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Route- and dose-dependent pharmacokinetics of hexobarbitone in the rat: a re-evaluation of the use of sleeping times in metabolic studies

M. VAN DER GRAAFF*, N. P. E. VERMEULEN† AND D. D. BREIMER*‡

*Center for Bio-Pharmaceutical Sciences, Divisions of *Pharmacology and †Pharmaceutical Analysis, University of Leiden, Sylvius Laboratories, P.O. Box 9503, 2300 RA Leiden, The Netherlands*

The metabolic clearance (CL) and half-life of racemic hexobarbitone and sleeping time were studied in rats following intra-arterial (i.a.), intraperitoneal (i.p.) and oral (p.o.) administration, at dose levels of 25 and 100 mg kg^{-1} of its sodium salt. $CL_{p.o.}$ was higher than $CL_{i.a.}$ at both 25 and 100 mg kg^{-1}. $CL_{i.a.}$ and $CL_{i.p.}$ values were much lower, but $CL_{i.p.}$ was higher than $CL_{i.a.}$ at 25 mg kg^{-1} and lower than $CL_{i.a.}$ at 100 mg kg^{-1}. There was no distinct dependency of the half-lives on route of administration, but a slight increase upon increasing the dose was observed. Hexobarbitone blood concentrations at which the rats awoke were significantly higher after 100 mg kg^{-1} i.p. than after 100 mg kg^{-1} i.a., although there was only a small difference in sleeping time. It is postulated that the rate of uptake of the barbiturates into the portal system after i.p. administration is so high that transient saturation of hepatic first-pass metabolism occurs. Therefore neither $CL_{i.p.}$ nor sleeping times can be used as an accurate reflection of drug-metabolizing enzyme activity in the rat; instead $CL_{p.o.}$ should be used.

Hexobarbitone is a widely used model substrate for the assessment of changes in the activity of hepatic drug-metabolizing enzymes, both in man and in experimental animals (Breimer et al 1977; Vermeulen et al 1983). Its disposition kinetics have been widely studied in the rat (Holcomb et al 1974; Hétu & Joly 1979; Vermeulen et al 1979; Igari et al 1982; Van der Graaff et al 1983a). The drug is almost entirely metabolized in the liver, with minimal contributions of the lungs (Lister & Virgo 1982) and the intestines (Pantuck et al 1976). For the rat, hexobarbitone has been classified as an 'intermediately high-clearance drug', undergoing a first-pass elimination of about 70% upon oral administration (Vermeulen et al 1983). It is preferentially oxidized by the major phenobaritone-inducible subspecies of cytochrome P-450, Cyt. P-450b (Ryan et al 1982). Quite often, changes in sleeping times (ST) following intraperitoneal (i.p.) administration of the drug to rodents, in doses in the order of 100 mg kg^{-1}, are used as an index of changes in hepatic drug metabolizing enzyme activity. The popularity of this relatively simple procedure is based on the assumption that there is some inverse relation between the duration of hypnotic effect and cytochrome P-450 activity responsible for the elimination of the drug. However, there is no experimental proof that either CL or ST after i.p. administration is a good reflection of drug metabolizing enzyme activity; on the other hand, CL after oral (p.o.) administration of low doses of hexobarbitone has been shown to be proportional to intrinsic clearance and hence enzyme activity (Vermeulen et al 1983; Van der Graaff et al 1983b).

The objective of the present rat experiments was to compare ST, CL and blood elimination half-lives following the often employed i.p. route of administration of hexobarbitone to corresponding data upon p.o. and i.a. administration. Since it is known to exhibit dose-dependent pharmacokinetics in the rat (Vermeulen et al, unpublished) both 25 and 100 mg kg^{-1} doses were used.

MATERIALS AND METHODS

Chemicals

Racemic hexobarbitone [1,5-dimethyl-5-(1′-cyclohexenyl)-barbituric acid] was used as its sodium salt (Evipan, Bayer, Leverkusen, GFR), Vinylbital [5-(1′-methylbutyl)5-vinylbarbituric acid] was kindly provided by Byk-Nederland (Zwanenburg, The Netherlands). Dichloromethane and light petroleum (b.p. 40–60 °C) were obtained from J. T. Baker (Deventer, The Netherlands) and were freshly redistilled.

Apparatus

The gas chromatograph used was a Hewlett Packard (HP) model 5710A, equipped with a nitrogen

† Correspondence.

selective detector and a HP 7671 automatic liquid sampler. The chromatographic data were processed by a HP 3390 reporting integrator. A glass capillary column (length 10 m; inside diameter 0·35 mm) was used, statically coated with OV-1701 (0·2% w/v in pentane). Column temperature was 215 °C, the injection port and detector temperature were kept at 300 °C. The carrier gas was helium, with a flow-rate of 5 ml min^{-1}.

