J. Pharm. Pharmacol. 2000, 52: 1065–1074
Received January 10, 2000
Accepted April 20, 2000



# Clearance of Fentanyl, Alfentanil, Methohexitone, Thiopentone and Ketamine in Relation to Estimated Hepatic Blood Flow in Several Animal Species: Application to Prediction of Clearance in Man

SVEN BJÖRKMAN AND FINN REDKE *

*Hospital Pharmacy and Department of Experimental Research, Malmö University Hospital, Malmö, Sweden*

**Abstract**

We have used estimated hepatic blood flow ($Q_{hep}$) as an aid to evaluate clearance (CL) values in animals and to predict clearance in man of five anaesthetic agents: fentanyl, alfentanil, methohexitone, thiopentone and ketamine.

The disposition of methohexitone was determined in rats and that of ketamine in rats, rabbits and pigs. Further data were compiled from the literature and supplemented experimentally as needed. Allometric interspecies scaling, according to three different methods, was used to estimate blood clearance and unbound clearance ($CL_u$) in man. The results of scaling according to the three different methods were evaluated in relation to estimated hepatic extraction ratio ($CL/Q_{hep}$) of the drugs.

In most animals the clearance of the drugs were comparable with or lower than estimated $Q_{hep}$. However, ketamine showed extensive extrahepatic clearance in rabbits. Prediction of clearance in man was successful by at least one method for all five drugs, while prediction of $CL_u$ generally failed. Estimates of $CL/Q_{hep}$ gave no indication as to the choice of the best method. Volume of distribution at steady state could be predicted for alfentanil, thiopentone and ketamine.

Comparison of clearance with $Q_{hep}$ should be used to evaluate clearance data in animals, however estimation of hepatic extraction ratios appears to be of little use for allometric scaling. The use of ketamine as an anaesthetic agent in rabbits is questionable, while the use of fentanyl in pigs, methohexitone in rats and ketamine in rats and pigs is well supported by the pharmacokinetic data.

Pharmacokinetic investigations in animals may serve either of two purposes; to predict pharmacokinetic properties of the drug in man, or to support actual use of the drug in the animal. For most drugs only the first objective may apply. However, anaesthetic agents are used in animal experimentation and so pharmacokinetic studies of these drugs may serve both purposes. Studies in small animals are hampered by the problem of how to obtain enough blood samples to characterize the whole area under the blood or plasma concentration curve (AUC). Underestimation of AUC gives an overestimation of clearance (CL). A theoretically obvious, but rarely used method to evaluate a calculated value for metabolic clearance would be to compare it with measured or estimated total hepatic blood flow ($Q_{hep}$) in the species. Interspecies scaling can then be used to predict the pharmacokinetics of a drug in man from data obtained in animals (Weiss et al 1977; Boxenbaum 1982; Boxenbaum & D'Souza 1990; Mahmood & Balian 1999). Theoretically, the clearance of a drug with a high hepatic extraction ratio should change with body weight (BW) according to the basic allometric function:

$$CL = a \times BW^x \quad (1)$$

where a and x are scaling parameters. The reason for this is that total blood clearance of these drugs is predominantly determined by $Q_{hep}$, which itself

*Present address: Department of Anaesthesia, Aalborg Hospital (Section South), DK-9000 Aalborg, Denmark.
Correspondence: S. Björkman, Hospital Pharmacy, Malmö University Hospital, S-205 02 Malmö, Sweden.

follows this allometric function (Boxenbaum & D'Souza 1990). The clearance of drugs with low hepatic extraction ratios is determined by other factors and should therefore not show this simple relationship with body weight. Several modified methods for allometric scaling of clearance have consequently been proposed. The most common modifications of equation 1 are the use of the product of clearance and maximum life-span potential (MLP) (Boxenbaum 1982) or of clearance and brain weight (BrW) (Mahmood & Balian 1996a, b) instead of just clearance as the independent variable.

Mostly the scaling methods have been evaluated retrospectively, with prior knowledge of clearance in man. However, a more realistic scenario in drug development is that the clearance in man is not known, and the problem then becomes to determine which (if any) of the different clearance values predicted by the various methods comes close to the true one. It has been suggested that the value of the exponent of equation 1 should be used as a guide in this choice (Mahmood & Balian 1996b). Estimation of hepatic extraction ratio ($CL/Q_{hep}$) has not been used in practice for this purpose.

In many cases, volume of distribution at steady state ($Vd_{ss}$) can be scaled to body weight by means of a basic allometric function analogous to equation 1 (Boxenbaum 1982; Mahmood & Balian 1996a, 1999). However, dividing $Vd_{ss}$ with the unbound fraction of drug in plasma ($f_u$) may improve predictions by compensating for differences in $f_u$ between species.

The aim of this study was to use estimated $Q_{hep}$ as an aid to evaluate clearance values in animals and to predict the clearance in man of five anaesthetic drugs, fentanyl, alfentanil, methohexitone, thiopentone and ketamine, which are or may also be used in animal experiments. Fentanyl and alfentanil are a pair of structural analogues (opioids) with very different hepatic extraction ratios in man, while methohexitone and thiopentone are another such pair (barbiturates). All five drugs are cleared by metabolism, with very little renal excretion. Two assumptions were tested, firstly that drugs with a high hepatic extraction ratio should be scalable by equation 1, and secondly that the exponential term of this equation could be used to indicate which alternative method should give the best prediction.

## Materials and Methods

*Animal pharmacokinetics*

The Ethics Committee on Animal Studies at Lund University approved the study protocols. Eleven rats, 13 rabbits and 18 pigs were used.

The pharmacokinetics of methohexitone and ketamine were determined in male Sprague-Dawley rats, using 2-h infusions. A central venous catheter for drug infusion and an arterial catheter for blood sampling were introduced under anaesthesia with chloral hydrate. The rats were allowed to recover overnight. Methohexitone was infused into five animals (270–360 g) at a rate of 22–30 $mg\,kg^{-1}\,h^{-1}$ and ketamine into another six animals (260–300 g) at a rate of 37–57 $mg\,kg^{-1}\,h^{-1}$ by means of a syringe infusion pump. Arterial blood samples were taken by a technique described by Björkman et al (1990) at the following times: blank (400 $\mu$L), at 5, 10, 30, 60, 90, 120 (100 $\mu$L), 125, 130, 150, 180 (200 $\mu$L), 240 (300 $\mu$L) and 300 (400 $\mu$L) min from the beginning of the infusion. The total blood loss before termination of the experiment at 300 min was thus 2·1 mL.

