Comparative Pharmacokinetics of Coumarin Anticoagulants II

Pharmacokinetics of Bishydroxycoumarin Elimination in the Rat, Guinea Pig, Dog, and Rhesus Monkey

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Low (1–2 mg./Kg.) and high (8–20 mg./Kg.) doses of bishydroxycoumarin (BHC) were administered intravenously in cross-over fashion to rats (Sprague-Dawley, male), guinea pigs (Hartley, male), dogs (beagle and mongrel, male and female), and monkeys (rhesus, male). BHC levels in the plasma declined exponentially with time in each species. The size of the dose had no significant effect on half-life in rats and guinea pigs. The half-life was variable in the dog and increased with increasing dose in the monkey. There was no pronounced change in the apparent volumes of distribution of BHC with dose in any of the animal species studied. The rat, guinea pig, and monkey eliminate BHC considerably more rapidly, while the dog eliminates the drug more slowly than man.

THE PHARMACOKINETICS of bishydroxycoumarin (BHC) in man are unusual in that the biologic half-life of the drug increases markedly with increasing dose, although plasma concentrations appear to decline exponentially at all dose levels (1, 2). It also has been suggested that the apparent volume of distribution of BHC in man decreases with increasing dose (2). These pharmacokinetic characteristics are encountered only infrequently, and a detailed study of the mechanism responsible for them is highly desirable. In view of the potential hazards associated with the use of BHC in man a search was instituted for a suitable animal species showing the same characteristics of BHC distribution and elimination as man. While there have been some studies of BHC elimination kinetics in the mouse (3–5), rat (6–8), rabbit (3–5, 9), and dog (4, 10), these investigations were carried out at only a single dose level of BHC and, therefore, provide no information concerning a possible dose dependency in the distribution and elimination kinetics of this drug. The distribution and elimination of BHC at several dose levels in rats, guinea pigs, dogs, and monkeys have been studied in this laboratory, and the results of this investigation are described in the present communication.

EXPERIMENTAL

Bishydroxycoumarin Solution for Injection—Bishydroxycoumarin was purchased from Nutritional Biochemicals Co., Cleveland, Ohio. One part by weight of the drug and 10 parts by weight of tris(hydroxymethylamino) methane (Nutritional Biochemicals Co.) were dissolved in a sufficient volume of freshly distilled water to yield a 0.5 or 1.0% (w/v) solution of BHC. The pH of the solutions was 9.2–9.3. They were stored in a refrigerator and were used within 12 hr. after preparation.

Animals—The following is a list of animal species, strain or its equivalent, sex, body weight, and the diet fed before and during the experiments: rat, Sprague-Dawley, male, 400–500 Gm., Big Red rat mouse diet;[1] guinea pig, Hartley, male, 400–500 Gm., Big Red guinea pig diet;[1] dog, mongrel male or female or beagle male, 10–25 Kg., Big Red dog foods expanded;[1] monkey, rhesus, male, 5–7 Kg., primate diet expanded.[2] The animals had unrestricted access to water and food before and during the experiments.

Drug Administration and Collection of Plasma Samples—The drug was administered intravenously in the morning and blood samples were withdrawn at appropriate intervals. Rats and guinea pigs were placed under light ether anesthesia, and blood was collected into a heparinized centrifuge tube[3] by retro-orbital puncture using a glass capillary tube (1.6–1.8 mm. o.d.). Blood withdrawal from the cephalic vein of dogs and the saphenous vein of monkeys was done without anesthesia, using a heparinized syringe.[4] After centrifugation of the blood at 1800 × g for 10 min., the plasma was separated and stored at −15° until assayed. Blank plasma was obtained from each animal immediately before every experiment. The volume of blood withdrawn from rats and guinea pigs did not exceed 0.6 ml. per sample and 3.0 ml. in 24 hr. Two-milliliter blood samples were obtained from dogs and monkeys.