Animals

Male, SPF Wistar rats (180–220 g) from the laboratory breed were used and were permitted free access to water and food. The animals were fed a commercially available diet (Standard Laboratory Rat, Mouse and Hamster Diets, RMH-TM, Hope Farms, Woerden, The Netherlands). From 16 h before the experiments until 24 h afterwards, only water was allowed.

Treatment

Doses of 25 or 100 mg kg^{-1} of racemic hexobarbitone-Na, dissolved in isotonic saline (0·9% NaCl) just before administration, were used. For i.a. administration, a solution containing 5·0 or 20·0 mg per 0·5 ml was given by linear infusion at a rate of 600 µl min^{-1}, which means that administration was completed in about 50 s. For p.o. administraton, a solution containing 5·0 or 20·0 mg per 1·0 ml was administered by stomach tube. For i.p. administration the solution contained 5·0 or 20·0 mg per 0·5 ml and was administered by injection (5 s).

Blood sampling

Animal operations and arterial blood sampling were performed as described previously (Van der Graaff et al 1983a). Except for the 25 mg kg^{-1} p.o. experiments, it was tried as much as possible to adhere to a tight blood sampling scheme in the initial phase of the experiments, with samples being taken at 2·5, 5, 7·5, 10, 15 and 20 min. For the terminal log-linear phase, samples were taken at appropriate times for up to 100 min. With i.a. and i.p. doses of 100 mg kg^{-1}, an additional sample was taken at return of the righting reflex.

Assay of hexobarbitone in blood

With some slight modifications, the gas chromatographic measurement of the drug in haemolysed whole blood was carried out as described previously (Van der Graaff et al 1983a). To 100 µl blood samples, 100 µl of dichloromethane–light petroleum (b.p. 40–60 °C) = 4 : 6 (v/v), containing 10 µg ml^{-1} of vinylbital as an internal standard, was added. After extraction for 20 s on a whirlmixer and centrifugation for 5 min at 3000 rev min^{-1}, 0·5 µl of the organic layer was injected into the gas chromatograph.

Sleeping times

Sleeping times (ST) were measured as the time elapsed between the loss of righting reflex and the moment the rats were able, when placed on their backs, to right themselves three times consecutively.

Data analysis

Blood elimination half-life ($t_{\frac{1}{2}}$) of hexobarbitone was determined by linear regression of the terminal part of the log blood concentration vs time curves (from 20 min after administration onwards). For each individual curve, the area under the curve (AUC) was calculated with the aid of the logarithmic trapezoidal rule method. A time-averaged, apparent extraction ratio at 25 mg kg^{-1} was calculated as: $E^{*}_{p.o.} = 1-(AUC_{p.o.}/AUC_{i.a.})$. Only this extraction ratio was calculated being the closest approximation of E under conditions of completely linear enzyme kinetics. The clearance (CL) was calculated as D/AUC, where D is the dose. It was assumed that absorption of hexobarbitone was complete in all cases (Van der Graaff et al 1983a). Sleeping times (ST), $t_{\frac{1}{2}}$ and CL data were compared using the unpaired Student's *t*-test.

RESULTS

Pharmacokinetics in blood

All blood concentration vs time curves in individual experiments exhibited a log-linear disappearance of hexobarbitone after 20 min, when absorption and/or distribution were apparently complete. In Fig. 1 mean data are displayed for the two dose levels administered via the three different routes. With 25 mg kg^{-1}, the highest concentrations were obtained after i.a. administration, whereas concentrations following p.o. administration were much lower as a result of the hepatic 'first-pass' effect. Upon i.p. administration of 25 mg kg^{-1}, concentrations were between i.a. and p.o. concentrations, whereas after 100 mg kg^{-1} concentrations were higher than following i.a. administration and substantially higher than expected on the basis of a linear dose-concentration proportionality relationship (Fig. 1B).

Elimination half-lives and clearance data

All data are presented in Table 1. At 25 mg kg^{-1}, $CL_{i.p.}$ was between $CL_{p.o.}$ and $CL_{i.a.}$. At 100 mg