The pharmacokinetics of ketamine were determined in New Zealand White rabbits, using six control animals (2·2–2·6 kg) and seven animals (2·1–3·1 kg) pretreated with *Escherichia coli* endotoxin, as previously described for methohexitone (Redke & Björkman 1994) and alfentanil (Björkman & Redke 1996). The rate of infusion was 9·7–11·4 $mg\,kg^{-1}\,h^{-1}$ for 2 h and blood sampling was at 5, 10, 30, 60, 90, 120, 123, 128, 135, 145, 160, 180, 210, 240, 300 and 360 min after the start of the infusion.

The clearance of ketamine was determined in 18 pigs (mixed Swedish domestic breed, 18–25 kg) during a pharmacological experiment, where a bolus loading dose of 10 $mg\,kg^{-1}$ followed by a 7-h infusion at a rate of 15 $mg\,kg^{-1}\,h^{-1}$ was used for anaesthesia. Arterial blood was sampled every 30 min. Åkeson et al (1993) described the animal model.

*Blood/plasma concentration ratios and unbound fractions in plasma*

The blood/plasma concentration ratios ($\lambda$) of fentanyl in pigs, methohexitone in rats and man, ketamine and norketamine in rats, rabbits and man, and of thiopentone in rabbits were determined in-vitro by a procedure previously described by Redke & Björkman (1994) and Björkman & Redke (1996).

The $f_u$ of fentanyl in plasma from eight pigs was determined by equilibrium dialysis against isotonic phosphate buffer at 37°C (Meuldermans et al 1982) at a total concentration of 15 $ng\,mL^{-1}$. The $f_u$ of methohexitone in plasma from 11 rats and 18 rabbits at concentrations of 0·5 and 10 $\mu g\,mL^{-1}$ was determined by ultracentrifugation (Redke et al 1991). The $f_u$ of ketamine was determined by

equilibrium dialysis (as for fentanyl) in plasma from 10 rats and seven pigs at concentrations of 0·3 and 6 $\mu g\,mL^{-1}$. In all determinations, human plasma from 2 to 3 volunteers was used as a reference medium.

*Analytical methods*

Blood, plasma and plasma water concentrations of the drugs were determined by gas-liquid chromatography according to published methods: fentanyl, Björkman & Stanski (1988); methohexitone, Redke et al (1991); ketamine and norketamine, Björkman et al (1992).

*Pharmacokinetic calculations*

The total blood clearance of methohexitone and ketamine in rats and the plasma clearance of ketamine in rabbits were calculated as dose/AUC, where AUC is the area under the blood concentration curve extrapolated to infinite time. Terminal half-life was estimated by non-linear regression of the post-infusion concentration data using the RSTRIP software (MicroMath, Salt Lake City, UT). MRT and $Vd_{ss}$ were calculated by standard model-independent procedures. The steady-state clearance of fentanyl in pigs (data from Åkeson et al 1992) was calculated as rate of infusion divided by the mean of the 4-, 4·5- and 5-h plasma concentrations in each animal. Similarly for ketamine, the steady-state clearance was calculated using the 6-, 6·5- and 7-h blood concentrations.

*Literature data*

Further pharmacokinetic data were compiled from the literature, based on several MEDLINE searches (Table 1). Only data from studies in unanaesthetized animals, or in a few cases where only anaesthetic gases were administered concomitantly, were used. In addition, literature data were included only if the blood sampling protocol appeared to be adequate to reliably characterize either a terminal half-life and an AUC of the curve after a bolus injection, or actual attainment of steady state during an infusion. A number of published studies were rejected for not fulfilling these criteria. For most species CL and $Vd_{ss}$ values based on plasma concentrations were converted to corresponding values based on blood concentrations by division with $\lambda$. In a few cases, data could not be obtained either experimentally or from the literature.

The literature data for man are shown in Table 2. In addition, literature values of $\lambda$ and $f_u$ in man were: fentanyl $\lambda = 0{\cdot}97$ and $f_u = 16\%$, alfentanil $\lambda = 0{\cdot}63$ and $f_u = 7{\cdot}9\%$ (Meuldermans et al 1982), methohexitone $f_u = 21\%$ (Redke et al 1991), thiopentone $\lambda = 1{\cdot}0$, 0·88 or 0·95 (Morgan et al 1981; Jung et al 1982; Wada et al 1997) and $f_u = 20\%$ (Jung et al 1982). These data were used to calculate total blood clearance or unbound clearance ($CL_u$) from literature data on plasma clearance.

*Allometric scaling*

$Q_{hep}$ ($mL\,min^{-1}$) was estimated by the allometric equation (Boxenbaum & D'Souza 1990):

$$Q_{hep} = 55 \cdot 4 \times BW^{0{\cdot}894} \qquad (2)$$

where body weight is given in kg. In man, however, a $Q_{hep}$ value of $1{\cdot}6\,L\,min^{-1}$ was assumed (Wada et al 1997; Björkman et al 1998) because the estimate given by the equation ($2{\cdot}5\,L\,min^{-1}$ in a 70-kg person) was obviously too high.

Allometric scaling of clearance (total blood CL or $CL_u$) was performed by linear regression of the non-human data according to three different equations. The first was the logarithmic form of equation 1:

$$\log CL = x \times \log BW + \log a \qquad (3)$$

The second equation included brain weight of the animals (Mahmood & Balian 1996a,b):

$$\log (CL \times BrW) = x \times \log BW + \log a \qquad (4)$$

Brain weights of the various species, as percent of body weight, were assumed to be: rats 0·75%, rabbits 0·39%, dogs 0·53%, sheep 0·19% and man 2·2% (Boxenbaum 1982), and pigs 0·34% (Björkman et al 1992).

The third equation included the MLP of the various species according to Boxenbaum (1982):

$$\log (CL \times MLP) = x \times \log BW + \log a \qquad (5)$$

The MLP of the various species were assumed to be: rats 4·7 years, rabbits 8·0 years, dogs 19·7 years, pigs 11·4 years, sheep 18·3 years and man 93·4 years (Boxenbaum 1982).

$Vd_{ss}$ was scaled according to the simple allometric equation:

$$\log Vd_{ss} = x \times \log BW + \log a \qquad (6)$$

and $Vd_{ss}/f_u$ was scaled analogously.