Assay Method—BHC in the plasma was determined by a modification of the method of Axelrod *et al.* (11). The assay consists essentially of adjustment of plasma to pH 3.2, extraction with heptane, transfer of the drug into 2.5 *N* NaOH, and spectrophotometry at 314 mμ. BHC metabolites in the plasma do not interfere in the assay (12).

Determination of Apparent Volume of Distribution and Biologic Half-Life—The apparent volume of distribution (V_d) was calculated by dividing the intravenous dose by the apparent initial concentration of BHC in the plasma. The latter was determined by extrapolating the linear portion of a log BHC concentration *versus* time plot to zero time. The biologic half-life ($t_{1/2}$) was determined graphically from the linear portion of the semilogarithmic plot. The small volume of the plasma samples

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[1] Country Best Foods, Agway, Inc., Syracuse, N.Y.

[2] Teklad, Inc., Monmouth, Ill.

[3] Prepared by placing 0.01 ml. sodium heparin solution (1000 units/ml., Upjohn Co.) in a centrifuge tube and allowing it to dry at room temperature overnight.

[4] Syringes were rinsed with sodium heparin solution (1000 units/ml.) and allowed to dry at room temperature overnight.

obtained from rats and guinea pigs made it necessary to use weighed rather than measured aliquots for analysis. Consequently, plasma concentration and V_d values for rats and guinea pigs are expressed in terms of mcg./Gm. and Gm./Kg., respectively. The specific gravity of the plasma averaged about 1.02.

RESULTS AND DISCUSSION

Figure 1 illustrates the elimination of BHC from the plasma in rats. Plasma concentrations of BHC declined exponentially and there was no pronounced difference in the apparent volume of distribution of BHC at the two dose levels. Unlike man, the rat did not show an increase in the biologic half-life of BHC with increasing dose.

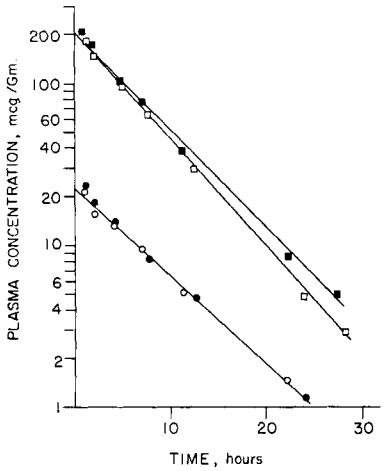

Fig. 1—Plasma concentration of bishydroxycoumarin (BHC) as a function of time after intravenous administration in rats. The dates listed in this and the other figure legends refer to the time of drug administration. Rat 1: □, 8-11-65, 20 mg./Kg.; ○, 8-4-65, 2 mg./Kg. Rat 2: ■, 8-4-65, 20 mg./Kg.; ●, 8-11-65, 2 mg./Kg.

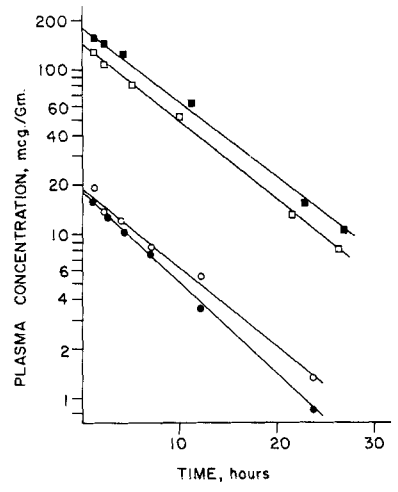

Fig. 2—Plasma concentration of BHC as a function of time in guinea pigs. Guinea pig 1: □, 8-18-65, 20 mg./Kg.; ○, 8-11-65, 2 mg./Kg. Guinea pig 2: ■, 8-11-65, 20 mg./Kg.; ●, 8-18-65, 2 mg./Kg.

