Oxyphenbutazone and Phenylbutazone Determination in Plasma and Urine by GLC

ROBERT B. BRUCE[x], WILLIAM R. MAYNARD, Jr., and LYNDA K. DUNNING

Abstract □ A method was developed for the determination of phenylbutazone and oxyphenbutazone in biological fluids using GLC. Plasma levels and urinary excretion following doses of 150 mg of phenylbutazone to dogs and 8.9 mg/kg to horses are presented. The average plasma half-lifes of phenylbutazone found were 2.5 hr in dogs and 4 hr in horses. No measurable hydroxy derivative could be detected in the plasma. The hydroxy derivative was present in horse urine for at least 48 hr but not after 72 hr.

Keyphrases □ Phenylbutazone—GLC determination in plasma and urine, compared to UV method □ Oxyphenbutazone—GLC determination in plasma and urine, compared to UV method □ GLC—determination, phenylbutazone and oxyphenbutazone in plasma and urine

The present study was undertaken to determine the bioavailability of new formulations of phenylbutazone, an anti-inflammatory and antiarthritic agent. Previously reported blood level and excretion studies were based on estimation methods using UV spectrophotometry (1, 2). In this study, GLC methods were developed and used. Such methods are more specific and easier to use than those involving spectrophotometry. Another GLC method using different conditions was reported (3) while this study was in progress. However, no results were presented in that study, and a method for the primary metabolite, oxyphenbutazone, was not given.

EXPERIMENTAL

Analytical Methods—Phenylbutazone was determined directly by GLC. However, the primary metabolite, oxyphenbutazone, had to be derivatized before it could be determined.

Materials—The tablets (Formulations I[1] and II[2]) contained 100 mg phenylbutazone/tablet. Some tablets from each preparation were weighed, and one-half tablet (within ±4%) was used so that 150-mg doses of phenylbutazone could be administered to the dogs.

Two tablets and a granulation of phenylbutazone were compared with a comparable dose of a solution in the horse studies. The tablets contained 1 g phenylbutazone/tablet, and the granules contained 2 g phenylbutazone/7.7 g. The solution was prepared as a 6% solution (w/v) in 5% $NaHCO_3$.

***In Vivo* Testing**—All animals were fasted overnight before dosing. Six male mongrel dogs were used to study Formulation I and the solution. Four dogs were given Formulation II. Six mares were used in the horse studies. The dogs were given doses of 150 mg of phenylbutazone as a single oral dose, and horses were given a single oral dose of 8.9 mg/kg body weight. Two weeks were allowed between studies to overcome enzyme induction effects.

Blood samples were taken at the intervals shown in the tables. Urine from dogs was pooled for 0–24 and 24–48 hr. Since it was not practical to collect 24-hr samples of urine from the horses, spot samples were collected at intervals as shown in the tables.

To 2.0 ml of plasma or 5.0 ml of urine, 5.0 ml of water and 0.5 ml of 2 *N* H_2SO_4 were added. This mixture was extracted three times with 20-ml portions of nanograde benzene, centrifuging after each extraction and drying the benzene through a layer of anhydrous sodium sulfate. The sodium sulfate was washed with an additional 5 ml of benzene, and the pooled benzene extracts were evaporated to dryness under a stream of nitrogen. Heptafluorobutyric anhydride (0.20 ml) was added to the residue, mixed, and allowed to stand for 10 min. The excess reagent was evaporated with a stream of nitrogen, and the residue was dissolved in 0.5 ml of methylene chloride. The methylene chloride was evaporated to 100 μl, and 5.0 μl was immediately injected into the gas chromatograph[3]. Delays may cause hydrolysis of the esterified products. The conditions for GLC were: flame-ionization detector temperature, 280°; column, 1.21 m (4 ft), stainless steel with 3% OV-210 on Gas Chrom Q at 185°; injector temperature, 300°; and flow rate, 40 ml nitrogen/min. The retention times were 5 min for phenylbutazone and 11 min for the heptafluorobutyrate of oxyphenbutazone.

If it were desired to determine only unchanged phenylbutazone, the esterification step was omitted and the residue was taken up in ether and injected.

UV Method—The results from the GLC method were compared with those obtained by a slight modification of the UV method originally described by Burns *et al.* (2). A baseline modification was used by measuring the maximum absorbance (265 nm) from a baseline drawn between the recorded absorbances at 255 and 280 nm. The background absorbance was eliminated by this procedure. A linear relationship, passing through the origin, between the amount of phenylbutazone added to blood and absorbance was obtained. The slope of the line was 0.231 μg/mm.