Parameter values in man predicted by the allometric regression lines were compared with reported data from the literature. Percent error between the predicted and the observed values was calculated as:

$$\%Error = ((Predicted - Observed)/Observed) \times 100 \qquad (7)$$

Table 1. Body weights and estimated hepatic blood flows ($Q_{hep}$) of the animals and the primary pharmacokinetic parameters, blood clearance (CL), volume of distribution at steady state ($Vd_{ss}$), referenced to blood, blood:plasma partition coefficients, ($\lambda$) and unbound fraction ($f_u$) in plasma of the five anaesthetics.

| Species | Body weight (kg) | $Q_{hep}$[a] ($L\,min^{-1}$) | CL ($L\,min^{-1}$) | $Vd_{ss}$ (L) | $\lambda$ | $f_u$ (%) | Sources of pharmacokinetic data |
|---|---|---|---|---|---|---|---|
| Fentanyl | | | | | | | |
| Rat | 0·40 | 0·024 | 0·027 | n.d. | 0·89 | 17 | Meuldermans et al 1982; Björkman et al 1990 |
| Dog | 15 | 0·62 | 0·53 | 158 | 1·00[b] | 22 | Meuldermans et al 1982; Murphy et al 1983 |
| Pig | 20 | 0·81 | 1·01 | n.d. | 0·95 | 24 | Åkeson et al 1993; this study |
| Alfentanil | | | | | | | |
| Rat | 0·39 | 0·024 | 0·019 | n.d. | 0·70 | 16 | Meuldermans et al 1982 & Björkman et al 1990 |
| Rat | 0·4 | 0·024 | 0·028 | 0·39 | 0·70 | 16 | Meuldermans et al 1982; Mandema & Wada 1995 |
| Rabbit | 2·6 | 0·13 | 0·12 | 2·5 | 0·77 | 32 | Björkman & Redke 1996 |
| Rabbit | 3·4 | 0·17 | 0·086 | 4·0 | 0·77 | 32 | Ilkiw & Benthuysen 1991; Björkman & Redke 1996 |
| Dog | 8·6 | 0·38 | 0·26 | 8·2 | 0·62 | 27 | Meuldermans et al 1982; Ilkiw & Benthuysen 1991 |
| Sheep | 37 | 1·40 | 0·69 | 37 | 0·70[b] | n.d. | Ilkiw & Benthuysen 1991 |
| Methohexitone | | | | | | | |
| Rat | 0·31 | 0·019 | 0·025 | 1·2 | 0·97 | 25 | This study |
| Rabbit | 2·5 | 0·13 | 0·18 | 3·3 | 0·68 | 24 | Redke & Björkman 1994; this study |
| Dog | 27 | 1·06 | 1·16 | 49 | 0·70[b] | n.d. | Sams et al 1985 |
| Thiopentone | | | | | | | |
| Rat | 0·35 | 0·022 | 0·00064 | 0·33 | 0·95 | 12 | Sharma et al 1970; Ebling et al 1994; Wada et al 1997 |
| Rabbit | 3·1 | 0·15 | 0·043 | 1·9 | 1·02 | n.d. | Ilkiw et al 1991; this study |
| Dog | 20 | 0·81 | 0·039 | 27 | 1·00[b] | 26 | Brandon & Baggot 1981; Baggot et al 1984 |
| Dog | 20 | 0·81 | 0·069 | 17 | 1·00[b] | 26 | Brandon & Baggot 1981; Ilkiw et al 1991 |
| Dog | 27 | 1·06 | 0·094 | 62 | 1·00[b] | 26 | Brandon & Baggot 1981; Sams et al 1985 |
| Sheep | 38 | 1·43 | 0·12 | 36 | 1·00[b] | 33 | Rae 1962; Ilkiw et al 1991 |
| Sheep | 43 | 1·60 | 0·16 | 43 | 1·00[b] | 33 | Rae 1962; Toutain et al 1983 |
| Ketamine | | | | | | | |
| Rat | 0·28 | 0·018 | 0·031 | 1·1 | 1·08 | 62 | This study |
| Rabbit | 2·6 | 0·13 | 0·67 | n.d. | 1·19 | n.d. | This study |
| Dog | 19 | 0·77 | 0·76 | 86 | 0·82 | 46 | Kaka & Hayton 1980 |
| Pig | 21 | 0·84 | 0·76 | n.d. | 0·97 | 66 | Björkman et al 1992; this study |
| Pig | 26 | 1·02 | 0·29 | 59 | 0·97 | 66 | Löscher et al 1990; this study |

[a]Estimated according to equation 2. [b]Assumed. n.d., not determined.

Predictions were considered to be accurate (or successful) if the error was less than 30% (Mahmood & Balian 1996c, 1999).

## Results

*New pharmacokinetic data*

The plasma clearance of fentanyl in 20-kg pigs was $0{\cdot}99 \pm 0{\cdot}20\,L\,min^{-1}$ (mean ± s.d.), the $\lambda$ value was $0{\cdot}95 \pm 0{\cdot}06$ and $f_u$ was $24 \pm 5{\cdot}6\%$.

The pharmacokinetic parameters of methohexitone in rats (referenced to total blood concentrations) were $CL = 25 \pm 5{\cdot}5\,mL\,min^{-1}$, $Vd_{ss} = 1{\cdot}2 \pm 0{\cdot}99\,L$, $MRT = 44 \pm 25$ min, terminal half-life $73 \pm 24$ min, $\lambda = 0{\cdot}97 \pm 0{\cdot}13$ and $f_u = 25 \pm 4{\cdot}0\%$. The $f_u$ of methohexitone in rabbits was $24 \pm 7{\cdot}5\%$ and the $\lambda$ value in man was $0{\cdot}74 \pm 0{\cdot}12$. The $\lambda$ value of thiopentone in rabbits was $1{\cdot}02 \pm 0{\cdot}055$.