TABLE I—PHARMACOKINETIC CONSTANTS FOR BISHYDROXYCOUMARIN ELIMINATION FROM PLASMA IN RATS AND GUINEA PIGS[a]

Animal No.	Body Wt., Gm.	Date Dosed	Dose, mg./Kg.[b]	$t_{1/2}$, hr.	V_d, Gm./Kg.[c]
			Rat		
1	470	8/4	2	5.6	89
	470	8/11	20	4.6	98
2	440	8/4	20	5.0	98
	430	8/11	2	5.6	89
			Guinea Pig		
1	450	8/11	2	6.3	106
	450	8/18	20	6.4	140
2	480	8/11	20	6.7	112
	500	8/18	2	5.5	111

[a] Rat, Sprague-Dawley, males; guinea pig, Hartley, males. [b] The drug was administered intravenously. [c] Apparent volume of distribution.

The elimination of BHC in the guinea pig followed a pattern similar to that found in the rat (Fig. 2). Drug elimination was exponential with the half-life slightly longer than in the rat. There was no apparent change in the half-life of BHC with increasing dose. The individual pharmacokinetic constants for BHC elimination in the rats and guinea pigs are listed in Table I.

The results of the study of BHC elimination in dogs are shown in Figs. 3, 4, and 5, and in Table II. The elimination followed apparent first-order kinetics, except for a small deviation from day 3 to day 7 after administration of the higher dose in dog *2*. The reason for this deviation is not known. There were appreciable differences in the plasma half-life of BHC between dogs. It appears that the half-life increased with increasing dose, but a definitive conclusion with respect to this apparent dose-dependency is not possible because of the occasional and unexplained pronounced changes in the half-life observed in dogs *4* and *5* (stippled lines in Figs. 4 and 5). These changes could not be ascribed to previous drug administration. There was no significant change in the apparent volume of distribution of BHC as a function of dose (Table II). Initial intensive blood sampling in one dog (No. *1*) during the first 2 hr. after BHC administration yielded an

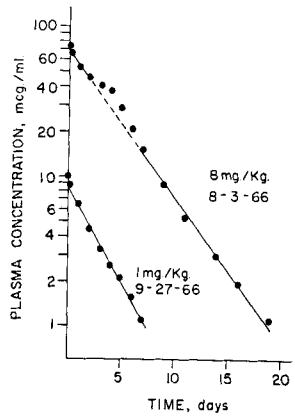

Fig. 3—Plasma concentration of BHC as a function of time in a female mongrel dog (No. 2) which received a high and a low intravenous dose of the drug.

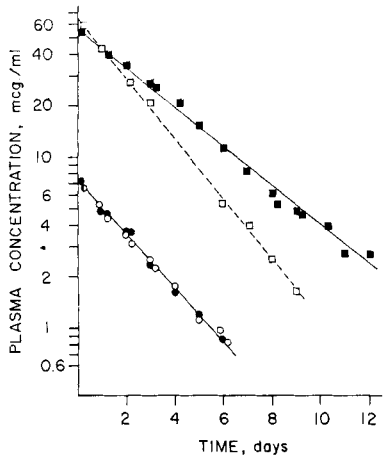

Fig. 4—Plasma concentration of BHC as a function of time in a male beagle dog (No. 4) which received two low and two high intravenous doses of the drug. Key: ●, 11-1-66, 1 mg./Kg.; ■, 11-29-66, 8 mg./Kg.; ○, 1-17-67, 1 mg./Kg.; □, 2-14-67, 8 mg./Kg.

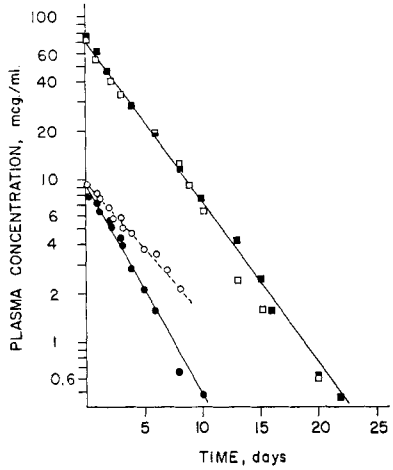

Fig. 5—Plasma concentration of BHC as a function of time in a male beagle dog (No. 5) which received two low and two high intravenous doses of the drug. Key: ○, 11-15-66, 1 mg./Kg.; ■, 12-13-66, 8 mg./Kg.; ●, 1-17-67, 1 mg./Kg.; □, 2-14-67, 8 mg./Kg.

