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Hepatic first-pass metabolism of hexobarbital in rabbits and dogs

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Summary

In rabbits, the elimination kinetics of hexobarbital followed a one-compartment open model and the rate constants did not depend on dose. AUC values for both intravenous and oral dosing increased linearly with dose. Systemic availability after oral administration or hepato-portal infusion was about 40% at every dose. On the other hand, in dogs, the elimination of hexobarbital followed capacity-limited kinetics. This capacity-limited elimination could be approximated by a simple one-compartment model with Michaelis-Menten metabolism. Systemic availability after oral administration and hepato-portal infusion increased with an increase in dose, and these values were nearly equal to each other at corresponding doses. It can be concluded, therefore, that the increase in systemic availability with an increase in dose in dogs is probably due to the greater degree of saturation at higher doses in the hepatic first-pass metabolism process.

Introduction

When a drug is administered orally, even if it is completely absorbed from the gastrointestinal tract into the the hepato-portal blood as unmetabolized drug, it may be metabolized and/or cleared by the liver before reaching systemic circulation.

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Thus, the systemic availability of orally administered drugs is frequently reduced by this first-pass effect in the liver (Harris and Riegelman, 1969; Shand and Rangno, 1972). The degree of this hepatic first-pass metabolism and the elimination rate of drugs should be largely affected by the activity of drug-metabolizing enzymes in the liver. Quinn et al. (1958) reported that the in vivo elimination half-life of hexobarbital was shortened in animal species with higher drug-metabolizing enzyme activity in the hepatic microsomes. If the metabolic pathways are saturated, systemic availability will then depend on dose. The present study was undertaken to clarify the effect of dose and route of administration on the elimination kinetics and the systemic availability of hexobarbital in rabbits and dogs, which have widely different hepatic ability to metabolize hexobarbital.

Materials and Methods

Bolus administration of hexobarbital and sampling

Adult male albino rabbits, weighing 2.5–3.0 kg, and adult male mongrel dogs, weighing 10 kg, were used. The weight of the animals was held constant by controlling food intake throughout the experimental period. Three rabbits and two dogs were used repeatedly at one-week intervals to test various doses and routes of administration. After being fasted for 24 h, with water available ad libitum, hexobarbital was administered intravenously (aural vein of rabbits and foreleg vein of dogs) and orally, in solution, to both animals in various doses. Enzyme induction by hexobarbital was not detected in either animal species under this dosage schedule. Blood was withdrawn at predetermined intervals from the congested aural vein of rabbits and from the foreleg vein of the dogs, and the plasma was separated. The drug solution was prepared at a concentration of 50 mg/ml for intravenous and 10 mg/ml for oral administration. This was done by adding the drug and an equimolar amount of NaOH to water shortly before each experiment.

Constant infusion of hexobarbital into peripheral or hepato-portal vein

Three other male albino rabbits, weighing 2.5–3.0 kg, and the same two dogs as used for the earlier experiments were used in the experiment for constant infusion of the drug into the peripheral or hepato-portal vein. For peripheral infusion, hexobarbital was infused into the aural veins of the rabbits and into the femoral veins of the dogs at one-week intervals. A week after the end of all experiments for peripheral vein infusion, hepato-portal cannulation was performed in all animals.

A polyethylene catheter (i.d. 1.14 mm, o.d. 1.57 mm) attached to a trifurcate cock was introduced into hepato-portal vein of dogs by the method of Harris and Riegelman (1969). The first hepato-portal infusion was performed at least 10 days after the surgery, and thereafter these cannulated dogs were used repeatedly at one-week intervals.

The abdomens of the 3 rabbits were locally anesthetized with 1% procaine hydrochloride and opened through a midline incision. A polyethylene catheter (i.d. 0.5 mm, o.d. 0.65 mm) was introduced into the hepato-portal vein through a

convenient branch of the pyloric vein near its origin, and the abdominal incision was sutured. The first hepato-portal infusion was performed immediately after suturing. After the end of the first infusion, the catheter, filled with fresh heparin solution, was implanted under the skin near the original incision site. After one week, the implanted catheter was again exposed and used for the second infusion.

The infusion of hexobarbital into the peripheral or hepato-portal vein was carried out at a constant rate of 0.086 ml/min for 60 min in the rabbits, and 0.246 ml/min for 120 min in the dogs by means of an infusion pump (Micro-feeder, Furue Scientific, Tokyo). Drug solutions for infusion were prepared by dissolving the appropriate amount of hexobarbital in water containing an equimolar quantity of NaOH just prior to infusion. The animals were fasted for 24 h, with water available ad libitum, before infusion. Blood was withdrawn at predetermined intervals from the congested aural vein of the rabbits and from the foreleg vein of the dogs.

Assay of hexobarbital in plasma and urine

The concentration of hexobarbital in plasma and urine was determined by gas chromatography with a hydrogen flame ionization detector, as described in a previous report (Kaneniwa et al., 1979).

