Bioavailability of Dicumarol from Different Commercial Tablets in Dogs

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Abstract ☐ The availability of dicumarol from three different commercial tablets was determined by measuring plasma levels in dogs and by an *in vitro* dissolution rate test. The products showed significant differences in plasma levels, areas under plasma level curves, peak prothrombin time responses, and rates of dissolution even though they contained the labeled amount of dicumarol.

Keyphrases ☐ Dicumarol—bioavailability from three different commercial tablets, dogs ☐ Bioavailability—dicumarol from three different commercial tablets, dogs ☐ Anticoagulants—bioavailability of dicumarol from three different commercial tablets, dogs

In 1960, Losinski (1) reported that different formulations of dicumarol (bishydroxycoumarin) tablets, although chemically equivalent, exhibited large differences in therapeutic response after administration to patients requiring anticoagulation therapy. On the basis of that report, speculation has arisen that dicumarol tablets from different manufacturers may not be equivalent in terms of drug bioavailability. The availability of dicumarol from three different commercial tablets is reported here. *In vivo* studies, performed by measuring plasma levels of dicumarol in dogs, and *in vitro* dissolution rate tests indicated that significant differences in the bioavailability of the anticoagulant from commercial products do exist.

EXPERIMENTAL

Products Tested—Three commercial tablets, designated Products A, B, and C[1], were purchased in June 1971 from local pharmacies. Each was labeled to contain 25 mg. of dicumarol.

Animals—Four healthy mongrel dogs, weighing between 10 and 21 kg., were used. They were housed in stainless steel cages under controlled temperature and lighting. The animals were fed[2] daily between 8 and 11 a.m. and allowed water *ad libitum*.

Protocol—Large interanimal differences have been observed in the plasma levels of dicumarol after oral administration to dogs on a milligram per kilogram basis. The plasma levels, however, are reproducible in an individual animal (2). Similar results have been observed in humans (2). In the clinical use of the drug, the dosage is adjusted in each patient to provide the desired therapeutic response.

Similar to the clinical use of dicumarol, the dosage used in this study was adjusted in each individual animal to give a desired response. The dosage required to provide a peak plasma level of 21–25 mcg./ml. and a doubling of normal prothrombin time was determined in preliminary experiments. This was accomplished by administering different dosages of dicumarol powder to each dog and measuring drug plasma levels and prothrombin times. After the desired dosage had been determined in an animal (either 4, 5, or 7 mg./kg.), the drug was administered in tablet form. Individual dogs received the same dose of whole 25-mg. dicumarol tablets

throughout the study. The animals were given Products A, B, and C on a randomized basis in a crossover design, and each dog received all three products using this procedure. At least a 2-week interval was allowed between doses of dicumarol to the same animal. During this period, the plasma levels of the drug fell to undetectable levels and prothrombin times returned to normal.

The animals were fasted from food for 8 hr. before and 24 hr. after drug administration. Blood samples were obtained from the external jugular vein at various times after the dose. The one-stage prothrombin time was determined on fresh, oxylated plasma. Plasma for dicumarol analysis was stored at −5°.

Analysis of Dicumarol—The spectrophotometric method of Weiner *et al.* (2) as revised by Nagashima *et al.* (3) was used for plasma. Tablets were analyzed for dicumarol by grinding a single tablet in a mortar and adding 25 ml. of 1 *N* NaOH. The solution was filtered and 0.1 ml. of the filtrate was added to 1.4 ml. of the citrate-phosphate buffer employed in the plasma assay for dicumarol. This solution was assayed for dicumarol by the same method used for plasma.

Rate of Dissolution—The USP XVIII method was used. One 25-mg. tablet was placed in a stainless steel basket immersed in 900 ml. of simulated intestinal fluid, pH 7.5 (without pancreatin), at 37°. The basket was about 4 cm. from the bottom of the beaker and rotated at 60 r.p.m. At appropriate times, a 5-ml. sample was withdrawn from the beaker by means of a sintered-glass filter pipet. An equal volume of fluid was added to the beaker. The sample was read directly at 373 nm. to determine dicumarol concentration.