Figure 1 shows the blood concentration curves of ketamine and norketamine in rats and rabbits. There was no significant influence of endotoxin pretreatment on the pharmacokinetics of ketamine, and consequently data from all rabbits were combined. The molar blood AUC ratio of norketamine to ketamine was $0{\cdot}52 \pm 0{\cdot}10$ in the rats and $2{\cdot}4 \pm 0{\cdot}51$ in the rabbits. The pharmacokinetic parameters obtained for ketamine in rats were $CL = 31 \pm 12\,mL\,min^{-1}$, $Vd_{ss} = 1{\cdot}1 \pm 0{\cdot}37\,L$, $MRT = 38 \pm 6{\cdot}1$ min, terminal half-life $44 \pm 14$ min, $\lambda = 1{\cdot}08 \pm 0{\cdot}07$ and $f_u = 62 \pm 6{\cdot}9\%$. For the rabbits $CL = 674 \pm 174\,mL\,min^{-1}$ and $\lambda = 1{\cdot}19 \pm 0{\cdot}06$, no other parameters could be determined. The mean blood concentration of ketamine in pigs after 6–7-h infusion was $7{\cdot}3 \pm 1{\cdot}9\,\mu g\,mL^{-1}$, as compared with $7{\cdot}5 \pm 2{\cdot}3\,\mu g\,mL^{-1}$ at 3–4·5-h infusion. Steady state had consequently been reached and the calculated blood clearance was $0{\cdot}76 \pm 0{\cdot}19\,L\,min^{-1}$. The $f_u$ value was $66 \pm 7{\cdot}5\%$. The molar concentration ratio of norketamine to ketamine was $2{\cdot}1 \pm 0{\cdot}40$ at 6–7 h. In man the $\lambda$ value was $1{\cdot}1 \pm 0{\cdot}05$ and $f_u$ was $72 \pm 11\%$.

*Allometric scaling of clearance*

Comparison of CL values with estimated $Q_{hep}$ suggested that fentanyl was a high-extraction drug in all species investigated except man. Predictions

Table 2. Total blood clearance (CL), unbound clearance ($CL_u$), volume of distribution at steady state ($Vd_{ss}$) and unbound $Vd_{ss}$ ($Vd_{ss}/f_u$) in man of five anaesthetics, as observed and as predicted by allometric scaling, with % prediction errors within parentheses.

| CL (L min$^{-1}$) | | | | $CL_u$ (L min$^{-1}$) | | | |
|---|---|---|---|---|---|---|---|
| Observed | Predicted, by method | | | Observed | Predicted, by method | | |
| | Body weight | Brain weight | MLP | | Body weight | Brain weight | MLP |
| Fentanyl | | | | | | | |
| 0·76 | 2·82 (272) | 0·53 (−30) | 0·70 (−7) | 4·74 | 10·3 (117) | 1·88 (−60) | 2·60 (−45) |
| 0·70 | 2·87 (309) | 0·54 (−23) | 0·72 (3) | 4·38 | 10·5 (139) | 1·95 (−56) | 2·66 (−39) |
| Alfentanil | | | | | | | |
| 0·57 | 1·13 (100) | 0·16 (−72) | 0·32 (−43) | 4·45 | 1·44 (−68) | 0·24 (−95) | 0·54 (−88) |
| 0·44 | 1·13 (158) | 0·16 (−63) | 0·32 (−27) | 3·45 | 1·44 (−58) | 0·24 (−93) | 0·54 (−84) |
| 0·48 | 1·11 (132) | 0·16 (−67) | 0·31 (−35) | 3·75 | 1·42 (−62) | 0·23 (−94) | 0·53 (−86) |
| Methohexitone | | | | | | | |
| 1·13 | 3·04 (168) | 0·62 (−45) | 0·85 (−25) | 4·00 | 6·60 (65) | 0·41 (−90) | 1·35 (−66) |
| 0·88 | 2·73 (209) | 0·56 (−36) | 0·73 (−17) | 3·11 | 6·01 (94) | 0·38 (−88) | 1·20 (−62) |
| Thiopentone | | | | | | | |
| 0·15 | 0·21 (42) | 0·04 (−76) | 0·05 (−64) | 0·75 | 0·61 (−19) | 0·10 (−86) | 0·16 (−79) |
| 0·23 | 0·20 (−11) | 0·03 (−85) | 0·05 (−78) | 0·99 | 0·58 (−41) | 0·10 (−90) | 0·16 (−84) |
| 0·25 | 0·24 (−2) | 0·04 (−84) | 0·06 (−74) | 1·18 | 0·69 (−42) | 0·12 (−90) | 0·19 (−84) |
| Ketamine | | | | | | | |
| 1·14 | 1·18 (4) | 0·17 (−85) | 0·23 (−80) | 1·70 | 1·79 (5) | 0·25 (−85) | 0·34 (−80) |
| 1·20 | 1·11 (−7) | 0·16 (−87) | 0·21 (−82) | 1·80 | 1·70 (−6) | 0·24 (−87) | 0·32 (−82) |

| $Vd_{ss}$ (L) | | $Vd_{ss}/f_u$ (L) | | Data source |
|---|---|---|---|---|
| Observed | Predicted | Observed | Predicted | |
| Fentanyl | | | | |
| 417 | –[a] | 2600 | –[a] | Hudson et al 1986 |
| 312 | –[a] | 1950 | –[a] | Lemmens et al 1994 |
| Alfentanil | | | | |
| 59 | 71 (20) | 463 | 95 (−79) | Maitre et al 1987 |
| 63 | 71 (13) | 494 | 95 (−81) | Van Beem et al 1989 |
| 46 | 69 (50) | 363 | 93 (−74) | Henthorn et al 1992 |
| Methohexitone | | | | |
| 230 | 94 (−59) | 810 | 27 (−97) | Hudson et al 1983 |
| 423 | 85 (−80) | 1490 | 26 (−98) | Le Normand et al 1988 |
| Thiopentone | | | | |
| 97 | 73 (−25) | 488 | 223 (−54) | Morgan et al 1981 |
| 91 | 69 (−24) | 402 | 213 (−47) | Jung et al 1982 |
| 181 | 85 (−53) | 860 | 252 (−71) | Hudson et al 1983 |
| Ketamine | | | | |
| 189 | 202 (7) | 283 | 341 (20) | Wieber et al 1975 |
| 258 | 186 (−28) | 388 | 313 (−19) | Domino et al 1982 |
| 120 | 210 (75) | 181 | 355 (96) | Domino et al 1984 |

[a]Insufficient animal data available.

of the CL of fentanyl in man are shown in Table 2. Scaling according to body weight only gave an allometric exponent of 0·89 and $r^2 = 0{\cdot}982$ for the animal data but a 4-fold over-prediction of CL in man. In contrast, prediction of total blood CL was successful (within ±30% of the true value) by the brain weight and MLP (Figure 2) methods. Prediction of $CL_u$ was not accurate by any of the methods used.

