Pharmacokinetics and Plasma Binding of Thiopental. I: Studies in Surgical Patients


The pharmacokinetics and plasma protein binding of thiopental were investigated in 5 female patients who received a bolus intravenous dose of the drug for induction of anesthesia for gynecologic surgery. Blood samples were collected for 3 to 4 days after the dose. Plasma protein binding determinations were also carried out by ultrafiltration and equilibrium dialysis on samples from a panel of healthy volunteers. Plasma concentrations of thiopental were determined by reverse-phase, high-performance liquid chromatography. The coefficient of variation of the method was 2.8 per cent (n = 10). In healthy volunteers, the plasma protein binding of thiopental was concentration dependent. Percentage bound ranged from 96.7 (n = 4, SD = 0.8) at a thiopental concentration of 0.2 μg/ml to 60.4 (n = 5, SD = 0.8) at 150 μg/ml. Therefore, saturation of binding sites on rapid administration of the drug may occur, exposing vital organs to unexpectedly high concentrations of free drug. Values of the fraction of thiopental bound in plasma obtained from the surgical patients during the hour following drug administration were similar to values obtained in healthy volunteers at comparable concentrations. Mean pharmacokinetic parameters obtained for thiopental in the surgical patients were as follows: initial distribution volume 13.81 (SD = 9.4), apparent volume of distribution 233 (SD = 98), volume of distribution at steady state 97.51 (SD = 40), elimination half-life 11.5 h (SD = 1.0) and systemic plasma clearance 0.150 l/min (SD = 0.065). None of these parameters correlated with body weight.

Values reported by other workers vary from ours and this variation may be explained by the much shorter duration of blood collection used in those studies. (Key words: Anesthetics, intravenous; thiopental. Pharmacokinetics. Protein: binding.)

THIOPENTAL has had widespread usage in anesthetics for many years, but a complete pharmacokinetic study of the drug in humans has only recently been reported. The elimination half-life of the drug reported in that study was ~5 hours, which was somewhat longer than values which had previously appeared in the literature. Subsequent to the report of Ghoneim and Van Hamme, Cloyd et al. reported much larger values for the elimination half-life of thiopental in two patients (1.6 and 3.6 days) and attributed the discrepancy in half-lives between their study and previous studies to differences in the duration of data collection. Discrepancies in volume of distribution and systemic plasma clearance were also apparent between the studies of Ghoneim and Van Hamme and of Cloyd et al. Values for the extent of plasma protein binding of thiopental reported in the literature vary, as does the extent to which binding varies with thiopental concentration.

In the present study, we have investigated: 1) the plasma protein binding of thiopental and the effect of surgery on binding; and 2) the pharmacokinetics of thiopental, using a longer duration of blood sample collection than that used previously, in an attempt to resolve the discrepancies in values reported by other workers.

Materials and Methods

Subjects

The disposition of thiopental was investigated in 5 nonpregnant female patients (age 27–69 years, mean 41 years; weight 49–73 kg, mean 60 kg). Informed consent was given by all patients and the project was approved by the Research Committee of the Hospital. These patients who were otherwise healthy and who were undergoing gynecologic surgery such as vaginal hysterectomy or tubal reanastomosis, received thiopental sodium (275–450 mg, mean 355 mg) by bolus (30 s) intravenous injection for induction of general anesthesia. Patients were premedicated with papa-veretum and scopolamine, and succinylcholine was administered to facilitate tracheal intubation. General anesthesia was maintained with nitrous oxide and halothane and patients remained supine for the duration of anesthesia. Each patient received 1 l of Compound Sodium Lactate Injection B.P. during operation. No patient lost clinically significant amounts of blood (i.e., >1 liter) and in no case was blood transfused. The patients also received routine drug therapy as required clinically (e.g., morphine for postoperative analgesia).

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Determinations of the extent of thiopental bound to plasma proteins and the blood/plasma concentration ratio of thiopental were carried out in vitro by spiking blood obtained from young, healthy, adult male and female volunteers with known amounts of the drug.

CLINICAL PROTOCOL

Prior to induction, an indwelling venous catheter was inserted into the antecubital vein of the arm contralateral to that used for the injection of the thiopental and a blank blood sample was collected (5 ml). Further 5-ml blood samples were collected at 2, 5, 15, 30, 45 min, and 1, 2, 4, 6, and 12 hours after thiopental administration. At this point the catheter was removed and subsequent blood samples (10 ml) were collected by venipuncture twice daily (morning and night) for approximately 4 days. Blood samples were collected in heparinised plastic tubes and centrifuged to provide plasma. In some cases, an aliquot of blood (1.0 ml) was removed prior to centrifugation for the determination of the whole blood concentration of thiopental. Blood and plasma samples were stored at −20°C until analysis.

