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IMPAIRED METABOLIC HANDLING OF DRUGS IN RATS WITH ARTHRITIS INDUCED BY 6-SULFONANILAMIDOINDAZOLE*

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Abstract—A possible explanation for the previously reported "synergistic toxicity" of normally non-lethal doses of anti-inflammatory drugs in rats with arthritis induced by 6-sulfonanilamidoindazole (SAI) was sought by examining the drug-metabolizing capability of these arthritic rats. Rats given SAI had prolonged (10-fold) sleeping times when challenged with pentobarbital. The apparent plasma half-life of phenylbutazone was increased from 4.2 to 17.4 hr in SAI-treated rats. The mean "steady state" plasma concentration of phenylbutazone was two to three times higher in SAI-treated rats. The *N*-demethylation of aminopyrine *in vitro* by liver 9000 *g* supernatant enzyme preparations proceeded at a rate of approximately 25 per cent that of control rats. The results suggest that SAI-treated rats are defective in their drug-metabolizing capability.

A periarticular inflammation of primarily the hind paws of old rats can be induced by repeated administration of 6-sulfonanilamidoindazole (SAI). Swelling of the hind paws is apparent after two or three oral doses of SAI, is maximal at about 2 weeks, and subsides at about 4 weeks [1–3]. The swelling can be prevented by appropriate doses of steroidal or non-steroidal anti-inflammatory drugs and has been suggested for use in the assessment of new drugs [2, 4]. A "synergistic toxicity" between SAI and normally non-lethal doses of drugs, such as phenylbutazone, 6-mercaptopurine and cyclophosphamide, has been reported [2, 5, 6]. An impairment in the liver microsomal drug-metabolizing system has been described for rats with arthritis induced by Freund's adjuvant [7–17] or *Mycoplasma arthritidis* [17]. The following report describes an impairment in the metabolic handling of certain drugs by SAI-arthritic rats.

EXPERIMENTAL

General design. SAI (furnished by the Sterling–Winthrop Research Institute) was administered by gavage as a suspension in 4% aqueous acacia to male Holtzman rats (Holtzman Rat Co., Madison, WI) weighing 430–480 g at the start of the experiment. The dose of SAI (250 mg/kg) was administered once daily for 10 consecutive days. Control rats were administered a like volume (5 ml/kg) of acacia. The design of the experiment was:

Days 1–10: Rats were administered either SAI or acacia.

Day 3: Ten arthritic and ten control rats were given pentobarbital-Na (25 mg/kg, i.p.) and sleep times recorded.

Days 8–10: Four arthritic and six control rats were given p.o., 15 mg/kg of phenylbutazone as a 4% aqueous suspension once a day for the 3 days. Four and 8 hr after the dose of phenylbutazone on day 10, heparinized blood was obtained from the abdominal aortas of three control and two arthritic rats for each interval and the plasma separated and frozen until use.

Day 12: The remaining rats were given a single oral dose of phenylbutazone (15 mg/kg) and heparinized blood was obtained from three or four arthritic and control rats at 1, 2, 4, 8, 12 and 24 hr after the dose. The plasma was separated and frozen until use. All rats were fasted overnight but the 12- and 24-hr groups were given food 3 hr after the dose of phenylbutazone. Livers were removed from the 4-hr group at the time of exsanguination and frozen until used for the *in vitro* metabolism studies.

Phenylbutazone determinations. The detailed procedure will be reported separately. Briefly, the concentration of phenylbutazone in plasma was determined by a high performance liquid chromatographic method. The drug and added internal standard (4-nitro-2-phenoxy-methanesulfonanilide) were extracted from acidified plasma into benzene. The benzene extract was evaporated to dryness and the residue immediately dissolved in a small volume of chloroform. The chloroform was chromatographed on a Silica gel column and the eluate monitored at 254 and 280 nm. Peak height ratios of phenylbutazone/internal standard were used to determine the concentration of phenylbutazone.

The apparent plasma half-life of phenylbutazone was estimated from the slope of a log plasma concentration vs time plot by the method of least squares.

In vitro *drug metabolism studies.* A 25% (w/v) homogenate of rat liver in 1.15% ice-cold KCl solution was prepared in a Waring blender operated for 60 sec. The liver homogenate was centrifuged at

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9000 *g* for 20 min at 0–4°, and the supernatant fraction was kept frozen until used.

One ml of the 9000 *g* supernatant fraction was added to 25-ml incubation flasks containing, in pH 7.4 phosphate buffer, nicotinamide (20 μM), $MgCl_2$ (10 μM), semicarbazide (37.5 μM), NADP (2 μM), glucose 6-phosphate (20 μM) and glucose 6-phosphate dehydrogenase (2 units) with or without aminopyrine (5 μM). The final volume of the incubation mixture was 5 ml. Samples were incubated for 15 min at 37° in a Dubnoff metabolic shaker. The reaction was terminated by the addition of 2 ml of 0.17 M $ZnSO_4$ and 2 ml of 0.14 M Ba $(OH)_2$. Formaldehyde (HCHO) was measured in the protein-free supernatant fraction by the method of Nash [18]. Results are expressed as μmoles formaldehyde formed/hr/g of liver.