Table I—GLC Determination of Known Quantities of Phenylbutazone and Oxyphenbutazone Added to 5.0 ml of Control Sample

Phenylbutazone, μg		Oxyphenbutazone, μg	
Added	Found	Added	Found
	Plasma		
2.5	2.3	10	9.5
5.0	4.9	15	14.0
10.0	10.2	20	19.0
15.0	15.1	40	44.0
15.0	14.7	40	42.0
	Urine		
10	10.8	10	10.2
20	19.2	20	19.0
30	30.8	30	28.6
40	42.1	40	39.0
50	50.0	50	44.9

RESULTS AND DISCUSSION

The recoveries of phenylbutazone and oxyphenbutazone when known amounts were added to plasma or urine are shown in

[1] A. H. Robins Co.
[2] Veterinary formulation purchased on the open market.
[3] Barber Coleman Series 5000.

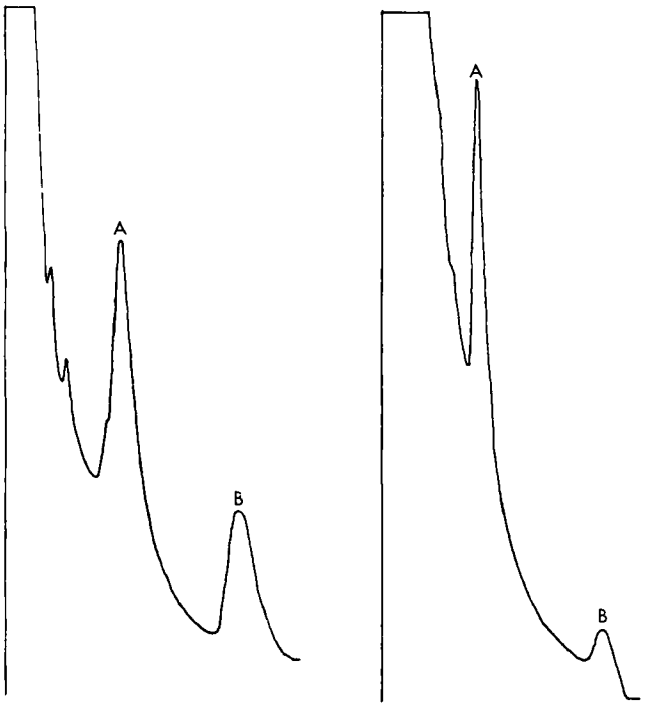

Figure 1—*Typical chromatograms found for plasma (left) and urine (right) samples. Key: A, phenylbutazone; and B, oxyphenbutazone heptafluorobutyrate.*

Table I. Chromatograms shown in Fig. 1 are typical of those obtained during the analyses. Sensitivities of the UV and GLC methods are approximately the same.

Dogs—Results obtained by the UV and GLC methods using plasma from the individual dogs that had received the solution are shown in Table II. In general, the values found by the UV method were considerably higher than those found by GLC. Mean values for the six dogs gave a maximum plasma concentration of 50.5 μg/ml by the UV method, whereas those found by the GLC method (Fig. 2) gave 30.5 μg/ml. The half-lives obtained from the mean values were approximately the same, being about 2.5 hr, and the mean maximum plasma level in each case occurred at 1 hr.

The difference in results obtained by the two methods warrants some discussion. Accuracy and precision as measured by the percent recovery and standard deviation were approximately the same, and the average percent recovery was 100.8% in each case. The standard deviations for (10 μg) 10 determinations were ±0.46 for GLC and ±0.67 for the UV method. Therefore, the difference must be due to the presence of some phenylbutazone-related material. The remaining plasma from one dog was pooled and extracted in the usual manner for the UV procedure. The solvent was evaporated to a small volume and subjected to TLC, using ethyl acetate–benzene (1:9) on silica gel. This procedure revealed three UV-absorbing spots at R_f 0.26, 0.68, and 0.89 in addition to phenylbutazone, which may have interfered with the UV method of analysis. These were not investigated further for lack of sufficient material.

Phenylbutazone appeared in the first samples of plasma taken after dosing (30 min) when the solution was administered, and the maximum plasma level occurred at this time or at 1 hr. When Formulation I was given, the first appearance of measurable plasma concentration was at 30 min for three dogs, at 1 hr for two dogs, and at 2 hr for the other dog. The maximum plasma concentration occurred at 2 hr in all dogs except one, where it occurred at 4 hr. With Formulation II, none of the dogs showed measurable plasma levels at 30 min; in two dogs the drug appeared at 1 hr, and in the other two dogs it occurred at 2 hr. The maximum plasma levels appeared only after 4 hr in all cases with Formulation II.