Results and Discussion

Pharmacokinetics of hexobarbital in rabbits

Plasma concentration–time curves after intravenous or oral administration of 20–60 mg/kg of hexobarbital to rabbits are shown in Fig. 1. As can be clearly seen in the left graph, the plasma concentration diminished over a period of 2–3 h after intravenous administration, and the slopes (straight lines in semi-logarithmic coordi-

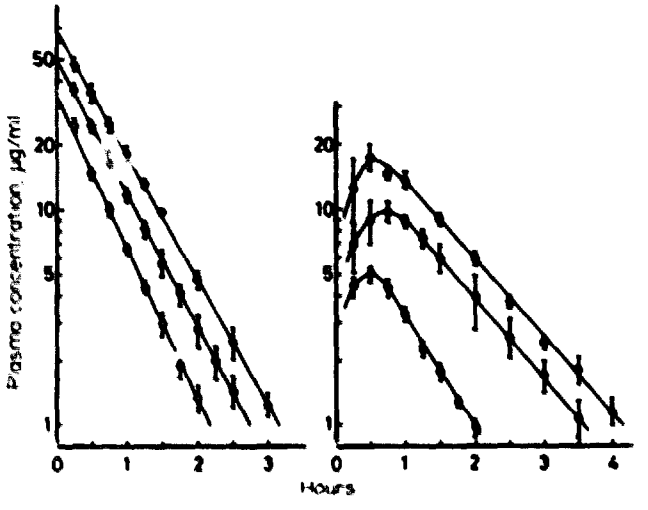

Fig. 1. Semi-logarithmic plot of plasma concentration of hexobarbital in rabbits after intravenous (left) and oral (right) administration at doses of 20 (○), 30 (◐), 40 (●), and 60 mg/kg (◑). Each point is the mean of 3 rabbits; vertical bars show standard error of the mean.

A and dog B differed little, but the V_m values in dog B were about twice that in dog A. Therefore, it may be considered that the affinity of hexobarbital for the metabolizing enzyme in the liver is similar in dog A and dog B, but the apparent activity or amounts of enzyme responsible for the metabolism of hexobarbital in dog B was two times larger than that in dog A.

The value of AUCpo/AUCiv underestimates the systemic availability of drugs which exhibit capacity-limited elimination, because the relative saturation in this elimination process is greater after intravenous than after oral administration. Thus, Eqn. 2 has been proposed to estimate the systemic availability (F) of drug exhibiting capacity-limited elimination (Martis and Levy, 1973; Jusko et al., 1976; Lettieri and Fung, 1979).

$$F = \frac{\int_0^{\infty} \frac{V_m \cdot C}{K_m + C} dt}{C_0} \quad (2)$$

where C is the plasma concentration observed after oral administration at any time t, and the parameters V_m, K_m, and C_0 are the values estimated previously from intravenous data at each corresponding dose (Table 2). As shown in Table 3, the systemic availability of oral hexobarbital, calculated from Eqn. 2, was less than unity at every dose, but nevertheless increased with dose in both dogs.

Effect of hepatic first-pass metabolism on systemic availability of hexobarbital in rabbits and dogs

As shown in Table 1, the area under the plasma concentration–time curves after infusion of hexobarbital into the peripheral vein (AUCsv) or the hepato-portal vein (AUChp) of rabbits was directly proportional to the infused dose, and was nearly equal to the values of AUCiv or AUCpo, respectively, in each dose. The systemic

TABLE 2

ESTIMATED MICHAELIS-MENTEN PARAMETERS AND MEASURE OF FIT OF PLASMA DATA AT VARIOUS INTRAVENOUS DOSES

	Dose (mg/kg)	Parameter estimated			Measure of fit (r^2)	V_m/K_m (h^{-1})
		C_0 (μg/ml)	V_m (μg/(ml·h))	K_m (μg/ml)		
Dog A	5	4.9	5.0	11.7	0.996	0.427
	10	11.6	4.8	11.5	0.999	0.417
	20	24.5	4.9	11.8	0.996	0.415
	30	39.8	5.2	11.9	0.998	0.437
Dog B	10	9.0	10.5	10.7	0.990	0.981
	20	22.2	9.7	10.9	0.997	0.890
	30	40.7	9.3	10.4	0.999	0.894

TABLE 3

SYSTEMIC AVAILABILITY OF HEXOBARBITAL AFTER ORAL (Fpo) AND HEPATO-PORTAL INFUSION (Fhp) TO DOGS AT VARIOUS DOSES, AND PARAMETERS NECESSARY TO CALCULATE THE SYSTEMIC AVAILABILITY BY EQN. 2

	Dose (mg/kg)	C_0 (μg/ml)	$(\int_0^\infty \frac{V_m \cdot C}{K_m + C} dt)_{po}$ (μg/ml)	$(\int_0^\infty \frac{V_m \cdot C}{K_m + C} dt)_{hp}$ (μg/ml)	Fpo	Fhp
Dog A	10	11.6	8.6 [a]	9.1 [a]	0.74	0.78
	20	24.5	20.0	21.2	0.82	0.87
	30	39.8	37.5	37.0	0.94	0.93
Dog B	10	9.0	5.6	6.4	0.62	0.71
	20	22.2	17.5	17.3	0.79	0.78
	30	40.7	32.5	35.0	0.80	0.86

[a] Calculated from the asymptote of the area under a plot of the value of $V_m \cdot C/(K_m + C)$ vs time t.

availability of hexobarbital infused into the hepato-portal vein was 41% (at 20 mg/kg) and 42% (at 40 mg/kg) of the corresponding doses infused into the peripheral vein, and was nearly equal to that of the corresponding oral dose. These findings show that oral hexobarbital in rabbits is probably completely absorbed from the gastrointestinal tract into hepato-portal blood as unmetabolized drug. Therefore, it may be concluded that about 60% of the infused or absorbed hexobarbital was cleared during the first-pass through the liver before reaching systemic circulation.