Comparison of clearance values with estimated $Q_{hep}$ suggested that alfentanil had a high hepatic extraction ratio in rats and possibly in rabbits, while it was a medium- to low-extraction drug in larger animals and man. Allometric scaling of the animal data according to body weight gave an exponent of 0·76 and $r^2 = 0{\cdot}971$. Prediction of total blood CL of alfentanil in man was, however, marginally successful by the MLP method only (Table 2 and Figure 2). $CL_u$ could not be predicted by any of the methods used.

Methohexitone appeared to be a high-extraction drug in all species investigated except man. As in

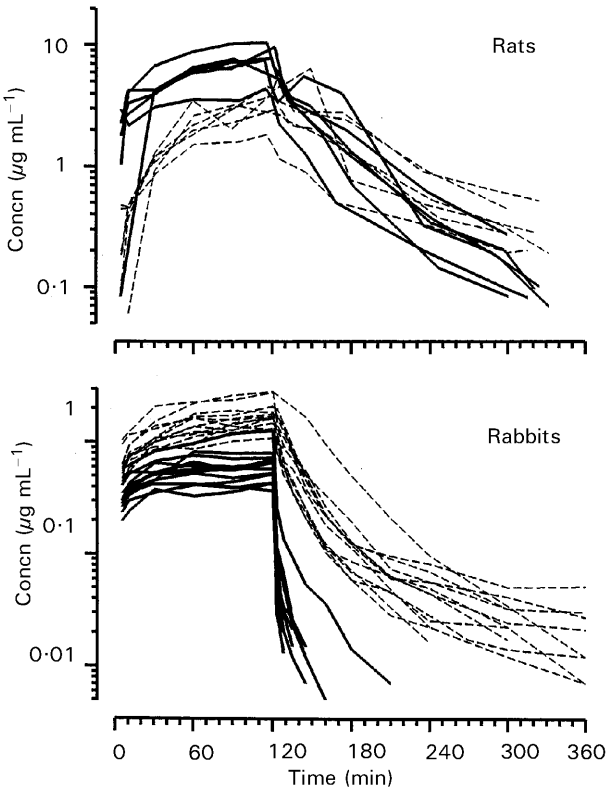


Figure 1. Comparison of the disposition of ketamine in rats and rabbits. Heavy curves are blood concentrations of ketamine and dashed curves are blood concentrations of norketamine. The curves are normalized to infusion rates of 45 mg kg$^{-1}$ h$^{-1}$ in rats and 10 mg kg$^{-1}$ h$^{-1}$ in rabbits.

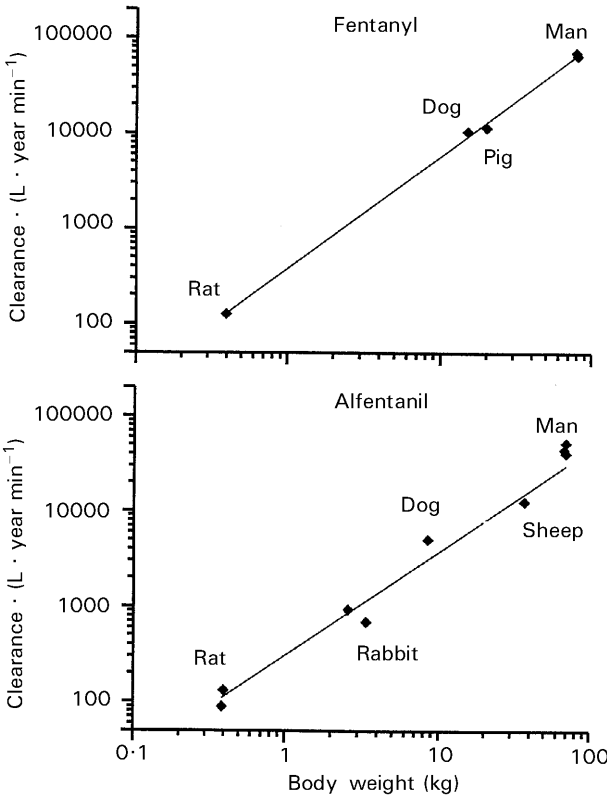


Figure 2. Allometric scaling of the total blood clearance of fentanyl and alfentanil by the MLP method.

the case of fentanyl and alfentanil, the total blood CL of methohexitone in man could be predicted by the MLP method (Figure 3) but not by simple allometric scaling according to body weight (Table 2). The latter procedure applied to animal data gave an exponent of 0·86 and $r^2 = 0{\cdot}997$. Predictions of $CL_u$ were very inaccurate.

Thiopentone differed from the other drugs in having a low hepatic extraction in all species. The clearance in rabbits was unexpectedly high. Prediction of total blood CL in man was accurate by simple allometric scaling according to body weight, the animal data giving an exponent of 1·002 and $r^2 = 0{\cdot}848$ (Figure 4), but not by the brain weight and MLP methods. Prediction of $CL_u$ was almost successful by the body weight method (Table 2).

Comparison of measured blood clearance values of ketamine with estimated $Q_{hep}$ suggested high hepatic extraction ratios and/or extrahepatic clearance in all species except man, with reservation for the discordant data from pigs. Prediction of total blood clearance (Figure 5), as well as of $CL_u$, in man was accurate by simple allometric scaling, provided that the obviously outlying data from the rabbits were excluded. For total blood clearance in the animals, the exponent was 0·65 and $r^2 = 0{\cdot}814$. Given the very diverging data on clearance in dogs and pigs, this apparently successful scaling could, however, be a chance finding.

*Allometric scaling of $Vd_{ss}$*

Allometric scaling of the $Vd_{ss}$ of fentanyl was not possible due to lack of animal data. Results for the other four drugs are given in Table 2. For most drugs there were larger variations in observed mean

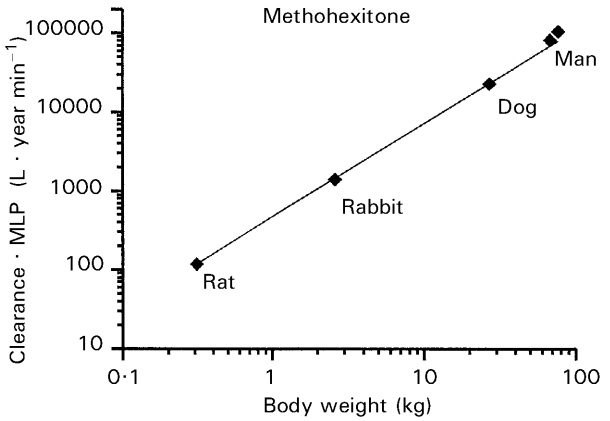


Figure 3. Allometric scaling of the total blood clearance of methohexitone by the MLP method.

