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Studies on Drug Metabolism. IV.[1] Effects of High Dose Administration of Pentobarbital and Phenylbutazone on the Plasma Biologic Half Lives in Various Species

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Effect of high dose administration of pentobarbital and phenylbutazone on the plasma biologic half lives were determined.

The remarkable effect on the plasma biologic half life was seen in the pentobarbital administration especially in the mouse, rabbit, cat, dog and monkey.

In the case of phenylbutazone administration, however, the prolongation of the half life in the high dose group was seen only in the dog.

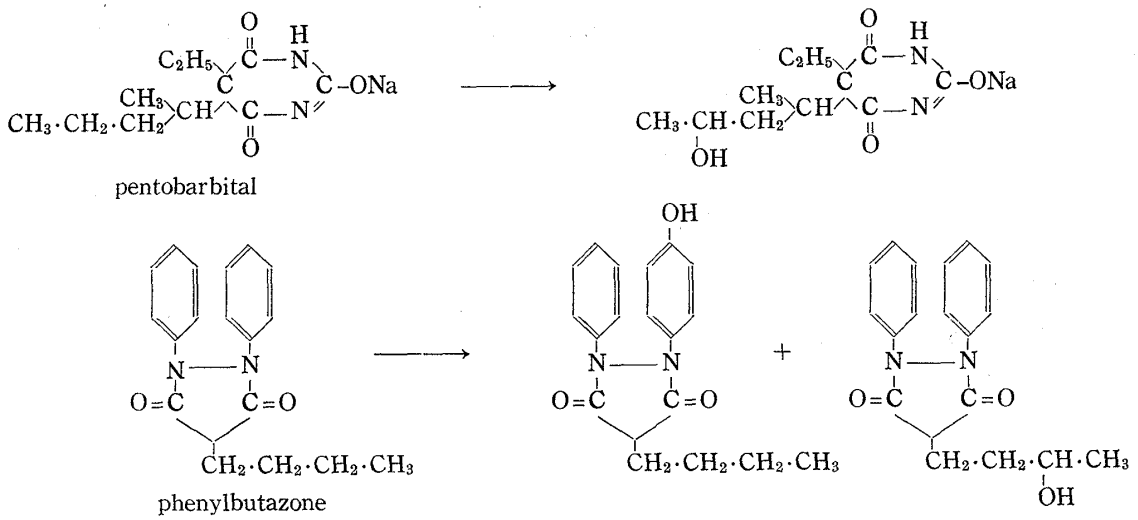
The liver microsomal pentobarbital and phenylbutazone metabolizing enzyme activities were, in general, in agreement with the plasma biologic half lives.

Introduction

Recently, Kitagawa[3] reported that the *in vivo* plasma biologic half life of pentobarbital, after high dose administration, was prolonged as consequence of respiratory imparement. Indications were that the prolongation of the biologic half life was caused by the reversible inactivation of liver microsomal drug metabolizing enzyme induced by the low blood oxygen tension resulting from the high level of pentobarbital.

Hydroxylation of the side chain leads to an inactive oxidated pentobarbital. Phenylbutazone is also hydroxylated at the aromatic ring and the side chain resulting in oxidated products.

The fates of these drugs *in vivo*[4–10] have been reported as follows;



pentobarbital

phenylbutazone

1) Part III: H. Kitagawa, K. Ono, S. Yoshida, T. Kamataki, and Y. Fukuda, *Yakugaku Zasshi*, **88**, 958 (1968).
2) Location: *a) 1–33 Yayoi-cho, Chiba-shi*; *b) 3–9–1 Izumi-cho, Narashino-shi, Chiba.*
3) H. Kitagawa, *Chem. Pharm. Bull.* (Tokyo), **16**, 1589 (1968).

Pentobarbital is one of central nervous depressants and is used as a sedatives, hypnotics of moderate duration of action and anticonvulsants. Respiratory repression is observed as a side effect.

On the other hand, phenylbutazone is an analgesic, antiinflammatory agent for gout, rheumatoid arthritis, acute bursitis and thrombophlebitis. Respiratory repression is not observed as a main or side effect even in high doses.

From the above observations, and from the findings that many drugs are oxidized by drug metabolizing enzymes of liver microsomes using aerobic oxygen,[11,12] it was thought possible to alter the biologic half life by high dose administration of pentobarbital, but not by the phenylbutazone administration because of lack of its respiratory effects.

