (CSF) measurements of the thiopentone enantiomers in patient 17 demonstrated values approximately 20% of those found concurrently in plasma as well as a higher concentration of R-(+)- compared to S-(-)-thiopentone which is opposite to that found in the serum of the majority of patients in studies 1 and 2. Figure 5 compares the unbound serum and total CSF concentrations of R-(+)- and S-(-)- enantiomers plotted against time. The higher R-(+):S-(-) ratio is consistent with a higher free fraction of R-(+)- than S-(-)-thiopentone in patient 17. There was only minimal protein binding (<10%) of both R-(+)- and S-(-)-thiopentone in the CSF of this patient.

Discussion

It is well established that significant differences may exist in the pharmacokinetic and pharmacodynamic properties of enantiomeric forms of the same drug [1, 2]. The pharmacokinetics and pharmacodynamics of *rac*-thiopentone have been extensively studied over several decades. There have been, however, few studies comparing differences between the enantiomers despite the findings of pharmacokinetic and pharmacodynamic differences two decades ago [6, 7]. Moreover, the pharmacokinetic and pharmacodynamic

Figure 2 The time course of a) median and b) spectral edge frequency EEG data for F4-F3 (averaged results for eight patients in study 1) respectively (right axes), compared with R-(+)- and S-(-)-thiopentone enantiomer serum concentrations for the same patients (left axes). S-(-)-thiopentone = O; R-(+)-thiopentone = ●; median frequency = ▲; spectral edge frequency = ◆.

properties of the enantiomers of thiopentone when the racemate or individual enantiomers are given as a single bolus injection or prolonged infusion in human subjects had not been described until recently.

The pharmacokinetic data obtained from our two patient groups in study 1 and 2 indicate there are only minor differences between the two thiopentone enantiomers when the drug is given in its pharmacopoeal form as the racemate. Furthermore a trend towards greater CL_T and V_{SS} of R-(+)-thiopentone relative to S-(-)-thiopentone in study 1 is no longer apparent when unbound fractions of the enantiomers are considered. The minor differences in CL_T and V_{SS} , hence, are most likely due to enantioselective serum protein binding. Similar differences in the pharmacokinetics of R-(+)- and S-(-)-thiopentone have also been recently described by Mather *et al.* [10] in sheep and by Nguyen *et al.* [11] in human subjects.

Figure 3 R-(+)- and S-(-)-thiopentone enantiomer serum concentrations ($\mu\text{g ml}^{-1}$) vs time (h) for patient 13 of study 2. Time 0 (h) is time of completion of *rac*-thiopentone infusion. S-(-)-thiopentone = O; R-(+)-thiopentone = ●.

Figure 4 R-(+)- and S-(-)-thiopentone enantiomer serum concentrations ($\mu\text{g ml}^{-1}$) vs time (h) for patient 17 of study 2. Time 0 (h) is time of completion of *rac*-thiopentone infusion. S-(-)-thiopentone = O; R-(+)-thiopentone = ●.

Figure 5 A comparison of the unbound serum and total CSF concentrations of R-(+)- and S-(-)-thiopentone enantiomers for patient #17 of study 2. S-(-)-thiopentone in serum = O; R-(+)-thiopentone in serum = ●; S-(-)-thiopentone in CSF = △; R-(+)-thiopentone in CSF = ▲.

The 24 h urinary excretion of unmetabolised thiopentone was small (<1%) following a single intravenous bolus injection and is consistent with the findings of Carroll *et al.*

[15] who reported a 0.3–0.4% recovery of unchanged forms of R-(+)- and S-(–)-thiopentone. The enantiomeric difference in renal excretion of R-(+)- and S-(–)-thiopentone found in our study, although minor, was statistically significant and is also most probably due to the higher free fraction of R-(+)- than S-(–)-thiopentone.

Analysis of R-(+)- and S-(–)-thiopentone CSF concentrations in patient 17 indicate that under infusion conditions the equilibration of R-(+)- and S-(–)- enantiomers across the blood-brain-barrier into CSF is essentially complete and is consistent with passive diffusion of unbound fractions. Overall, no clinically significant difference in CSF distribution was noted between R-(+)- and S-(–)-thiopentone.