ANALYTICAL TECHNIQUES

Thiopental was assayed in biological fluids using a modification to a method described previously. To blood or plasma (1–4 ml) 25 μl of internal standard solution (carbamazepine 10 μg/ml for low or 50 μg/ml for high expected thiopental concentration, respectively) was added in a glass centrifuge tube. The pH was adjusted to 3 with HCl (2m) and saturated ammonium phosphate solution and the mixture extracted with ether (7 ml) by vortex mixing. The phases were separated by centrifugation and the ether phase was transferred to another glass centrifuge tube with a tapered base. The ether was concentrated to about 50 μl on a water bath. Ten to 25 μl of this solution was then injected into a Perkin Elmer® Series 2 high-performance liquid chromatograph fitted with a thermostatically-controlled oven, a Perkin Elmer® LC-55 ultraviolet detector and a Perkin Elmer® M-2 calculating integrator. The column was Whatman Partisil PXS 10/25 ODS operated at 50°C, the mobile phase was 0.2 per cent ammonium phosphate buffer-methanol (60:40) at pH 3.5, the flow rate was 1 ml/min and the detection wavelength was 290 nm. A pre-column (3 cm x 2.8 mm i.d., containing Perkin Elmer® Pellicular ODS media 25–37 μm), which was periodically repacked, was employed to prolong the life of the main column. The retention times of thiopental and carbamazepine were approximately 9 and 14 min, respectively.

A standard curve was obtained using peak area measurements. Analysis of 10 samples (5 μg/ml) extracted simultaneously gave a coefficient of variation of 2.8 per cent, while the day to day coefficient of variation was 6.2 per cent for 10 samples. The within day coefficient of variation at 0.1 μg/ml was 4.6 per cent (n = 5). Extraction efficiency of thiopental was 70 per cent.

Plasma protein binding of thiopental was determined by ultrafiltration using a method described previously, and also by equilibrium dialysis of plasma (5 ml) against sterile M/15 Sorensen's phosphate buffer (5 ml), pH 7.4. Dialysis was carried out at 37°C in pairs of glass dialysis cells separated by Visking cellulose membrane over a period of 24 hours. Preliminary experiments showed that thiopental did not bind to the cellulose membrane used in the ultrafiltration and dialysis studies. Binding determinations on plasma samples from the surgical patients were carried out only by ultrafiltration because of its greater convenience. The filtrate represented about 5 per cent of the plasma volume.

PHARMACOKINETIC AND STATISTICAL ANALYSIS

Using the computer program Autoan, the unweighted plasma concentration-time data for each subject were fitted to the following polyexponential equation:

\[ C_p = \sum_{i=1}^{n} C_i e^{-\lambda_i t} \]

where \( C_p \) is the plasma concentration of thiopental at time \( t \), \( C_i \) is a zero-time intercept, and \( \lambda_i \) is a disposition rate constant. The estimates obtained from the CSTRIP part of the Autoan program were accepted because in all cases \( r^2 > 0.98 \).

Using methods described previously, coefficients and exponents from fitted functions were used to calculate the following pharmacokinetic parameters; initial distribution volume (\( V_d \)), volume of distribution at distribution pseudo-equilibrium (\( V_{ae} \)), volume of distribution at steady-state (\( V_{ss} \)), elimination half-life (\( t_{1/2} \)) and total systemic plasma clearance (\( Cl_p \)). Total systemic blood clearance was obtained by dividing \( Cl_p \) by the ratio blood concentration/plasma concentration of thiopental. The rate of change of volume of distribution at zero time (\( RV_{ae} \)) was calculated according to Niazi.

