Inhibition of Drug Metabolism by Hydroxylated Metabolites: Cross-Inhibition and Specificity

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Abstract □ Inhibition of drug metabolism was studied in adult male Sprague–Dawley rats. A hydroxylated metabolite of phenylbutazone (oxyphenbutazone) inhibited the elimination of phenytoin, which is metabolized by oxidative pathways. The biotransformation of a relatively polar and only slightly plasma protein-bound drug, antipyrine, was subject to product inhibition by a hydroxylated metabolite, 4-hydroxyantipyrine. Neither oxyphenbutazone nor 4-hydroxyantipyrine measurably affected the elimination kinetics or metabolic fate of a drug (sulfanilamide) that is not metabolized by oxidative pathways.

Keyphrases □ Metabolism—oxyphenbutazone, phenytoin, antipyrine, sulfanilamide, effect of hydroxylated metabolites, rats □ Oxyphenbutazone—effect on metabolism of phenytoin and sulfanilamide, rats □ Antipyrine—biotransformation, effect of 4-hydroxyantipyrine, rats □ Sulfanilamide—biotransformation, effect of oxyphenbutazone and 4-hydroxyantipyrine, rats □ Phenytoin—biotransformation, effect of oxyphenbutazone, rats □ 4-Hydroxyantipyrine—effect on metabolism of antipyrine and sulfanilamide, rats

Several studies have shown that the hydroxylated metabolites of certain drugs can inhibit the biotransformation of their precursors. Such inhibitory effects were demonstrated *in vitro* with 5-(*p*-hydroxyphenyl)-5-phenylhydantoin (hydroxyphenytoin) and 2-hydroxydesmethylimipramine (1, 2), the metabolites of phenytoin and desipramine, respectively. *In vivo* studies revealed the inhibitory effect of injected hydroxyphenytoin and oxyphenbutazone on the elimination of phenytoin and phenylbutazone, respectively (3, 4). Accumulation of endogenously formed hydroxyphenytoin in the body by blocking its glucuronidation also has caused inhibition of phenytoin elimination (5). Computer simulations indicate that product inhibition may account for the dose-dependent change in the half-life of some drugs (6).

More recently, it was shown *in vitro* that the hydroxylated metabolite of one drug can inhibit the biotransformation of another drug. Such cross-inhibition was reported for the following metabolite–drug systems: hydroxyphenytoin–phenylbutazone (7), oxyphenbutazone–phenytoin (7), hydroxyphenytoin–hexobarbital (8), hydroxyphenytoin–ethylmorphine (8), hydroxyphenytoin–zoxazolamine (9), and hydroxyphenytoin–aniline (9). It also has been shown that hydroxyphenytoin prolongs hexobarbital sleeping time in mice (8) and zoxazolamine paralysis time in rats (9), but there has been no direct pharmacokinetic demonstration of cross-product inhibition *in vivo*.

The purposes of this investigation were to determine: (*a*) if a product inhibitory effect can be demonstrated for a relatively hydrophilic and negligibly protein-bound drug (antipyrine), (*b*) if hydroxylated metabolites with a demonstrated inhibitory effect on the biotransformation of their precursors can affect the elimination of a drug metabolized by other than oxidative pathways (sulfanilamide), and (*c*) if the hydroxylated metabolite of one drug (oxyphenbutazone) can inhibit the *in vivo* elimination of another drug metabolized by oxidative pathways (phenytoin).

EXPERIMENTAL

All studies were performed on male Sprague–Dawley rats[1], 300–450 g. A two-piece cannula of silicone rubber–polyethylene was implanted in the right jugular vein under light ether anesthesia (3, 10), 3 days before the first experiment. Crossover experiments were carried out 2 weeks after the first experiment. Blood samples were obtained through one outlet of a three-way stopcock after withdrawal of 0.5 ml of blood (which was subsequently reinjected) to ensure against artifacts due to sample dilution by heparin or blood trapped in the cannula (3).

4-Hydroxyantipyrine–Antipyrine Experiments—Three rats received 4-hydroxyantipyrine[2], 100 mg/kg iv, 30 min before an intravenous injection of ^{14}C-antipyrine, 70 mg/kg, and additional doses of 4-hydroxyantipyrine, 50 mg/kg, every 45 min during the experiment. The hydroxylated metabolite was dissolved in a small volume of 1.2 *M* NaOH, which was diluted immediately with 0.2 *M* phosphate buffer, pH 7.4, to yield a 100-mg/ml solution of pH