The phenylbutazone dose was prepared as an aqueous solution

Table II—Phenylbutazone Plasma Levels in Six Dogs following Oral Administration of 150 mg of Phenylbutazone in Solution

	Phenylbutazone, μg/ml					
Hours	Dog 1 (15.2 kg)	Dog 2 (11.1 kg)	Dog 3 (8.0 kg)	Dog 4 (10.9 kg)	Dog 5 (8.3 kg)	Dog 6 (8.2 kg)
			GLC			
0	0	0	0	0	0	0
0.5	30.0	33.2	23.4	25.3	26.6	37.1
1.0	31.4	18.3	24.7	36.4	30.6	47.8
2.0	16.9	14.3	20.8	14.1	14.8	23.2
4.0	12.2	7.8	13.8	9.6	6.2	10.7
6.0	9.7	2.8	7.4	7.8	2.0	5.4
8.0	6.1	0.7	2.8	5.8	0.4	4.9
24	0	0	0	0	0	0
			UV			
0	0	0	0	0	0	0
0.5	45.9	54.0	34.0	31.6	49.3	60.7
1.0	35.5	32.6	59.2	68.3	50.6	56.9
2.0	41.6	32.6	63.6	32.9	29.1	49.3
4.0	32.6	18.5	42.2	20.2	14.6	15.2
6.0	18.5	5.9	19.2	13.9	5.1	15.2
8.0	14.1	3.7	10.4	13.9	0	10.1
24	3.7	2.2	2.6	0	0	0

Table III—Excretion of Phenylbutazone and Oxyphenbutazone in Urine by Dogs Receiving 150 mg as Measured by GLC Analysis

	Phenylbutazone, mg			
	Dog 1	Dog 2	Dog 3	Dog 5
Formulation II:				
0–24 hr	0.009	0.69	1.18	9.30
24–48 hr	2.43	1.12	4.15	2.37
Solution:				
0–24 hr	0.56	0.27	0.33	0.52
24–48 hr	1.53	1.57	0.32	0.87
Formulation I:				
0–24 hr	2.40	5.43	0.76	2.43
24–48 hr	0	1.37	0.58	Lost
	Oxyphenbutazone, mg			
	Dog 1	Dog 2	Dog 3	Dog 5
Formulation II:				
0–24 hr	0	3.06	3.87	8.84
24–48 hr	4.25	8.10	3.01	30.60
Solution:				
0–24 hr	0.54	0.40	0.23	0.67
24–48 hr	4.65	1.39	1.88	6.08
Formulation I:				
0–24 hr	0.95	8.05	1.35	8.47
24–48 hr	3.68	6.01	3.15	Lost

Table IV—Concentrations (Micrograms per Milliliter) of Phenylbutazone in Plasma after Orally Dosing 8.9 mg of Phenylbutazone/kg as a Solution to Horses

Hours after Dosing	Horse					
	1	2	3	4	5	6
0	0	0	0	0	0	0
0.5	3.5	3.1	14.9	4.8	5.4	7.1
1.0	4.8	4.0	19.3	6.3	6.3	15.8
2.0	3.6	4.4	11.5	11.5	8.2	9.5
4.0	2.5	3.9	4.1	12.0	8.3	4.8
6.0	2.1	2.4	3.9	12.2	6.5	5.4
8.0	1.8	1.8	3.7	5.8	4.2	3.7
12.0	0.9	0.6	1.0	1.3	3.4	1.6
24.0	0	0	1.1	0.8	0.9	0.8
36.0	0	0	0.9	0.6	0	0
48.0	0	0	0.6	0.4	0	0