Materials and Methods

Animals——Male mice weighing about 15—25 g, rats weighing 150—200 g, guinea pigs weighing 200—300 g, adult albino rabbits, adult cats, adult dogs and adult monkeys were used. Duration of sleep was determined by eyelid reflex in the cat, dog and monkey and by the righting reflex in the mouse, rat and guinea pig.

Materials Sodium pentobarbital was solubilized in water, and injected intraperitoneally. Phenylbutazone was solubilized in a small volume of 2.5 N sodium hydroxide and after dilution with water, the pH was adjusted to about 7.5 with 0.1 N hydrochloride solution. The final phenylbutazone concentration was obtained after dilution in water.

Method of Blood Letting——In the mouse, rat and guinea pig, the blood was collected into heparinized centrifuge tubes after cutting their carotid arteries. In larger animals, such as rabbits, cats, dogs and monkeys, the blood samples were obtained by heparinized injector or cannula from the femoral veins and after each blood collection, 2 volumes of plasma expander (Alginon, Nippon Eiyokagaku Co., Ltd.)[13] was injected intravenously per unit of blood collected.

Estimation of Pentobarbital and Phenylbutazone——Estimation of pentobarbital and phenylbutazone for measuring plasma biologic half lives and for determinations of metabolizing enzyme activities were as follows:

1) Preparation of Liver Microsomal Fraction: The procedure for obtaining the enzyme has been described previously.[14]

2) Estimation of Pentobarbital: The estimation of pentobarbital was also described in this series I.[3]

3) Estimation of Phenylbutazone: The estimation of phenylbutazone was according to the method of Burns, *et al.*[9] that is, 1 ml of the incubation mixture or plasma was pipeted into a 60 ml glass stoppered bottle containing 0.5 ml of 3 N hydrochloride and 20 ml of *n*-heptane. After shaking for 30 min and centrifugation at 2000 rpm for 5 min, 15 ml of the organic phase was transfered to an another bottle containing 5 ml of 2.5 N sodium hydroxide, shaken for 5 min and centrifuged at 2000 rpm for 5 min. The heptane phase was removed by a finetipped pipet. The 2.5 N sodium hydroxide phase was transfered to a quartz cuvette, and the optical density was read at 260 mμ and 310 mμ by Shimazu spectrophotometer.

From the calibration curve obtained by pure phenylbutazone, the concentration in plasma or the unmetabolized concentration in the incubation mixture was determined.

Results and Discussions

Sleep induced by low and high dose of pentobarbital to various species and plasma level of the drug during activity are shown in Table I.

4) E.W. Maynert and H.B. van Duke, *Science*, **110**, 661 (1949).
5) E.W. Maynert and J.M. Dawson, *J. Biol. Chem.*, **195**, 389 (1952).
6) B.B. Brodie, J.J. Burns, Lester C. Mark, Philip A. Lief, Elemore Bernstein, and E.M. Papper, *J. Pharmacol. Exptl. Therap.*, **109**, 26 (1953).
7) E. Titus and H. Weiss, *J. Biol. Chem.*, **214**, 807 (1955).
8) J.R. Cooper and B.B. Brodie, *J. Pharmacol. Exptl. Therap.*, **120**, 75 (1955).
9) J.J. Burns, Rose K.Rose, Theodore Chenkin, A. Goldman, Arthur Schulert, and Bernard B. Brodie, *J. Pharmacol. Exptl. Therap.*, **109**, 346 (1953).
10) J.J. Burns, Rose K. Rose, Sidney Goodwin, Jules Reichenthal, Evan C. Horning, and Bernard B. Brodie, *J. Pharmacol. Exptl. Therap.*, **113**, 481 (1955).
11) H.S. Mason, *Advance in Enzymol.*, **19**, 79 (1957).
12) H.S. Mason, *Science*, **125**, 1185 (1957).
13) It contains sodium alginate 4.0 g, glucose 50.0 g, sodium chloride 3.0 g, citric acid 0.015 g and sodium phosphate 0.113 g in 1000 ml of Alginon.
14) H. Kitagawa, S. Yoshida, and T. Kamataki, *Yakugaku Zasshi*, **88**, 954 (1968).