Analysis of Data—Plasma concentrations of dicumarol, areas under plasma level curves, and peak prothrombin times were subjected to an analysis of variance randomized complete block design. Statistical differences between means were computed using the least significant difference method (4). The level of significance was chosen at $p < 0.05$.
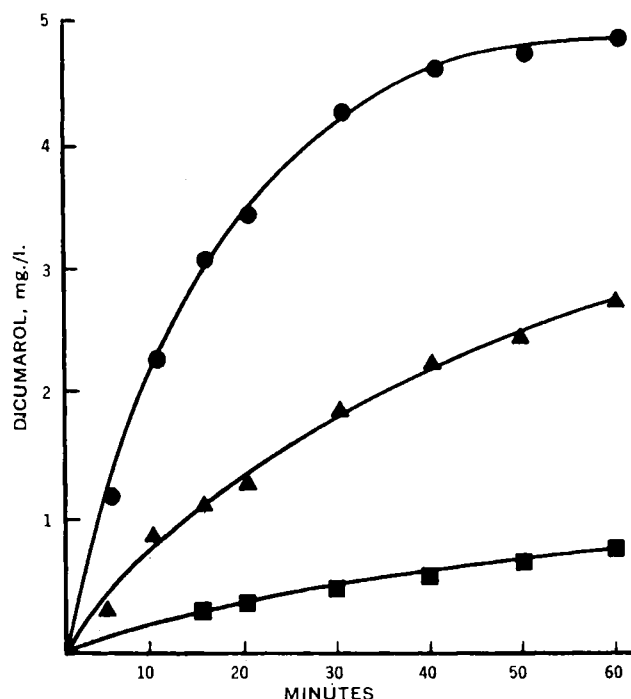

Figure 1—*Dissolution rate of dicumarol from three commercial tablets. Key:* ▲, *Product A;* ●, *Product B; and* ■, *Product C.*

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[1] Product A was Dicoumarol, 25 mg. (Abbott Laboratories, North Chicago, Ill.), Lot No. 822-1764. Product B was Blue Cross Bishydroxycoumarin, 25 mg. (Halsey Drug Co., Brooklyn, N. Y.). No lot number was listed. Product C was Bishydroxycoumarin, 25 mg. (Columbia Medical Co., New York, N. Y.), Lot 1817 (only number visible on label).
[2] Purina dog chow.

Table I—Plasma Levels of Dicumarol (Micrograms per Milliliter) and Peak Prothrombin Times Produced by Commercial Tablets

Hours	Product B Dog Number[a] 1	2	3	4	$\bar{X}$	Product A Dog Number[a] 1	2	3	4	$\bar{X}$	Product C Dog Number[a] 1	2	3	4	$\bar{X}$
1	9.5	40.3	35.2	16.6	25.4[b]	1.0	17.5	16.6	1.0	9.0	9.5	13.9	2.6	3.9	7.4
2	31.9	34.1	39.0	30.9	33.9[b]	3.4	17.5	19.7	17.2	14.5	8.6	11.8	12.2	18.4	12.7
4	28.0	34.3	39.2	29.0	32.6[b]	16.9	14.3	19.9	23.9	18.7	7.7	12.4	12.3	16.7	12.2
8	28.0	38.5	37.0	28.5	33.0[b]	23.1	13.9	19.0	23.9	19.9	11.6	13.4	12.7	15.0	13.1
12	27.2	31.3	35.9	31.0	31.3[b]	21.0	14.8	19.7	21.4	19.2[c]	9.3	12.7	13.7	12.6	12.0
24	27.7	38.5	33.1	31.8	32.7[b]	19.4	18.1	21.3	19.3	19.5[c]	9.4	14.1	13.0	12.6	12.2
48	21.0	28.2	32.5	27.1	27.2[b]	13.3	16.2	17.8	14.0	15.3[c]	5.3	9.4	12.6	9.6	9.2
72	17.2	22.0	25.5	22.8	21.8[b]	8.6	13.3	13.7	10.0	11.4	6.6	6.9	11.4	5.2	7.7
96	12.2	15.2	19.3	18.4	16.2[b]	5.6	10.8	11.1	6.1	8.4	4.1	4.1	7.2	1.7	4.2
Area under curve 0–96 hr. (mcg./ml.) (hr.) (10²)	20.3	26.8	28.7	25.0	25.2[b]	12.6	14.2	16.1	13.6	14.1[c]	6.7	9.2	11.1	8.4	8.9
Peak prothrombin time (sec.)[d]	20.4	18.3	14.5	21.4	18.6	15.0	15.8	16.9	17.4	16.2[c]	12.4	10.8	10.9	10.9	11.2
Tablet analysis for dicumarol (% of labeled amount)			95					104					110		