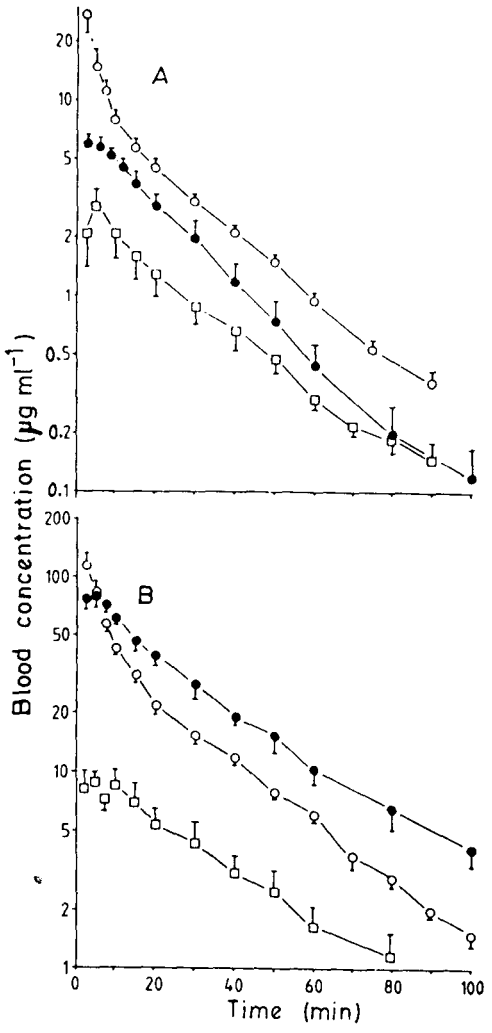

FIG. 1. Log blood concentration vs time curves after administration of hexobarbitone-Na at dose levels of (A) 25 mg kg^{-1}, (○), i.a., n = 6; (●), i.p., n = 6; (□), p.o., n = 5 and (B) 100 mg kg^{-1}, (○), i.a., n = 8; (●), i.p., n = 8; (□), p.o., n = 6.

kg^{-1}, all clearance values were lower than after 25 mg kg^{-1}. In this case, $CL_{i.p.}$ was even lower than $CL_{i.a.}$. The apparent extraction ratio $E_{p.o.}$ at 25 mg kg^{-1} was 0·82 ± 0·03.

Table 2. Sleeping times (min) and concentrations at return or righting reflex C_{awake} (μg ml^{-1}).

Route	Sleeping time (min) at 25 mg kg^{-1}	Sleeping time (min) at 100 mg kg^{-1}	C_{awake} (μg ml^{-1}) at 100 mg kg^{-1}
p.o.	0 (n = 5)	0 (n = 6)	—
i.p.	0 (n = 6)	29·4 ± 4·2 (n = 8)	23·2 ± 1·7 (n = 8)
i.a.	0† (n = 6)	25·9 ± 1·0 (n = 8)	16·7 ± 1·3 (n = 8)*

Results are given as mean ± s.e.m.
† Transient loss or righting reflex during injection.
* $P < 0·01$ compared with 100 mg kg^{-1} i.p.

Sleeping times

No loss of righting reflex was observed following i.p. or p.o. administration of 25 mg kg^{-1}, or after 100 mg kg^{-1} p.o. With 25 mg kg^{-1} i.a., a transient loss of righting reflex was observed during injection in some animals. Upon i.a. administration of 100 mg kg^{-1}, ST was 25·9 ± 1·0 min (n = 8), and after 100 mg kg^{-1} i.p., a longer ST was observed, viz 29·4 ± 4·2 min (n = 8). The difference, however, was not statistically significant. The blood hexobarbitone concentration at which the rats awoke was 16·7 ± 1·3 μg ml^{-1} with the i.a. route, which was significantly ($P < 0·01$) lower than after i.p. administration, 23·2 ± 1·7 μg ml^{-1}. These data are presented in Table 2.

DISCUSSION

The kinetic data obtained in the present study at a dose of 25 mg kg^{-1}, were in good agreement with previously published data for 50 mg kg^{-1} of hexobarbitone (Vermeulen et al 1983). $CL_{p.o.}$ was much greater than $CL_{i.a.}$; since an oral dose is absorbed into the portal circulation and subsequently passes through the liver, elimination by the hepatic enzymes during the first passage is likely to prevent a large fraction of the dose from reaching the systemic circulation. On the other hand, elimination of an i.a. dose of a high-extraction drug is much more dependent upon the rapidity of transport (portal blood flow) to the eliminating organ (Wilkinson & Shand 1975). Qualitatively, the processes of drug uptake and elimination following p.o. and i.p. administra-

Table 1. Elimination half-lives ($t_{\frac{1}{2}}$) and clearances (CL) of hexobarbitone.