In general, the pharmacokinetics of *rac*-thiopentone may be adequately described by first-order kinetic principles under conditions of an intravenous injection or brief infusion [4, 14, 16]. Studies have reported a decrease in steady state clearance with increasing plasma concentrations such that nonlinear or Michaelis-Menten kinetics apply after prolonged or high-dose intravenous infusions [17–19]. Similar observations were apparent in our two patient populations in that CL_T was much lower at high steady-state concentrations during a prolonged intravenous infusion than after a single intravenous dose of *rac*-thiopentone (see Tables 2 and 3). Our study 2 also showed that differences in the absolute values of R-(+)- and S-(–)- enantiomers were minor under conditions of prolonged infusion and thus unlikely to be of clinical significance.

The present study also determined the time course of EEG effects of *rac*-thiopentone in relation to the serum concentrations of its enantiomers. Avram *et al.* [20] found a threefold full dose range for clinical and EEG end-points in a group of 60 patients receiving *rac*-thiopentone for induction of anaesthesia. The EEG effect, however, was not compared to serum thiopentone concentrations. Variables influencing EEG end-points in that study included age, weight, lean body mass and cardiac output [20]. Buhrer *et al.* [21] demonstrated a biphasic relationship of EEG frequency and total serum thiopentone concentration in six young, healthy, subjects aged 25 to 39 years. By targeting different pseudo-steady state concentrations it was shown that EEG activation from a baseline of 5–8 Hz to a peak activation of 15–18 Hz occurred at a serum *rac*-thiopentone concentration of $13 \mu\text{g ml}^{-1}$, EEG slowing to 10.6 Hz at a concentration of $31 \mu\text{g ml}^{-1}$, further slowing to less than 50% baseline awake state at $40 \mu\text{g ml}^{-1}$ and loss of EEG activity at $50 \mu\text{g ml}^{-1}$.

The EEG data in our study 1 were subject to potential influence by concomitant drug administration, varying patient age and *rac*-thiopentone dosage as well as limitations accompanying intravenous bolus drug injections including rapid changes in drug serum concentrations and EEG effects over time. Despite these variables we were able to demonstrate a clear change from EEG slowing to peak activation at 3 min post-administration of *rac*-thiopentone at corresponding mean R-(+)- and S-(–)-thiopentone serum concentrations of 5.6 and $5.7 \mu\text{g ml}^{-1}$ respectively or *rac*-thiopentone concentration of $11 \mu\text{g ml}^{-1}$. This finding is comparable with the serum concentration of $13 \mu\text{g ml}^{-1}$ described by Buhrer *et al.* [20] when thiopentone infusion rate was increased in a step-wise fashion under pseudo-steady state conditions. However there were only minor

differences in the absolute levels of R-(+)- and S-(–)-thiopentone enantiomers following *rac*-thiopentone induction in our patient population including during the first few minutes post-induction and hence this study was unable to distinguish whether one or other enantiomer contributed more to the pharmacological EEG effect.

In conclusion, our study indicates that there are minor differences in the time course of the R-(+)- and S-(–)- enantiomers when *rac*-thiopentone is administered as an intravenous injection or under conditions of prolonged intravenous infusion. Moreover, a lack of intrinsic enantioselective pharmacokinetic differences between R-(+)- and S-(–)-thiopentone is seen when CL_T and V_{SS} are determined for unbound fractions of both enantiomers. Studies such as this, however, are unable to address the issue of whether there is a pharmacodynamic advantage of one or other enantiomer and further research into this area may enhance our understanding of the actions and clinical application of *rac*-thiopentone and its enantiomers.

The authors wish to acknowledge the assistance and cooperation of Dr Chris Sparkes and Dr Ian Farey. The authors also wish to acknowledge the technical assistance of Ms Xiao Qing Gu and Ms Victoria Jordan.

The financial support of the Australian and New Zealand College of Anaesthetists, the National Health and Medical Research Council of Australia and the University of Sydney is gratefully acknowledged.