RESULTS

PLASMA PROTEIN BINDING

The plasma protein binding of thiopental was examined in samples from healthy young volunteer
TABLE I. Plasma Protein Binding of Thiopental in Normal Subjects Determined by Ultrafiltration (Mean ± SD)

<table>
<thead>
<tr>
<th>Thiopental Concentration (µg/ml)</th>
<th>Percentage Bound Thiopental</th>
<th>Number of Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.2</td>
<td>96.7 ± 0.8</td>
<td>4</td>
</tr>
<tr>
<td>1.0</td>
<td>83.9 ± 5.6</td>
<td>4</td>
</tr>
<tr>
<td>10.0</td>
<td>79.6 ± 3.6</td>
<td>6</td>
</tr>
<tr>
<td>50.0</td>
<td>76.7 ± 2.6</td>
<td>6</td>
</tr>
<tr>
<td>150.0</td>
<td>60.4 ± 0.8</td>
<td>5</td>
</tr>
</tbody>
</table>

subjects. With ultrafiltration, the percentage thiopental bound ranged from 96.7 ± 0.8 (mean ± SD) at a plasma concentration of 0.2 µg/ml to 60.4 ± 0.8 at 150 µg/ml (table 1). Using equilibrium dialysis, concentration dependence of thiopental plasma binding was also observed (table 2), although values for fraction of thiopental bound using this method were higher than the respective values obtained using ultrafiltration.

BLOOD/PLASMA CONCENTRATION RATIO

The blood/plasma concentration ratio of thiopental was determined in blood samples from the volunteer subjects and surgical patients. There was no significant difference between the two groups in the values obtained. No concentration dependence of the blood/plasma ratio was observed over the range 0.036–113 µg/ml, and the mean value (±SD) obtained for 19 subjects was 1.01 ± 0.20.

PHARMACOKINETICS IN SURGICAL PATIENTS

The plasma concentration-time data of 2 of the 5 surgical patients were best fitted by a bi-exponential equation while the data of the other 3 patients were best fitted by a tri-exponential equation. A typical plasma concentration-time profile is shown in figure 1. The pharmacokinetic parameters calculated for the subjects are shown in table 3. None of the parameters was found to correlate with body weight. This may be due to the small number of patients studied and the limited weight range of the patients.

The percentage of thiopental bound in each patient was determined in each case in a plasma sample obtained by pooling plasma collected during the first hour following thiopental administration. The mean value (±SD) for the 5 patients was 83.4 ± 4.8 (concentration range 6.4–11.5 µg/ml) and was similar to the values obtained in healthy volunteers.

Discussion

In the present study, over the 10–50 µg/ml range of thiopental plasma concentrations, the fraction of thiopental bound was approximately 78 per cent with ultrafiltration, and 85 per cent with equilibrium dialysis. These values are in broad agreement with those reported previously over a similar concentration range (72–88 per cent). The large variation among reports in the extent of binding of thiopental may be due to both differences in plasma samples and to differences in methodology. The data in tables 1 and 2 suggest that equilibrium dialysis yielded systematically higher values than ultrafiltration. This was confirmed by replicate determinations on a pooled plasma sample spiked with 50 µg/ml thiopental which showed that equilibrium dialysis gave significantly higher binding values than ultrafiltration. This suggests that the large variation in values reported in the literature for the extent of binding of thiopental may arise predominantly from the binding technique rather than the thiopental analysis as suggested by Becker.

Although plasma binding of thiopental was essentially constant over the 10–50 µg/ml range, marked concentration dependence in binding was observed with both ultrafiltration and dialysis over the wider range of 0.05 to 150 µg/ml. Concentration dependence was observed at higher thiopental concentrations by Goldbaum and Smith and at lower concentrations by Dayton et al. (90–91 per cent at 0.54 to 4.4 µg/ml), but no concentration dependence was reported by Becker in patient samples over the range 4.2 to 134 µg/ml.

The concentration dependence of thiopental plasma protein binding may result in the occurrence of transiently high unbound thiopental concentrations at the high total plasma concentrations of thiopental resulting from rapid intravenous injection of the drug. Venous plasma concentrations of thiopental were in the 20–30 µg/ml range, 5–7 min following drug administration and arterial concentrations may be expected to exceed venous concentrations in this early period. Whether arterial concentrations greater

TABLE 2. Plasma Protein Binding of Thiopental in Normal Subjects Determined by Equilibrium Dialysis (Mean ± SD)