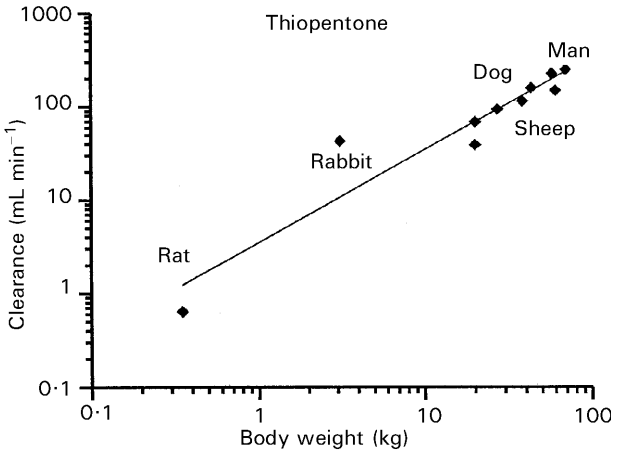


Figure 4. Allometric scaling of the total blood clearance of thiopentone by the body weight method.

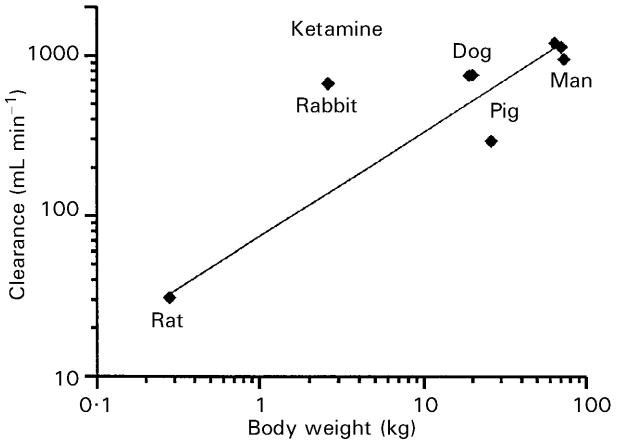


Figure 5. Allometric scaling of the total blood clearance of ketamine by the body weight method.

$Vd_{ss}$ than in mean CL between the clinical studies. Therefore, predictions of the $Vd_{ss}$ of alfentanil, thiopentone and ketamine in man were variously successful depending on which observed data were used for comparison. Predictions were unsuccessful for methohexitone. Allometric scaling of $Vd_{ss}/f_u$ invariably failed to give accurate predictions for man (Table 2).

## Discussion

For fentanyl, alfentanil, methohexitone and thiopentone all measured clearance values in the animals were comparable with or lower than the estimated $Q_{hep}$. The use of these anaesthetics in animals is supported by the pharmacokinetic findings. Ketamine, however, showed extensive extrahepatic clearance in the rabbit. The mean AUC ratio of norketamine to ketamine was also 4·6-times higher than in the rat. Due to its very high clearance and short half-life, ketamine does not appear to be a good anaesthetic agent for rabbits. An extensive extrahepatic clearance of ketamine in the rabbit is in agreement with the finding that microsomes from rabbit lung had comparable capacities with those from liver to metabolize ketamine to norketamine (Pedraz et al 1986). Also the clearance of thiopentone was much higher than expected in the rabbit, as compared with the other animal species. Whether this reflects extra hepatic metabolism is not known. For these drugs the rabbit was a poor experimental model for prediction of clearance in man.

Comparison of clearance to estimated $Q_{hep}$ is a good (but seldom used) way to evaluate findings of pharmacokinetic studies. Neglecting this may lead to spurious conclusions. It was, for example, reported by Simons et al (1996) that the mean clearance of diphenhydramine in rabbits decreased from 500 to 259 mL min$^{-1}$ during concomitant treatment with cimetidine, while the mean clearance of chlorpheniramine decreased from 361 to 270 mL min$^{-1}$. Those observations were ascribed to competitive inhibition of hepatic metabolism. However, all clearance values, also during cimetidine treatment, vastly exceeded the $Q_{hep}$ of a rabbit (Table 1) and consequently inhibition of hepatic enzymes cannot be the full explanation. Since the blood sampling protocol appeared to be adequate to characterize the AUC, a very high $\lambda$ (causing total blood clearance to be much lower than apparent plasma clearance) or extrahepatic clearance of both drugs must be invoked.

Previously, we have used the rabbit as an animal model to study the effects of pyrogen-induced fever on the pharmacokinetics of methohexitone (Redke & Björkman 1994) and alfentanil (Björkman & Redke 1996). A positive correlation of the clearance of methohexitone with body temperature resembled observations in patients with postoperative fever (Redke et al 1991). No similar influence of endotoxin on the clearance of ketamine could be discerned.

The comparison of clearance with the estimated $Q_{hep}$ also demonstrated an important qualification to the assumption that the clearance of a drug with a high hepatic extraction ratio should be scalable according to the basic allometric function. Hepatic extraction ratios appear to be species-dependent and lower in man and big animals than in small ones. Other results of this study also contradicted the assumption; in the pairs of analogues, the clearance of the two high-extraction drugs (in animals) fentanyl and methohexitone could not be

scaled according to the basic allometric equation, while the clearance of the low-extraction drug thiopentone could be scaled in this way. The ketamine data, which conform to the assumption, should be interpreted with caution. Comparison of clearance with $Q_{hep}$ was therefore no guide to the choice of scaling method.

The discussion above assumes that $Q_{hep}$ is reasonably accurately known in the various species. As previously discussed (Redke & Björkman 1994) $Q_{hep}$ is seldom known during the conditions of the pharmacokinetic experiments, and discrepant values in a species are often found in the literature. Therefore, $Q_{hep}$ values given by an allometric function (Boxenbaum & D'Souza 1990) were used for all species except man, and only large differences between clearance and $Q_{hep}$ in an animal were used to draw conclusions about hepatic extraction ratios or extrahepatic clearance. Using estimated $Q_{hep}$ would also be the practical approach in drug development, since actual determination of $Q_{hep}$ in every pharmacokinetic experiment would be far too laborious.