[a] Doses used were: Dog 1 (10 kg.), 50 mg.; Dog 2 (18 kg.), 125 mg.; Dog 3 (21 kg.), 100 mg.; and Dog 4 (20 kg.), 100 mg. [b] Significantly different from Products A and C. [c] Significantly different from Products B and C. [d] In all cases the peak prothrombin response occurred at 48 hr. after the dose. Normal prothrombin times of 7–8 sec. were not subtracted from the values presented.

RESULTS AND DISCUSSION

Significant differences were observed among the mean plasma levels of dicumarol produced by the three different products. As shown in Table I, Product B produced significantly higher plasma levels than Product A at all time points. Product C produced the lowest mean plasma levels among the three products tested. Peak plasma levels after oral administration of Product B were almost three times higher than those produced by Product C.

The mean areas under the plasma level curves from 0 to 96 hr. also reflected product differences: Product B produced a significantly larger area than Product A or C. A semilogarithmic plot of the mean values for the 48-, 72-, and 96-hr. plasma levels showed that the mean plasma half-life for dicumarol was approximately the same after each product. The observed differences in plasma levels were probably due to differences in the availability of the drug for absorption.

Also shown on Table I are the mean peak prothrombin time responses attained after a single dose of each product. These values are directly related to the dicumarol plasma level attained with each product. Product B produced a 60% greater anticoagulant response than Product C, with the response from Product A occupying an intermediate position.

A characteristic of the dicumarol plasma concentrations observed in these experiments was the rather constant level produced between 4 and 24 hr. after the dose. The plasma levels could not be adequately fit by simple one- or two-compartment models using single zero- or first-order absorption rate constants. The absorption process was undoubtedly more complex. Since all products contained the labeled amount of drug (Table I), the different plasma concentrations but similarly shaped plasma level curves suggest that incomplete absorption may be primarily responsible for the observed inequivalency among products.

The USP dissolution rate test correlated well with the plasma level results. At the end of 1 hr., Product B allowed the dissolution of five times more dicumarol than was observed with Product C. Product A occupied an intermediate position in its availability for in vitro dissolution. The USP dissolution rate test, using simulated intestinal fluid, may be of use to the formulation scientist in predicting the relative bioavailabilities of experimental products.

Large differences existed in the bioavailability of the three commercial products. Only one lot of each product was tested so the reliability of the products cannot be assessed. The highly insoluble dicumarol undoubtedly represents a challenge to the formulation scientist. One would have to conclude from both the in vivo and in vitro data that the challenge had not been met for at least two of the products.

O'Reilly et al. (5) showed that dicumarol tablets are often poorly absorbed from the GI tract of humans. If it can be assumed that similar differences in absorption of dicumarol from the tablets studied here could occur in human patients undergoing anticoagulant therapy, then consideration of these products as biologically equivalent could lead to potentially dangerous clinical situations. It should be emphasized, however, that the present study is limited in scope and that the clinical use of dicumarol tablets should not be based on these results alone. The reporting of this data is meant to alert pharmaceutical and clinical scientists to possible differences in the commercial brands of dicumarol and to serve as an impetus for further studies in man.

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