Route	25 mg kg^{-1} t (min)	25 mg kg^{-1} CL (ml min^{-1} kg^{-1})	n	100 mg kg^{-1} t (min)	100 mg kg^{-1} CL (ml min^{-1} kg^{-1})	n
p.o.	19·0 ± 1·1	406 ± 56	5	20·8 ± 2·4	284 ± 35	6
i.p.	15·0 ± 0·6	188 ± 18	6	20·6 ± 1·2	46·3 ± 4·9	8
i.a.	17·2 ± 0·3	72·9 ± 5·9	6	20·9 ± 1·2	57·8 ± 5·7	8

tion are thought to be comparable (Lukas et al 1971), and thereby these routes are often thought to be interchangeable. In the present experiment, $CL_{i.p.}$, which was comparable to previous data reported at a dose of 125 mg kg^{-1} (Drew et al 1977) was found to be much lower than $CL_{p.o.}$; even at the 25 mg kg^{-1} dose level, i.p. administration was not comparable to p.o. administration, indicating that the route and rate of drug delivery to the liver are at least partially different. At 100 mg kg^{-1}, maximal blood concentrations were in the order of 100 μg ml^{-1} (i.p. and i.a.). These concentrations, which subsequently decline via redelivery to the liver (systemic clearance) were higher than the in-vitro affinity constant K_m of hexobarbitone, 25–35 μg ml^{-1} (Küpfer & Rosenfeld 1973; Heinemeyer et al 1980; Dvorchick & Hartman 1982; Igari et al 1982). Consequently, there was some saturation of hexobarbitone elimination.

Following 100 mg kg^{-1} i.p., blood concentrations in the order of 100 μg ml^{-1} also meant that saturation of hepatic first-pass elimination had occurred to a large extent, as a result of very high concentrations in the portal vein during absorption. In general, for all routes of administration it can be stated that going from the low dose to the high dose, there was a progressive increase in the degree of enzyme saturation. Since the terminal log concentration vs time curves were always linear, probably primarily the hepatic 'first-pass' elimination is transiently saturated and to a lesser extent systemic elimination. This is in agreement with the observation that the difference in $CL_{i.a.}$ between the two dose levels (which may be regarded as a rough index of the extent of saturation of systemic elimination) was much smaller than both the differences in $CL_{p.o.}$ and $CL_{i.p.}$. The difference in $CL_{i.p.}$ between the two dose levels was more pronounced than the corresponding difference in $CL_{p.o.}$. This may be explained by assuming that uptake of hexobarbitone following i.p. administration into the portal system and the liver is much more rapid than after p.o. administration. Correspondingly, maximal concentrations were reached earlier following i.p. than following p.o. administration (Fig. 1).

As a result of this rapid uptake of drug a more pronounced saturation of drug metabolizing enzymes during the first pass through the liver might occur, thus enabling more HB to reach the general circulation intact. Previously, similar observations have been made for hexobarbitone (Joeres et al, private communication) and another high-extraction drug, propranolol (Suzuki et al 1981), the CL of which decreased with increasing rate of portal infusion.

The sleeping time data following administration of hexobarbitone were in accordance with its high E*. Only at 100 mg kg^{-1} i.p. and i.a. could sleeping times be assessed. Although sleeping time after i.p. administration was only slightly longer than upon i.a. administration, the concentrations at waking were much higher following i.p. administration. This might be explained by the fact that in the rat, the intrinsic CL of the pharmacologically more active *S*(+)-enantiomer is higher than the intrinsic CL of *R*(−)-hexobarbitone (Van der Graaff et al 1983a). Although the extent of saturation of first-pass metabolism at higher doses is high, still a larger fraction of *S*(+)-hexobarbitone will be metabolized following i.p. administration as compared to i.a. administration. It is likely that the hypnotic effect of the same overall concentration of hexobarbitone depends on the ratio *S*(+)/*R*(−), which may vary depending on the route of administration.

In conclusion it can be stated that the best reflection of hepatic enzyme activity is in principle provided by the intrinsic clearance of hexobarbitone (Van der Graaff et al 1983b) which is proportional or even equal to $CL_{p.o.}$, at least under conditions where linear enzyme kinetics apply. This can be illustrated also from the present results. At both dose levels, the highest clearance values were obtained following p.o. administration. The procedure might even be improved if only *R*(−)-hexobarbitone were to be employed, since its K_m is higher than that of the *S*(+)-isomer (Furner et al 1969), thus minimizing the dose-dependency of $CL_{p.o.}$. $CL_{i.p.}$ is a much less useful reflection of hepatic enzyme activity. The elimination kinetics depend both on portal blood flow and hepatic enzyme activity, i.e. both perfusion and elimination rate limitations play a role, the dose level determining which of these exerts the predominant effect on the i.p. disposition kinetics. Thus although hexobarbitone sleeping times have for many years been successfully employed as a simple technique for demonstrating rough changes in hepatic drug metabolizing enzyme activity, it is very difficult to interpret such changes quantitatively in terms of changes in the concentrations or activity of the hepatic cytochrome P-450 system.

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