The present results confirmed, in four cases out of five, that the value of the allometric exponent (according to equation 1) can be used to identify the best method for predicting clearance in man, as proposed by Mahmood & Balian (1996b). According to that report, a value of 0·71–1·0 indicates that the MLP method would be the best one, and this was true for fentanyl, alfentanil and methohexitone. If the value was between 0·55 and 0·70 scaling according to body weight only should apply, and this was true for ketamine, with the stated reservation about the dispersion of the data. If the value exceeds 1·0, then the brain weight method would give the best prediction. However, despite the high value found for thiopentone only scaling according to body weight gave good predictions of clearance in man.

Predictions of $CL_u$ generally failed or were less accurate than the corresponding prediction of total blood clearance. This was unexpected from a theoretical point of view, since correction for differences in $f_u$ should diminish variation between animal species. However, it has been observed that in practice the $CL_u$ of a drug cannot be predicted any better than the total clearance (Mahmood & Balian 1999). The poor predictions of $CL_u$ were not due to missing data (dog and sheep plasma were not available, and determination of the $f_u$ of thiopentone and ketamine in rabbits was deemed unnecessary, for the reasons discussed below). In the case of fentanyl, $f_u$ was known or determined in all species. For the other drugs, comparisons were made in which total blood clearance was predicted after omission of those animal data that did not include a value for $f_u$.

Omitting the sheep data in the prediction of total clearance of alfentanil actually increased the accuracy, giving errors of −12%, −23% and −1% by the MLP method. Similarly for methohexitone, omitting the dog data improved predictions, giving errors of −8% and −17% by the MLP method. The clearance values for thiopentone and ketamine in rabbits were clearly outliers in the scaling and did not contribute to the estimation of clearance in man. No realistic values of $f_u$ would bring the $CL_u$ in line with data from the other species.

Previously the $Vd_{ss}$ of thiopentone, fentanyl and alfentanil have been scaled from rats to man by means of physiologically based pharmacokinetic models (Wada et al 1997; Björkman et al 1998). In accordance with this, predictions of the $Vd_{ss}$ of thiopentone and alfentanil by allometric scaling were also successful. On the other hand, predictions of $Vd_{ss}/f_u$ were not accurate. As in the case of $CL_u$, this discrepancy was not due to missing values of $f_u$ (data not shown).

In conclusion, we have shown that the clearance of fentanyl, alfentanil, methohexitone and thiopentone conform to expectations based on estimated $Q_{hep}$ and reasonable hepatic extraction ratios, in a number of representative animal species. This was true also for ketamine in some species, but extrahepatic clearance in the rabbit makes the use of this drug in this species questionable.

Comparison of clearance with $Q_{hep}$ should be used to evaluate pharmacokinetic data in animals. Prediction of clearance in man was generally successful, however different methods of allometric scaling gave very different results and comparison of clearance with $Q_{hep}$ was no guide to the choice of scaling method.

*Acknowledgements*

The authors wish to thank Mrs Bodil Roth and Ms Åsa Fyge at the Hospital Pharmacy and Dr Zhongquan Qi at the Department of Experimental Research for skilful technical assistance during various phases of this study. The secretarial assistance of Mrs Ewa Vorwerk is greatly appreciated.

## References

Åkeson, J., Messeter, K., Rosén, I., Björkman, S. (1993) Cerebral haemodynamic and electrocortical $CO_2$ reactivity in pigs anaesthetized with fentanyl, nitrous oxide and pancuronium. Acta Anaesthesiol. Scand. 37: 85–91

Baggot, J. D., Toutain, P. L., Brandon, R. A., Alvinerie, M. (1984) Effect of premedication with acetylpromazine on the disposition kinetics of thiopental. J. Vet. Pharmacol. Ther. 7: 197–202

Björkman, S., Redke, F. (1996) Influence of *Escherichia coli* endotoxin on the pharmacokinetics and respiratory depressant effect of alfentanil in rabbits. J. Pharm. Sci. 85: 680–684

Björkman, S., Stanski, D. R. (1988) Simultaneous determination of fentanyl and alfentanil in rat tissues by capillary column gas chromatography. J. Chromatogr. 433: 95–104

Björkman, S., Stanski, D. R., Verotta, D., Harashima, H. (1990) Comparative tissue concentration profiles of fentanyl and alfentanil in humans predicted from tissue/blood partition data obtained in rats. Anesthesiology 72: 865–873

Björkman, S., Åkeson, J., Nilsson, F., Messeter, K., Roth, B. (1992) Ketamine and midazolam decrease cerebral blood flow and consequently their own rate of transport to the brain: an application of mass balance pharmacokinetics with a changing regional blood flow. J. Pharmacokin. Biopharm. 20: 637–652

Björkman, S., Wada, D. R., Stanski, D. R. (1998) Application of physiologic models to predict the influence of changes in body composition and blood flows on the pharmacokinetics of fentanyl and alfentanil in patients. Anesthesiology 88: 657–667

Boxenbaum, H. (1982) Interspecies scaling, allometry, physiological time, and the ground plan of pharmacokinetics. J. Pharmacokin. Biopharm. 10: 201–227

Boxenbaum, H., D'Souza, R. W. (1990) Interspecies pharmacokinetic scaling, biological design and neoteny. Adv. Drug Res. 19: 139–196

Brandon, R. A., Baggot, J. D. (1981) The pharmacokinetics of thiopentone. J. Vet. Pharmacol. Ther. 4: 79–85

Domino, E. F., Zsigmond, E. K., Domino, L. E., Domino, K. E., Kothary, S. P., Domino, S. E. (1982) Plasma levels of ketamine and two of its metabolites in surgical patients using a gas chromatographic mass fragmentographic assay. Anesth. Analg. 61: 87–92

Domino, E. F., Domino, S. E., Smith, R. E., Domino, L. E., Goulet, J. R., Domino, K. E. Zsigmond, E. K. (1984) Ketamine kinetics in unmedicated and diazepam-premedicated subjects. Clin. Pharmacol. Ther. 36: 645–653

Ebling, W. F., Wada, D. R., Stanski, D. R. (1994) From piecewise to full physiologic pharmacokinetic modeling: applied to thiopental disposition in the rat. J. Pharmacokin. Biopharm. 22: 259–292

Henthorn, T. K., Krejcie, T. C., Avram, M. J. (1992) The relationship between alfentanil distribution kinetics and cardiac output. Clin. Pharmacol. Ther. 52: 190–196

Hudson, R. J., Stanski, D. R., Burch, P. G. (1983) Pharmacokinetics of methohexital and thiopental in surgical patients. Anesthesiology 59: 215–219