In the case of dogs, the disposition of hexobarbital followed capacity-limited kinetics after hepato-portal infusion (Fig. 4). Thus, the systemic availability of infused hexobarbital was calculated by Eqn. 2. The parameters V_m, K_m, and C_0 have been estimated from the intravenous data. These estimated values were used in

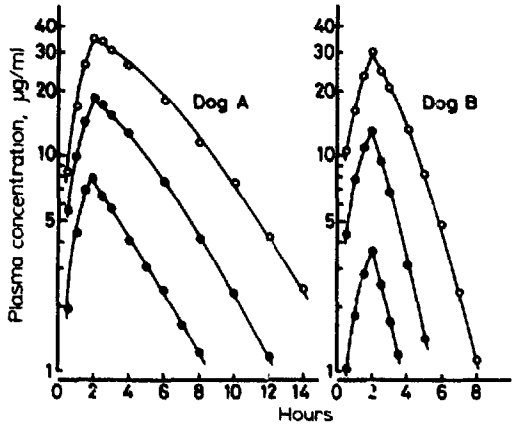

Fig. 4. Semi-logarithmic plot of plasma concentration of hexobarbital in 2 dogs (A and B) after hepato-portal infusion for 120 min at doses of 10 (◑), 20 (●), and 30 mg/kg (○).

TABLE 4

AREA UNDER THE PLASMA CONCENTRATION–TIME CURVES (AUC) AND RATIO OF AUChp/AUCsv OF HEXOBARBITAL AFTER CONSTANT-RATE INFUSION TO DOGS AT VARIOUS DOSES

Dose (mg/kg)	Dog A ($\mu g \cdot ml^{-1} \cdot h$)			Dog B ($\mu g \cdot ml^{-1} \cdot h$)		
	AUChp	AUCsv	AUChp/AUCsv	AUChp	AUCsv	AUChp/AUCsv
10	33.6	48.7	0.69	9.0	17.6	0.51
20	95.5	110.7	0.86	33.5	53.2	0.63
30	211.7	222.3	0.95	98.2	118.4	0.82

calculation of the systemic availability of infused hexobarbital. As shown in Table 3, the systemic availability of hexobarbital after hepato-portal infusion increased with an increase in dose, and differed little from that of the corresponding oral doses for each dog. Therefore, hexobarbital is absorbed almost completely from the gastrointestinal tract as unmetabolized drug with a major fraction of the oral dose available for the first-pass metabolism by the liver before reaching systemic circulation. If there is no hepatic first-pass metabolism of drug, AUC_hp should equal AUCsv at the same infusion rate and dose. As shown in Table 4, however, the AUChp/AUCsv was lower than unity and increased with an increase in dose in both dogs. Accordingly, the reduction of systemic availability from unit (Table 3) is probably due to hepatic first-pass metabolism. The increase in systemic availability with an increase in dose (Table 3) is probably due to the greater degree of saturation in hepatic first-pass metabolism which occurs at higher doses.

Since an insignificant amount of hexobarbital was excreted in the urine as unmetabolized drug in both rabbits and dogs, the apparent first-pass elimination rate constants, calculated from the slope of the plasma decline curves in semi-logarithmic plot, probably reflect the in vivo rate of drug metabolism in the liver of the animals tested. In dogs, the slope of the terminal phase of the decline curves with capacity-limited elimination is approximated by the value of V_m/K_m, as plasma concentrations become very small compared to K_m in the model used. These values were twice as large in dog B as they were in dog A (Table 2). Accordingly, dog B had a greater ability to hepatically metabolize hexobarbital than dog A, so that the greater reduction of systemic availability in dog B is due to greater hepatic first-pass metabolism compared to dog A at corresponding doses. On the other hand, the elimination rate constants in rabbits did not show individual variations, and the means of these values were much larger than those for dogs (Tables 1 and 2).

Conclusion

The disposition and the degree of hepatic first-pass metabolism of hexobarbital in rabbits with high hepatic hexobarbital-metabolizing ability were not dependent on

dose. In dogs the elimination of hexobarbital followed capacity-limited kinetics after administration in both intravenous and oral routes, and the degree of hepatic first-pass metabolism of hexobarbital decreased with an increase in dose. Furthermore, as expected, the degree of hepatic first-pass metabolism of hexobarbital was higher in a dog with higher hepatic hexobarbital-metabolizing ability. It may be concluded that the variations of disposition and the degree of hepatic first-pass metabolism of hexobarbital are probably due to the inter- and intra-species differences in hepatic-metabolizing capabilities of animals.

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