<table>
<thead>
<tr>
<th>Thiopental Concentration (µg/ml)</th>
<th>Percentage Bound Thiopental</th>
<th>Number of Subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td>0.05</td>
<td>96.1</td>
<td>1</td>
</tr>
<tr>
<td>0.2</td>
<td>94.5 ± 1.0</td>
<td>3</td>
</tr>
<tr>
<td>0.5</td>
<td>90.5 ± 0.5</td>
<td>3</td>
</tr>
<tr>
<td>1.0</td>
<td>86.4 ± 4.7</td>
<td>3</td>
</tr>
<tr>
<td>10.0</td>
<td>84.2 ± 3.3</td>
<td>4</td>
</tr>
<tr>
<td>25.0</td>
<td>86.0 ± 2.3</td>
<td>4</td>
</tr>
<tr>
<td>50.0</td>
<td>85.1 ± 2.1</td>
<td>4</td>
</tr>
<tr>
<td>100.0</td>
<td>74.7 ± 1.8</td>
<td>5</td>
</tr>
<tr>
<td>150.0</td>
<td>70.6 ± 2.0</td>
<td>4</td>
</tr>
</tbody>
</table>

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THIOPENTAL DISPOSITION IN SURGICAL PATIENTS

Fig. 1. Plasma concentrations of thiopental in subject 3 following bolus intravenous administration of 500 mg. The curve was fitted by means of the Autoan program.14

than 50 μg/ml actually occur in patients after bolus intravenous injection of clinically used doses requires further investigation. The concentration-dependent binding may also be particularly relevant to the use of thiopental in obstetrics where a decrease in binding at high total thiopental concentrations will facilitate transfer of the drug to the fetus because it is the unbound moiety which is transferred across the placenta.13 Furthermore, thiopental is eliminated mainly by the liver,16,19 its hepatic extraction ratio is low19 and it appears that only unbound drug is available for hepatic elimination. Therefore, it is possible that the elimination of thiopental may be dose-dependent as a result of the nonlinear plasma protein binding.20 However, extensive data on the time-course of tissue binding of thiopental as well as on plasma binding would be required to investigate this point further.21

The extent of binding of thiopental to plasma proteins, measured in samples obtained from patients in the hour following thiopental administration, was similar to that observed in healthy volunteers. The potential displacement of thiopental from binding sites, due to elevated plasma free fatty acids which may accompany surgery,22 was not evident.

The plasma concentration-time profile of thiopental was bi-exponential in 2 of the surgical patients and tri-exponential in the other 3 patients. Ghoneim and Van Hamme’s reported tri-exponential thiopental kinetics in 6 patients undergoing eye, ear or oral surgery and in 3 volunteers and bi-exponential kinetics in 3 other volunteers. These authors performed a classical compartment analysis on their data, but this was not necessary in the present study because all of the parameters required to describe the pharmacokinetics of thiopental were calculated by model independent methods.15,16 The parameter, rate of change of distribution at time zero (RV₀),16 was used to describe the rate of distribution of thiopental rather than the fast disposition rate constants because comparison among individuals using rate constants is made ambiguous by the difference in the number of distribution phases of the drug among subjects. In addition, RV₀ may be a more useful parameter to correlate with various pharmacologic, physiologic and toxicologic parameters.16 Ghoneim and Van Hamme’s

| Table 3. Pharmacokinetic Parameters of Thiopental Following Intravenous Administration to Patients Undergoing Gynecologic Surgery |
|---|---|---|---|---|---|---|---|---|
| Subject | Body Weight (kg) | Body Height (cm) | t₁/₂ (h) | V₁ (liters) | Vss (liters) | Vα (liters) | RV₀ (liters/minute) | Clp (liters/minute) |
| 1 | 57 | 160 | 11.5 | 31.2 | 263 | 175.0 | 0.53 | 0.264 |
| 2 | 65 | 166 | 9.7 | 13.7 | 126 | 90.3 | 0.82 | 0.150 |
| 3 | 49 | 161 | 11.7 | 8.2 | 304 | 67.7 | 0.79 | 0.086 |
| 4 | 55 | 159 | 12.1 | 12.2 | 366 | 86.9 | 0.22 | 0.156 |
| 5 | 73 | 164 | 12.7 | 3.65 | 104 | 67.7 | 1.28 | 0.095 |
| Mean ± SD | 59.8 ± 8.4 | 162 ± 2.6 | 11.5 ± 1.0 | 13.8 ± 9.4 | 233 ± 98 | 97.5 ± 40 | 0.73 ± 0.36 | 0.150 ± 0.063 |
found that neither enflurane with nitrous oxide anesthesia nor the stress of surgery affected the distribution of thiopental. As a matter of interest, our mean values of the half-lives of the fast and slow distribution processes (4.6 min and 38 min, respectively) were similar to values obtained by the above authors in both their subject groups.