Hudson, R. J., Thomson, I. R., Cannon, J. E., Friesen, R. M., Meatherall, R. C (1986) Pharmacokinetics of fentanyl in patients undergoing abdominal aortic surgery. Anesthesiology 64: 334–338

Ilkiw, J. E., Benthuysen, J. A. (1991) Comparative study of the pharmacokinetics of alfentanil in rabbits, sheep, and dogs. Am. J. Vet. Res. 52: 581–584

Ilkiw, J. E., Benthuysen, J. A., Ebling, W. F., McNeal, D. (1991) A comparative study of the pharmacokinetics of thiopental in the rabbit, sheep and dog. J. Vet. Pharmacol. Ther. 14: 134–140

Jung, D., Mayersohn, M., Perrier, D., Calkins, J., Saunders, R. (1982) Thiopental disposition in lean and obese patients undergoing surgery. Anesthesiology 56: 269–274

Kaka, J. S., Hayton, W. L. (1980) Pharmacokinetics of ketamine and two metabolites in the dog. J. Pharmacokin. Biopharm. 8: 193–202

Lemmens, H. J. M., Dyck, J. B., Shafer, S. L., Stanski, D. R. (1994) Pharmacokinetic-pharmacodynamic modeling in drug development: application to the investigational opioid trefentanil. Clin. Pharmacol. Ther. 56: 261–271

Le Normand, Y., de Villepoix, C., Pinaud, M., Bernard, J. M., Fraboul, J. P., Athouel, A., Ribeyrol, M., Beneroso, N., Larousse, C. (1988) Pharmacokinetics and haemodynamic effects of prolonged methohexitone infusion. Br. J. Clin. Pharmacol. 26: 589–594

Löscher, W., Ganter, M., Fassbender, C. P. (1990) Correlation between drug and metabolite concentrations in plasma and anesthetic action of ketamine in swine. Am. J. Vet. Res. 51: 391–398

Mahmood, I., Balian, J. D. (1996a) Interspecies scaling: predicting pharmacokinetic parameters of antiepileptic drugs in humans from animals with special emphasis on clearance. J. Pharm. Sci. 85: 411–414

Mahmood, I., Balian, J. D. (1996b) Interspecies scaling: predicting clearance of drugs in humans. Three different approaches. Xenobiotica 26: 887–895

Mahmood, I., Balian, J. D. (1996c) Interspecies scaling: a comparative study for the prediction of clearance and volume using two or more than two species. Life Sci. 59: 579–585

Mahmood, I., Balian, J. D. (1999) The pharmacokinetic principles behind scaling from preclinical results to phase I protocols. Clin. Pharmacokin. 36: 1–11

Maitre, P. O., Vozeh, S., Heykants, J., Thomson, D. A., Stanski, D. R. (1987) Population pharmacokinetics of alfentanil: the average dose-plasma concentration relationship and interindividual variability in patients. Anesthesiology 66: 3–12

Mandema, J. W., Wada, D. R. (1995) Pharmacodynamic model for acute tolerance development to the electroencephalographic effects of alfentanil in the rat. J. Pharm. Exp. Ther. 275: 1185–1194

Meuldermans, W. E. G., Hurkmans, R. M. A., Heykants, J. J. P. (1982) Plasma protein binding and distribution of fentanyl, sufentanil, alfentanil and lofentanil in blood. Arch. Int. Pharmacodyn. 257: 4–19

Morgan, D. J., Blackman, G. L., Paull, J. D., Wolf, L. J. (1981) Pharmacokinetics and plasma binding of thiopental. I: Studies in surgical patients. Anesthesiology 54: 468–473

Murphy, M. R., Hug, C. C., McClain, D. A. (1983) Dose-independent pharmacokinetics of fentanyl. Anesthesiology 59: 537–540

Pedraz, J. L., Lanao, J. M., Hdez, J. M., Domínguez-Gil, A. (1986) The biotransformation kinetics of ketamine in vitro in rabbit liver and lung microsome fractions. Eur. J. Drug Metab. Pharmacokin. 11: 9–16

Rae, J. H. (1962) The fate of pentobarbitone and thiopentone in sheep. Res. Vet. Sci. 3: 399–407

Redke, F., Björkman, S. (1994) Endotoxin-induced fever increases the clearance of methohexitone in rabbits. J. Pharm. Pharmacol. 46: 887–891

Redke, F., Björkman, S., Rosberg, B. (1991) Pharmacokinetics and clinical experience of 20-h infusions of methohexitone in intensive care patients with postoperative pyrexia. Br. J. Anaesth. 66: 53–59

Sams, R. A., Muir, W. W., Detra, R. L., Robinson, E. P. (1985) Comparative pharmacokinetics and anesthetic effects of methohexital, pentobarbital, thiamylal, and thiopental in Greyhound dogs and non-Greyhound, mixed-breed dogs. Am. J. Vet. Res. 46: 1677–1683

Sharma, R. P., Stowe, C. M., Good, A. L. (1970) Studies on the distribution and metabolism of thiopental in cattle, sheep, goats and swine. J. Pharmacol. Exp. Ther. 172: 128–137

Simons, K. J., Chen, X., Fraser, T. G., Simons, F. E. R. (1996) Effect of cimetidine on the pharmacokinetics and pharmacodynamics of chlorpheniramine and diphenhydramine in rabbits. Pharm. Res. 13: 301–304

Toutain, P. L., Brandon, R. A., Alvinerie, M., Baggot, J. D. (1983) Thipentone pharmacokinetics and electrocorticogram pattern in sheep. J. Vet. Pharmacol. Ther. 6: 201–209

Van Beem, H., Van Peer, A., Gasparini, R., Woestenborghs, R., Heykants, J., Noorduin, H., Van Egmond, J., Crul, J. (1989) Pharmacokinetics of alfentanil during and after a fixed rate infusion. Br. J. Anaesth. 62: 610–615

Wada, D. R., Björkman, S., Ebling, W. F., Harashima, H., Harapat, S. R., Stanski, D. R. (1997) Computer simulation of the effects of alterations in blood flows and body composition on thiopental pharmacokinetics in humans. Anesthesiology 87: 884–899

Weiss, M., Sziegoleit, W., Förster, W. (1977) Dependence of pharmacokinetic parameters on the body weight. Int. J. Clin. Pharmacol. 15: 572–575

Wieber, J., Gugler, R., Hengstmann, J. H, Dengler, H. J. (1975) Pharmacokinetics of ketamine in man. Anaesthesist 24: 260–263