References

- 1 Mather LE, Rutten AJ. Stereochemistry and its relevance in anaesthesiology. *Curr Opin Anaesthesiol* 1991; **4**: 473–479.
- 2 Eichelbaum M, Gross AS. Stereochemical aspects of drug action and disposition. *Adv Drug Research* 1996; **28**: 1–64.
- 3 Shanks CA, Avram MJ, Krejcie TC, Henthorn TK, Gentry WB. A pharmacokinetic-pharmacodynamic model for quantal responses with thiopental. *J Pharmacokin Biopharm* 1993; **21**: 309–321.
- 4 Stanski DR, Maitre PO. Population pharmacokinetics and pharmacodynamics of thiopental: the effect of age revisited. *Anesthesiology* 1990; **72**: 412–422.
- 5 Christensen HD, Lee IS. Anesthetic potency and acute toxicity of optically active disubstituted barbituric acids. *Toxicol Appl Pharmacol* 1973; **26**: 495–503.
- 6 Haley TJ, Gidley JT. Pharmacological comparison of R (+), S (–) and racemic thiopentone in mice. *Eur J Pharmacol* 1976; **36**: 211–214.
- 7 Mark LC, Brand L, Perel JM, Carroll FI. II. Barbiturate stereoisomers: Direction for the future? *Excerpta Medica. Int Congr Ser* 1977; **399**: 143–146.
- 8 Huang JL, Mather LE, Duke C. High-performance liquid chromatographic determination of thiopentone enantiomers in sheep plasma. *J Chromatog B: Biomed Applic* 1995; **673**: 245–250.
- 9 Jones DJ, Nguyen KT, McLeish MJ, Crankshaw DP, Morgan DJ. Determination of R-(+)- and S-(–)-isomers of thiopentone in plasma by chiral high-performance liquid chromatography. *J Chromatog B: Biomed Applic* 1996; **675**: 174–179.
- 10 Mather LE, Upton RN, Huang JL, Ludbrook GL, Gray E, Grant C. The systemic and cerebral kinetics of thiopental in sheep: enantiomeric analysis. *J Pharmacol Exp Ther* 1996; **279**: 291–297.

- 11 Nguyen KT, Stephens DP, McLeish MJ, Crankshaw DP, Morgan DJ. Pharmacokinetics of thiopental and pentobarbital enantiomers after intravenous administration of racemic thiopental. *Anesth Analg* 1996; **83**: 552–558.
- 12 Sharbrough FW. Electrical fields and recording techniques. In *Current Practice of Clinical Electroencephalography* Second Edition, eds Daly DD, Pedley TA, New York: Raven Press, 1990: 29–49.
- 13 Seibalt RJ, Kreeft JH. Efficient pharmacokinetic modelling of complex clinical dosing regimens; the universal elementary dosing regimen and computer algorithm EDFAST. *J Pharm Sci* 1987; **76**: 93–100.
- 14 Burch PG, Stanski DR. The role of metabolism and protein binding in thiopental anesthesia. *Anesthesiology* 1983; **58**: 146–152.
- 15 Carroll FI, Smith D, Mark LC, Brand L, Perel JM. Determination of optically active thiopental, thioamylal, and their metabolites in human urine. *Drug Metab Disp* 1977; **4**: 343–354.
- 16 Homer TD, Stanski DR. The effect of increasing age on thiopental disposition and anesthetic requirement. *Anesthesiology* 1985; **62**: 714–724.
- 17 Le Corre P, Mallendant Y, Tanguy M, Le Verge R. Non-linear disposition of thiopentone following long-term infusion. *Eur J Drug Metab Pharmacokin*, 1993; **3**: 255–259.
- 18 Turcant A, Delhumeau A, Premel-Cabic A *et al.* Thiopentone pharmacokinetics under conditions of long-term infusion. *Anesthesiology* 1985; **63**: 50–54.
- 19 Russo H, Bressolle F, Bres J, Duboin M-P. Nonlinear pharmacokinetics of high-dose thiopental following long term treatment. *Clin Drug Invest* 1996; **11**: 32–42.
- 20 Avram MJ, Sanghvi R, Henthorn TK, *et al.* Determinants of thiopental induction dose requirements. *Anesth Analg* 1993; **76**: 10–17.
- 21 Buhner M, Maitre PO, Hung OR, Ebling WF, Shafer SL, Stanski DR. Thiopental Pharmacodynamics. 1. Defining the pseudo-steady-state serum concentration-EEG effect relationship. *Anesthesiology* 1992; **77**: 226–236.

(Received 31 July 1996,
accepted 25 November 1996)