apparent first-order distribution rate constant of 1.1 hr.$^{-1}$. Dog *3* expired due to massive internal hemorrhage on the 9th day after receiving a 10 mg./Kg. dose of BHC which had been administered 54 days after a 1 mg./Kg. dose. It is of interest that this dog exhibited the longest half-life for BHC (130 hr. at a 1 mg./Kg. dose) observed in all of the experiments (Table II).[5] Dog *1* had a considerably larger apparent volume of distribution for BHC than any of the other dogs; unfortunately, this animal was lost for reasons unrelated to BHC administration before additional experiments could be carried out.

The elimination of BHC by the monkeys showed consistent dose-dependency in that half-life increased with increasing dose (Figs. 6 and 7, Table II). There was no significant change in the apparent volume of distribution as a function of dose. An initial downward curvature in the elimination of BHC observed in monkey *1* after the first 1 mg./Kg. dose prompted a repetition of this experiment about 1 month later. Exactly the same phenomenon was observed in the second experiment. While a single occurrence of this type might be ascribed to an accidental extravascular injection of part of the dose, the exact reproduction of the plasma level curve in

[5] The $t_{1/2}$ and V_d for the 10 mg./Kg. dose were essentially the same as for the 1 mg./Kg. dose based on data collected up to the time of death.

TABLE II—PHARMACOKINETIC CONSTANTS FOR BISHYDROXYCOUMARIN ELIMINATION FROM PLASMA IN DOGS AND MONKEYS[a]

Animal No.	Body Wt., Kg.	Date Dosed	Dose, mg./Kg.[b]	$t_{1/2}$, hr.	V_d, ml./Kg.[c]
			Dog		
1	11.8	7/13	2	47	210
2	10.0	8/3	8	72	114
	10.9	9/27	1	57	118
3	23.9	8/4	1	130	106
4	10.0	11/1	1	45	133
	10.9	11/29	8	62.5	144
	12.7	1/17	1	45	133
	13.6	2/14	8	41	123
5	12.5	11/15	1	88	103
	12.5	12/13	8	72	113
	11.8	1/17	1	57	109
	12.0	2/14	8	72	113
			Monkey		
1	5.0	11/1	1	6.2	70
	5.0	11/15	4	7.4	81
	5.0	12/6	1	6.2	70
	5.0	12/13	10	10.0	76
2	6.4	12/28	1	5.5	83
	6.4	1/4	10	8.0	85
	6.4	1/17	1	5.5	83

[a] All the animals used were male except for dog *2*. Dogs *1* to *3*, mongrel; dogs *4* and *5*, beagle; monkeys, rhesus. [b] The drug was administered intravenously. [c] Apparent volume of distribution.

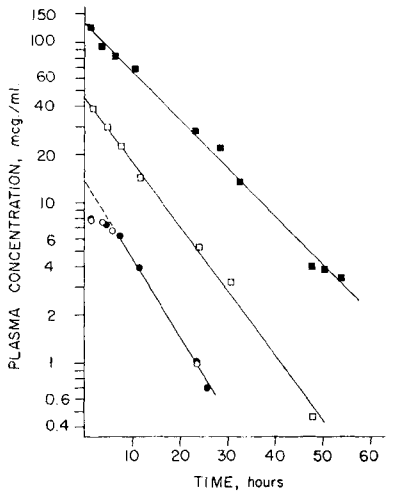

Fig. 6—Plasma concentration of BHC as a function of time in a male rhesus monkey (No. 1) which received one high, one intermediate, and two low intravenous doses of BHC. Key: ○, 11-1-66, 1 mg./Kg.; □, 11-15-66, 4 mg./Kg.; ●, 12-6-66, 1 mg./Kg.; ■, 12-13-66, 10 mg./Kg.