RESULTS

Pentobarbital sleeping times. Swelling of the hind paws was apparent in most SAI-treated rats after the third dose and occurred in all treated rats by day 6. As indicated in Table 1, rats given SAI for 3 days had prolonged (about 10-fold) sleeping times after a dose of pentobarbital-Na. The rats which were used for the sleeping time determinations were marked for future identification. There was no apparent difference between these rats and those not so treated in their responses in subsequent aspects of the study.

Plasma half-life of phenylbutazone. Results obtained after administration of a single oral dose of phenylbutazone to SAI-treated and control rats are given in Table 2 and Fig. 1. The mean plasma concentrations of phenylbutazone were approximately the same during the first 2 hr after dosing for the two groups. Beyond 2 hr, the mean plasma concentrations in SAI-treated rats were always significantly higher than in the control rats during the same time period (Table 2). The lack of a difference in plasma concentrations of drug during the first 2 hr suggests that SAI treatment has no effect on the absorption of phenylbutazone.

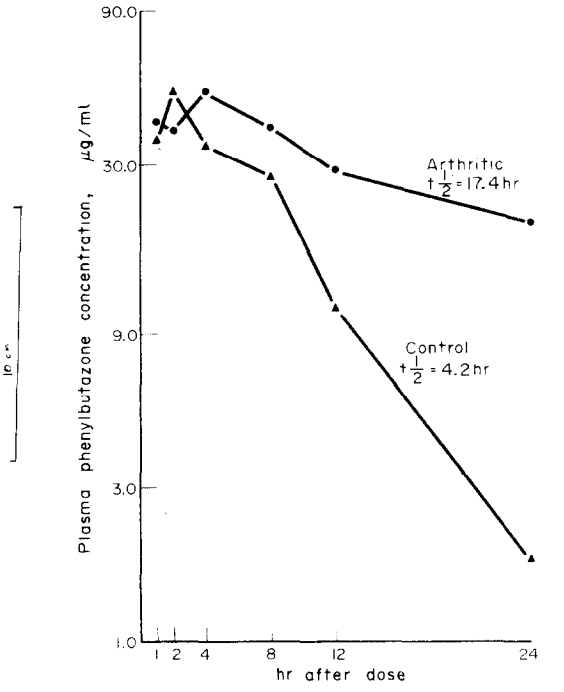

Fig. 1. Decline of mean concentration of phenylbutazone in the plasma after a single oral dose (15 mg/kg) of drug in control and SAI-arthritic rats. Mean values are based on three or four rats. The apparent plasma half-life of phenylbutazone was determined by the method of least squares.

Table 1. Effect of SAI treatment on pentobarbital sleeping times of rats*

Treatment	Sleep time (min ± S.E.)†	Range (min)
Control	27.9 ± 8.2	0–65
SAI	297.8 ± 17.1	156–358

* Pentobarbital-Na (25 mg/kg, i.p.) was administered 3 hr after the third daily dose of SAI or vehicle.
† Mean values are based on ten rats.

Table 2. Plasma concentration of phenylbutazone after a single oral dose (15 mg/kg) in SAI-arthritic and control rats

Time after dose (hr)	Phenylbutazone concentration (μg/ml ± S.D.)*		P†
	Control	SAI	
1	35.9 ± 5.1	41.1 ± 9.3	0.3
2	50.1 ± 12.8	38.9 ± 7.5	0.2
4	34.5 ± 4.8	51.1 ± 11.3	0.05
8	27.8 ± 6.6	39.5 ± 4.1	0.05
12	10.9 ± 6.0	29.1 ± 8.9	0.02
24	1.8 ± 1.2	20.0 ± 5.2	0.01

* Values are based on four rats except for the 24-hr groups in which three rats were used.
† P values were obtained by Student's '*t*' test.

The estimated apparent half-life of phenylbutazone in SAI-treated rats was 17.4 hr and is about four times that determined for control animals (Fig. 1).

"Steady state" plasma concentrations of phenylbutazone. The plasma concentrations of phenylbutazone in SAI-treated and control rats 4 and 8 hr after the last of three daily doses of drug are provided

Table 3. Plasma concentrations of phenylbutazone in SAI-arthritic and control rats after three daily oral doses of 15 mg/kg

Time from last dose (hr)	Phenylbutazone concentration (μg/ml ± S.D.)	
	Control	SAI
4	40.5 ± 3.3 (39.0, 44.2, 38.2)*	93.2 ± 16.6 (81.4, 104.9)
8	22.9 ± 12.6 (11.6, 20.6, 36.5)	72.1 ± 1.6 (73.2, 70.9)

* Individual values.