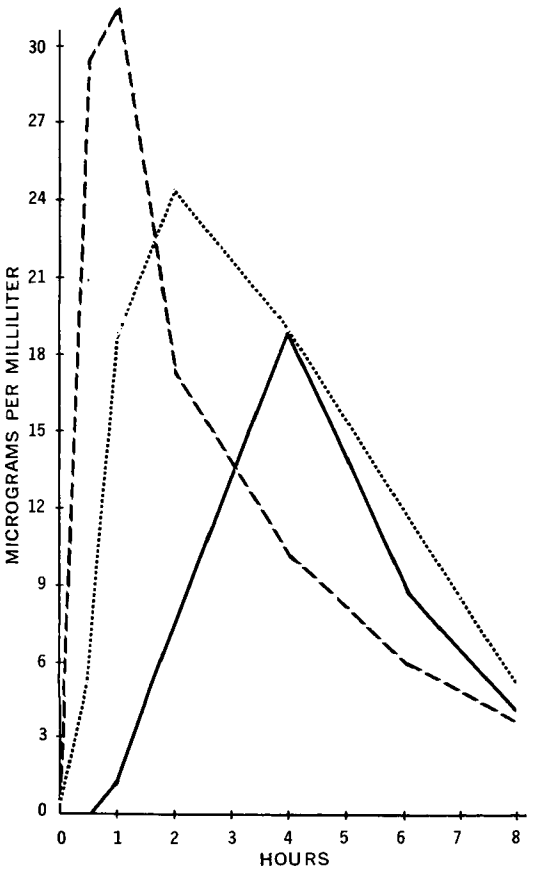

Figure 2—*Average phenylbutazone plasma levels found by GLC analysis after the administration of phenylbutazone to dogs. Key: – –, solution; - - -, Formulation I; and ——, Formulation II.*

in sodium bicarbonate because of its insolubility in water. This results in the formation of a sodium salt of the drug which is more rapidly absorbed than the free drug (4). The results presented in Fig. 2 show this effect. Perhaps a better comparison in the rates of plasma level rise and decrease would have been shown by the administration of the drug in a capsule. However, this procedure also has drawbacks since gelatin capsules often disintegrate slowly. A comparison of the total bioavailability can still be made from the results obtained by the methods used here.

The average values obtained by GLC analysis are shown in Fig. 2. These curves reflect the observations made previously. As another estimation of the relative bioavailability, the areas under the curves for the first 8 hr were calculated by triangulation, yielding the values of 71.8, 100.0, and 120.5 for Formulation II, the solution, and Formulation I, respectively. Each plasma sample obtained from the dogs after administration of the solution was analyzed for oxyphenbutazone, but none could be detected. Therefore, the remaining plasma samples were not analyzed for this compound.

The excretions of phenylbutazone and oxyphenbutazone are shown in Table III. The total amount of these compounds excreted is similar for various doses, but larger amounts of the hydroxy derivative were excreted from the solution and Formulation I than from Formulation II.

Horses—The plasma levels of phenylbutazone in horses (Table IV) show much greater variation than those found in dogs. No

Table V—Concentrations of Phenylbutazone and Oxyphenbutazone Found in Horse Urine after Dosing with 8.9 mg/kg as a Solution

Hours after Dosing	Horse 1	2	3	4	5	6
Phenylbutazone, μg/ml						
4.0	3.8	12.8	13.0	1.3	7.0	0
8.0	9.5	11.5	6.0	0	2.5	1.0
12.0	7.0	3.5	8.0	0	2.3	0
24.0	1.5	2.0	1.8	2.0	0	1.0
36.0	0	0	0	0	0	0
48.0	0	0	0	0	0	0
72.0	0	0	0	0	0	0
Oxyphenbutazone, μg/ml						
4.0	5.0	16.0	31.5	8.0	54.8	0
8.0	15.5	14.5	35.3	7.3	62.8	1.8
12.0	21.0	8.5	63.0	21.5	90.3	0
24.0	13.5	18.0	51.0	2.0	29.8	4.5
36.0	11.3	26.5	13.5	7.3	2.8	0
48.0	7.3	4.5	2.8	2.5	5.5	2.3
72.0	0	0	0	0	0	0

correlations could be found between plasma levels with either dosage form or with different animals. The estimated half-life of the drug using the average plasma level values found when the solution was given was about 4 hr. Measurable concentrations of phenylbutazone were present in the plasma of two of the horses for 48 hr. As in the case of the dogs, no measurable hydroxy metabolite could be determined in the plasma of horses.

The total excretion or excretion rates of phenylbutazone and oxyphenbutazone could not be determined in the horses because a suitable method of continuous collection was not available. Disappearance of the two compounds from the analyses of intermittent samples is shown in Table V. These figures indicate that, following a single oral dose of 8.9 mg/kg, oxyphenbutazone persists in the urine for at least 48 hr as measured by the described methods. The 72-hr samples gave negative results in all cases, and the unchanged drug could not be detected in the 36-hr samples.

The results of these studies indicate that GLC analysis of blood and urine for phenylbutazone and oxyphenbutazone is simple and specific. The method should be adaptable to the analysis of race horse urine in cases of suspected doping. Methods involving UV spectrophotometry have recently come under criticism (5) for such use.

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[x] To whom inquiries should be directed.