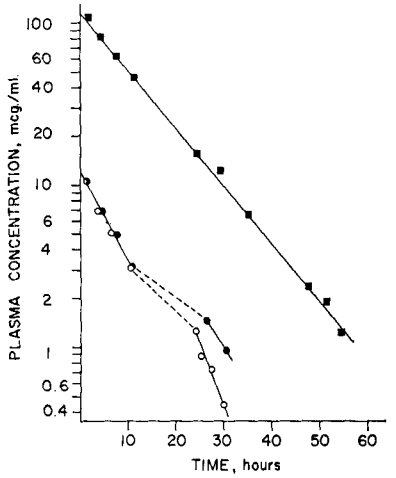

Fig. 7—Plasma concentration of BHC as a function of time in a male rhesus monkey (No. 2) which received one high and two low intravenous doses of BHC. Key: ●, 12-28-66, 1 mg./Kg.; ■, 1-4-67, 10 mg./Kg.; ○, 1-17-67, 1 mg./Kg.

TABLE III—AVERAGE PHARMACOKINETIC CONSTANTS FOR BISHYDROXYCOUMARIN ELIMINATION FROM PLASMA IN VARIOUS ANIMAL SPECIES

Species	Dose, mg./Kg.	Av. $t_{1/2}$, hr.	Av. Vd, ml./Kg.
Rat	2	5.6	89[f]
	20	4.8	98[f]
Guinea pig	2	5.9	109[f]
	20	6.6	126[f]
Dog[a]	1	58	119
	8	64	121
Monkey	1	5.9	77
	10	9.0	81
Man[b]	2.1	10	200
	8.6	32	130
Man[c]	2.0	23.6 ± 5.8	...
Man[d]	100 mg. total dose	22 ± 6	...
	150 mg. total dose	32 ± 7	...
Man[e]	5.0	40 (18–190)	~140

[a] Data on dogs *1* and *3* were excluded. [b] Data on one subject from O'Reilly *et al.* (2) after intravenous administration. [c] From Motulsky (15) after oral administration. [d] From Schrogie and Solomon (16) after oral administration. [e] From Weiner *et al.* (1) after intravenous administration. [f] Gm./Kg. (≃ml./Kg.).

the subsequent experiment makes this extremely unlikely.

Monkey *2* showed an interesting and reproducible discontinuity in the plasma concentration decline of BHC after administration of the lower dose (Fig. 7). Again, no explanation can be offered for this phenomenon other than to suggest the possibility of a postural effect, such as described recently by Levy (13). Diurnal variations were observed occasionally also in the dogs (see Fig. 4), although the long half-life of BHC in the latter makes these variations difficult to recognize. The value of intensive sampling and repetitive experiments is evident from the results of this study in that they illustrate the occurrence of interesting and apparently reproducible changes in individual elimination kinetics. A search for the mechanisms of such changes may lead to a better understanding of the effect of certain physiologic variables on drug elimination.

The findings of this study are summarized in Table III. The rat, guinea pig, and monkey showed similar half-lives for BHC elimination. The half-life of BHC in the dog is considerably longer. This is consistent with the previously observed unusually long half-life of another coumarin anticoagulant, biscoumacetate, in the dog (14). The limited number of animals studied in each group does not permit recognition of possible genetic or strain differences; the possibility of such effects should be recognized, particularly since a pronounced genetic effect has been noted in the rabbit (4, 5). Of the four species studied, only the rhesus monkey showed definite evidence of a dose dependency in the elimination kinetics of BHC, similar to that found in man. Differences in apparent volume of distribution as a function of dose were relatively minor in each species. In particular, there was no indication of a decreased volume of distribution for BHC with increasing dose, as reported for man (2). The possible reasons for this apparent difference are presently being studied.

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Keyphrases

Coumarin anticoagulants
Bishydroxycoumarin determination in plasma
Heptane extraction
UV analysis
Biologic half-life of bishydroxycoumarin
Volume of distribution—apparent
Pharmacokinetics of bishydroxycoumarin elimination