The mean elimination half-life obtained for thiopental in our 5 surgical patients (11.5 h) was at least twice that reported in several earlier studies. The widely quoted value of 15 per cent per hour for the first-order elimination rate constant of thiopental, which corresponds to a half-life of 4.6 h, was reported originally by Brodie et al. who measured thiopental plasma concentrations for 5 hours after intravenous administration of 1–4 g to volunteers. Subsequently, Plough et al. reported a value of 2.8 h for the elimination half-life of thiopental, but blood samples were collected for only 2 hours. Rahn et al. reported a value of 2.6 h with an 8- to 12-hour blood collection period, and Ghoneim and Van Hamme reported values of 5.74 h in volunteers and 5.14 h in surgical patients with a 12-hour blood collection period. In the present study, the blood collection period ranged from 80–100 hours, however calculation of an apparent elimination half-life using only those plasma concentrations up to 12 h yielded a mean value (±SD) of only 4.9 ± 1.3 h. This value is similar to those reported in the earlier studies; therefore, the shorter half-lives reported by the other workers are presumably due to the relatively short blood collection times employed. Longer elimination half-lives than those obtained in the present study have also been reported for thiopental. Analysis of data of Brodie in one subject with a 27-hour blood collection period resulted in a half-life of approximately 1.4 days, but the validity of this value is doubtful because the elimination phase was characterised by only two data points which were 19 hours apart. Cloyd et al. investigated the pharmacokinetics of thiopental in two patients who were being treated for uncontrollable seizures. The half-life in one of the patients who received 35 g over 11.4 days was 3.6 days, and that in the other patient (19.7 g over 3.3 days) was 1.6 days. The blood collection periods after cessation of thiopental were 72 hours and 48 hours, respectively. Cloyd et al. suggested that the discrepancy in the half-lives between their study and previous studies could be accounted for by differences in the duration of data collection. However, it may not be valid to view the pharmacokinetic parameters of thiopental obtained from these two patients as "normal values" because both were extremely ill and the doses used were extremely large.

As pointed out by Cloyd et al., with single-dose administration of thiopental for induction of anesthesia the magnitude of the elimination half-life is unimportant; however if thiopental is administered by prolonged infusion, the time to achieve the maximum response and the duration of response after termination of the infusion are determined by the magnitude of the half-life.

Another significant finding of Cloyd et al. was that the difference in half-life of thiopental in their two subjects was approximately proportional to the difference in volume of distribution, suggesting that the disappearance of thiopental from the body may be controlled by its distribution characteristics. Ghoneim and Van Hamme made a similar conclusion based on the values of the transfer rate constants between compartments of a three-compartment pharmacokinetic model. Interestingly, in the present study there was no correlation between the half-life of thiopental and its volume of distribution.

The volume of distribution of thiopental (Vd, Vss) obtained in the present study was greater and the mean plasma clearance was less than those of Ghoneim and Van Hamme. These discrepancies can also be accounted for by the differences in the duration of blood sample collection.

In view of the high and concentration-dependent plasma protein binding of thiopental, concentration dependence of the blood/plasma concentration ratio would be expected because only unbound thiopental would be available to penetrate the membrane of the red blood cell. In a single determination of the blood/plasma ratio in each of 19 subjects, no concentration dependence was observed. Possibly, intersubject differences in the parameters of plasma protein binding of thiopental and in hematocrits may account for this. The mean blood/plasma concentration ratio obtained in the present study was 1.01 which is somewhat greater than the value calculated from the data previously reported. Therefore, the blood clearance of thiopental may be equated with plasma clearance (mean 0.15 l/min). Assuming that the mean hepatic blood flow is 1.5 l/min and that thiopental is eliminated entirely by the liver, the calculated hepatic extraction ratio of thiopental is 0.10. Insignificant first-pass metabolism of thiopental would therefore be expected after oral administration of the drug which is contrary to the conclusion of Saidman and Eger who used a value for the hepatic extraction ratio of thiopental equal to 0.30 for their analogue simulation studies.

We conclude that the values of the pharmacokinetic parameters of thiopental reported herein represent the most reliable values reported to date due to the extended duration of blood sample collection.
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References


15. Wagner JG: Linear pharmacokinetic equations allowing direct calculation of many needed pharmacokinetic parameters from the coefficients and exponents of polyexponential equations which have been fitted to the data. J Pharmacokin Biopharm 4:443–467, 1976