Table 4. *N*-demethylation *in vitro* of aminopyrine by liver microsomal preparations from control and SAI-arthritic rats

Treatment	HCHO production (μmoles/hr/g ± S.E.)
Control	1.15 ± 0.20 (1.36, 1.33, 0.76)*
SAI	0.28 ± 0.07 (0.25, 0.25, 0.33)

* Separate experiments. There were 2–6 replicates/experiment.

in Table 3. SAI treatment resulted in plasma concentrations two to three times greater than in the control rats. The mean steady state plasma concentrations of phenylbutazone in the control rats at 4 and 8 hr were 40.5 and 22.9 μg/ml, respectively (Table 3), and these are not markedly different from the concentrations obtained at these time intervals after a single dose of the drug (34.5 and 27.8 μg/ml, respectively; compare Table 2). Such results are anticipated because of the relatively short half-life of phenylbutazone in control rats. However, in SAI-treated rats the plasma concentrations of drug after repeated dosing were 93.2 and 72.1 μg/ml, respectively, for the 4- and 8-hr intervals (Table 3), and these are about twice those determined at these intervals after a single dose of phenylbutazone in diseased animals (51.1 and 39.5 μg/ml, respectively; compare Table 2). These results suggest that the effect of SAI treatment on the pharmacokinetics of plasma phenylbutazone after a single dose of drug is enhanced in a multiple dosing schedule due to the relatively large increase in apparent half-life produced by SAI treatment.

N-demethylation of aminopyrine in vitro. The results obtained from three separate experiments are given in Table 4. Liver microsomal preparations from SAI-treated rats *N*-demethylate aminopyrine at a rate approximately 25 per cent that of preparations from normal rats. These results obtained *in vitro* support the data obtained *in vivo* for pentobarbital and phenylbutazone and suggest that an impairment in drug-metabolizing capability exists in SAI-treated rats.

DISCUSSION

The finding that rats with SAI-induced arthritis have a prolonged sleeping time after administration of pentobarbital, an extended plasma half-life for phenylbutazone, and an impairment in the *N*-demethylation of aminopyrine *in vitro* by liver microsomal preparations argues strongly for a defect in the drug-metabolizing capability of such rats. We feel that this defect at least partly explains the increased sensitivity of these rats to normally non-lethal doses of certain anti-inflammatory drugs. Other factors might contribute to the "synergistic toxicity", however. The possibility that the defect in the drug-metabolizing capability of arthritic rats is due to restricted food intake [19] has not been excluded. Rats given SAI suffer a large (approximately 100 *g*) loss in body weight. However, restricted food intake by rats with adjuvant-induced arthritis was shown not to be the cause of the impairment in the drug-metabolizing system in these rats by Cawthorne *et al.* [17] who included pair-fed controls in their studies. The possibility of a defect at the renal level has not been ruled out and SAI-arthritic rats excrete increased amounts of protein in the urine. Renal histology has been reported to be normal, however, as has been blood urea nitrogen and serum uric acid in these rats [3]. The possibility of a quantitative or qualitative impairment of drug binding to plasma proteins might also contribute to the observed increased toxicity of phenylbutazone and other drugs. If there is more free, as opposed to bound, phenylbutazone in the plasma of SAI-arthritic rats, coupled with a defect in the ability of these rats to metabolize (detoxify) the drug, then the toxicity of phenylbutazone would be expected to be compounded. Ward *et al.* [20] have demonstrated that SAI prepares rats for the Shwartzman reaction (endotoxin-induced vascular necrosis). If the preparative properties of SAI include an alteration of the vascular endothelium, which appears likely, then drugs or substances other than endotoxin might express a toxic effect which is qualitatively different in SAI-arthritic rats than in normal rats.

The fact that 6-mercaptopurine, gold, and cyclophosphamide have been reported by others [2, 5] to be more toxic in SAI-arthritic than normal rats suggests that mechanisms in addition to impairment of the microsomal drug-metabolizing system are involved. 6-Mercaptopurine is metabolized chiefly to 6-thiouric acid by xanthine oxidase [21], a cytoplasmic enzyme. Cyclophosphamide requires activation by the microsomal system [22] and the report of increased sensitivity to this drug of animals with an impaired activating system would appear to be paradoxical unless there occurs a selective alteration in the microsomal enzyme system. Beck and Whitehouse [14] reported a selective impairment in the microsomal system of rats injected with the edemagen, carrageenan. In these rats, the activation of cyclophosphamide was decreased but not the *N*-demethylation of aminopyrine. Gold preparations are not known to be extensively metabolized [23] but their capacity to produce nephrotoxicity may be aggravated in "SAI-prepared" renal tissue. In this regard, the generalized Shwartzman reaction includes as its prominent component a renal cortical necrosis [20].

For the studies reported in this paper, phenylbutazone seemed to be a logical choice for the drug to be studied since (a) it is widely used as a "standard" anti-inflammatory drug in this as well as other models of inflammation, (b) it is extensively metabolized by the liver microsomal enzyme system, and (c) a biological correlate (increased toxicity) had already been demonstrated in the model.

An awareness that certain models being used to evaluate potential anti-inflammatory drugs may affect the metabolic disposition of these drugs is important for the proper interpretation of results.

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