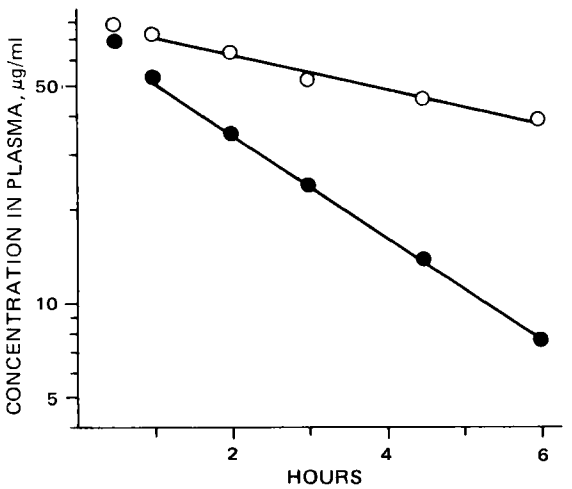

Figure 1—*Effect of 4-hydroxyantipyrine on the elimination of antipyrine, 70 mg/kg iv, in Rat 2-I. Key:* ●, *control experiment; and* ○, *with 4-hydroxyantipyrine, 100 mg/kg, 30 min before antipyrine injection and 50 mg/kg every 45 min thereafter for eight doses.*

[1] Blue Spruce Farms, Altamont, N.Y.
[2] Aldrich Chemicals Co., Milwaukee, Wis.

Table I—Effect of 4-Hydroxyantipyrine on the Elimination of Antipyrine in Rats

Rat	Half-Life, hr	
	Control	With 4-Hydroxyantipyrine
1-I	1.80[a]	4.05
2-I	1.70	5.20[a]
3-I	1.83	13.0[a]
4-I	1.60[a]	4.70
5-I	1.46[a]	5.60
6-I	1.30	4.58[a]
Mean	1.65	6.18
SD	0.23	3.38
Statistical significance of difference[b]	$p < 0.02$	

[a] First experiment in the crossover study. [b] Paired *t*-test.

9.5–10. This solution was injected slowly (3–5 min) to prevent irritation and precipitation. Three control animals received the solvent only. Crossover studies were performed on all six rats. The antipyrine injection solution was prepared by adding 5 μCi of ^{14}C-antipyrine, 12.1 mg/mCi labeled in the *N*-methyl position[3], to 0.5 ml of a 70-mg/ml antipyrine[2] solution in water.

Blood samples (0.5 ml) were obtained at 0.5, 1.0, 2.0, 3.0, 4.5, and 6.0 hr after antipyrine injection. A 200-μl portion of plasma with 0.05 ml of 1 *M* NaOH was extracted with 2 ml of chloroform by shaking on a reciprocating shaker (200 cpm) for 10 min. The phases were separated by centrifugation at 400×*g* for 5 min, and the aqueous phase was removed by aspiration. A 1.8-ml portion of the chloroform phase was evaporated to dryness in 2-ml vials[4] under nitrogen at room temperature. The sides of the vessel were washed twice with chloroform which was also evaporated.

The residue was redissolved in 50 μl of chloroform and applied to a TLC plate of silica gel G with fluorescent indicator F-254[5], which had been activated (1 hr at 100°) immediately before use. The vial was rinsed twice with 20-μl portions of chloroform, which were also applied on the plate. In addition to the plasma extracts, a chloroform solution of "cold" antipyrine was spotted as an R_f reference. The plate was developed immediately with chloroform–ethanol (9:1) for 45–60 min in a chamber which had been equilibrated with the solvent system (11).

The spots on the dried plate were visualized under UV light,

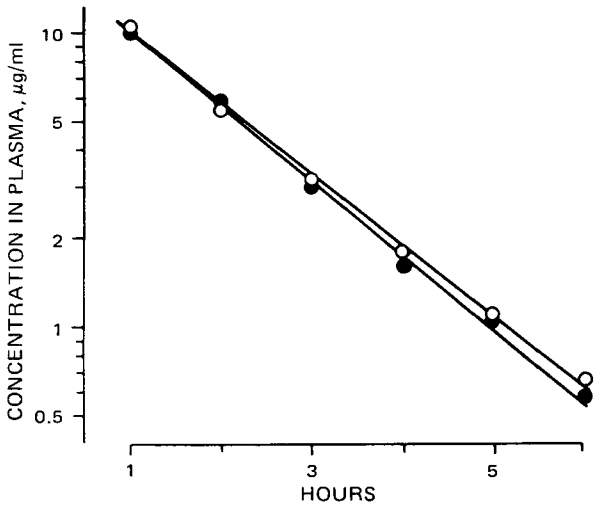

Figure 2—*Effect of 4-hydroxyantipyrine on the elimination of sulfanilamide, 20 mg/kg iv, in Rat 1-II. Key: ●, control experiment; and ○, with 4-hydroxyantipyrine, 100 mg/kg, 30 min before sulfanilamide injection and 50 mg/kg every 45 min thereafter for eight doses.*

[3] New England Nuclear Corp., Boston, Mass.
[4] Reactivials.
[5] EM Laboratories, Elmsford, N.Y.