Table I. Sleep induced by Low and High Dose of Pentobarbital to Various Species and Plasma Level of the Drug While Awake

Species	Dose (mg/kg)	Duration of sleep (min)	Plasma level while awake (μg/ml)
Mouse	35	0.0	—
	70	74.9	7.0
Rat	35	75.5	17.0
	70	212.7	18.5
Guinea pig	20	128.2	9.6
	40	207.9	10.5
Cat	35	123.3	28.5
	70	357.5	42.0
Dog	30	42.5	23.0
	65	45.0	43.0
Monkey	15	60.0	8.0
	30	12—24 hr	3.1—6.0

In the rat and guinea pig, the variation of the sleep period between low and high dose administration was about 2—3 fold, whereas the plasma levels during activity in both cases were similar. The plasma level during activity, in the case of low dose administration was higher than that of high dose in the cat and monkey. In contrast, monkey administered low dose awoke at high plasma level of the drug. Indications are then that the blood level of the drug is not always only one factor in determining the duration of sleep.

The effect of the low and high dose administration of pentobarbital on the plasma biologic half life is shown in Table II.

Table II. Effect of the Low and High Dose Administration of Pentobarbital on the Plasma Biologic Half Life

Species	Dose (mg/kg)	Biologic half life (hr)	Dose (mg/kg)	Biologic half life (hr)
Mouse	35	—	70	0.8
Rat	35	2.3	70	2.1
Guinea pig	20	2.35	40	2.7
Rabbit	40	1.0	80	3.0
Cat	35	6.75	70	long, 9.3
Dog	30	4.5	65	34.4,12.2
Monkey	15	3.6	30	10.6

The half life of pentobarbital when administered at a low dose in mice was less than 0.1 hr. When administered in high dosage, the half life was 0.82 hr, thus the half life in the group administered a high dose was longer than that of the low dose.

No difference of half lives between the low and high dose groups were found in the rat and the guinea pig, whereas prolongation of the half life was observed when a high dose of the drug was administered to the rabbit and the monkey. In the dog, the disappearance of the drug from the blood was similar with that in the cat, that is, a two phase type half life was encountered.

The species difference of plasma biologic half life of phenylbutazone administered in low and high doses is shown in Table III.

Table III. Effect of the Low and High Dose Administration of Phenylbutazone on the Plasma Biologic Half Life

Species	Dose (mg/kg)	Biologic half life (hr)	Dose (mg/kg)	Biologic half life (hr)
Mouse	100	1.5	200	3.0
Rat	100	6.5	200	7.9
Guinea pig	50	9.7	100	10.6
Rabbit	100	10.8	200	7.8
Cat	50	19.4	100	21.1
Dog	50	8.8	100	26.0
Monkey	10	long	40	long

The variations in plasma biologic half lives between the low and high dose groups were not as great as compared to the case of low and high dose of pentobarbital administration. Only in the dog, the prolongation of the half life by high dose was observed.

From the findings above, the prolongation of the half life was seen in most of the species in the high dose of pentobarbital but not in the high dose of phenylbutazone administration except in the dog.

The species differences of pentobarbital and phenylbutazone metabolizing enzyme activity by liver microsomal enzymes are shown in Fig. 1 and Fig. 2.

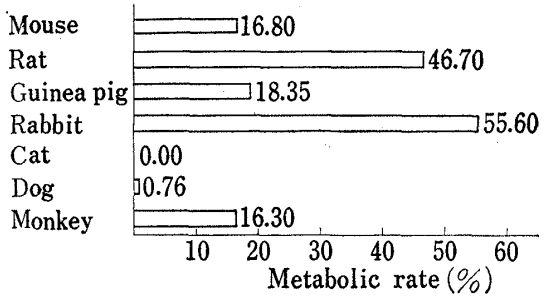


Fig. 1. Liver Microsomal Pentobarbital Metabolizing Enzyme Activity of Various Animals (*in Vitro*)

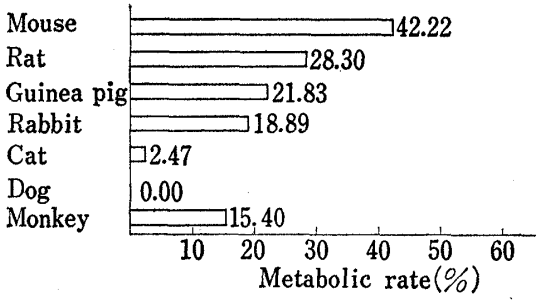


Fig. 2. Liver Microsomal Phenylbutazone Metabolizing Enzyme Activity of Various Animals (*in Vitro*)

The metabolic rate in the figures represent percent of disappearance of the parent substrates used for the assay, 1 mg of pentobarbital and 2 mg of phenylbutazone.

The pentobarbital metabolizing enzyme activity was high in the rat and the rabbit and the tendency appeared to correlate with the biologic half lives. The phenylbutazone metabolizing activity was high in the mouse, the rat and the guinea pig and also seemed related with the biologic half lives.

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