Table II—Effect of 4-Hydroxyantipyrine on the Elimination of Sulfanilamide in Rats

Rat	Half-Life, hr	
	Control	With 4-Hydroxyantipyrine
1-II	1.15	1.26[a]
2-II	1.32[a]	1.38
3-II	1.17	1.22[a]
4-II	0.86[a]	0.85
Mean	1.13	1.18
SD	0.19	0.23
	N.S.	

[a] First experiment in the crossover study.

scraped off, and placed into vials containing 10 ml of scintillation fluid for subsequent counting in a spectrometer with an external standard. Recovery of antipyrine from spiked samples was 88 ± 3.7% (mean ± *SD*) and was not affected by the presence of 4-hydroxyantipyrine and its metabolite(s) (plasma from rats given 4-hydroxyantipyrine).

4-Hydroxyantipyrine–Sulfanilamide and Oxyphenbutazone–Sulfanilamide Experiments—Crossover studies were carried out on four rats for each of the two hydroxylated metabolites. The same dosage schedules used in the other experiments were used in this study. A control blood sample (for determination of blank value) was obtained immediately before injection of sulfanilamide, 20 mg/kg iv. A solution of the latter was prepared by dissolving 100 mg in 0.5 ml of 1 *M* NaOH, adjusting the pH to 8 with hydrochloric acid, and adding water to yield 5 ml.

Blood samples (0.5 ml) were obtained at 1, 2, 3, 4, 5, and 6 hr after sulfanilamide injection. The animals were housed individually in plastic metabolism cages for 2 days. Sulfanilamide in plasma and urine and sulfanilamide metabolites in urine were assayed by a modification of the Bratton–Marshall method (12). Two hundred microliters of plasma was shaken with 1 ml of 15% trichloroacetic acid and 0.5 ml of concentrated hydrochloric acid for 3 min. The precipitated proteins were separated by centrifugation at 400×*g*. To 1 ml of the supernate was added 0.1 ml of 0.2% sodium nitrite solution. After 15 min, 0.1 ml of 10% ammonium sulfamate solution was added. Five minutes later, 0.1 ml of 0.1% naphthylethylenediamine dihydrochloride solution was added; after 20 min, the absorbance was determined at 545 nm, using microcells.

Assays of urine were carried out with 2 ml of appropriately diluted urine to which 5 ml of 15% trichloroacetic acid and 1 ml of concentrated hydrochloric acid had been added. Sulfanilamide metabolites were assayed by determining the difference between the concentration of total and unmetabolized drug. Total sulfonamide was determined after hydrolysis of the urine–trichloroacetic

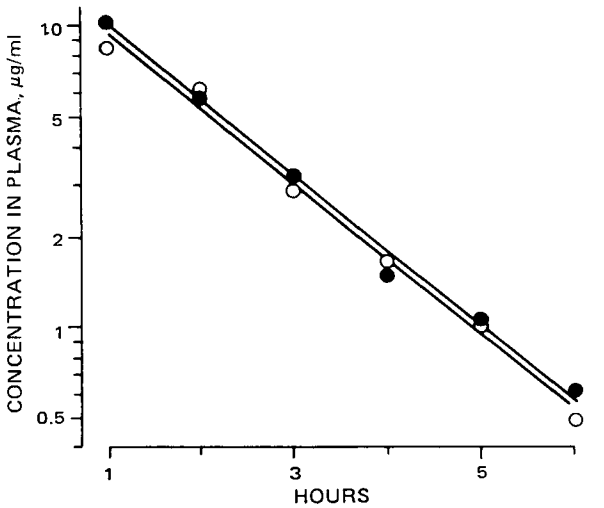

Figure 3—*Effect of oxyphenbutazone on the elimination of sulfanilamide, 20 mg/kg iv, in Rat 4-III. Key: ●, control experiment; and ○, with oxyphenbutazone, 80 mg/kg, at −1 hr and 40 mg/kg at +2 hr relative to the time of sulfanilamide injection.*

Table III—Effect of Oxyphenbutazone on the Elimination of Sulfanilamide in Rats

Rat	Half-Life, hr: Control	Half-Life, hr: With Oxyphenbutazone
1-III	1.23	1.12[a]
2-III	1.15[a]	1.12
3-III	1.35	1.38[a]
4-III	1.21[a]	1.19
Mean	1.24	1.20
SD	0.08	0.12
	N.S.	

[a] First experiment in the crossover study.

Table IV—Effect of Oxyphenbutazone and 4-Hydroxyantipyrine on the Metabolic Fate of Sulfanilamide in Rats

Experiment	Recovery in 48-hr Urine, % of Dose[a]: Sulfanilamide	Recovery in 48-hr Urine, % of Dose[a]: Metabolites
Oxyphenbutazone		
Without	22.4 ± 3.4	46.4 ± 5.4
With	25.8 ± 4.7[b]	49.9 ± 3.6[b]
4-Hydroxyantipyrine		
Without	23.9 ± 3.6	45.5 ± 7.7
With	25.2 ± 8.2[b]	46.3 ± 1.9[b]

[a] Mean ± *SD* for four animals in each group. [b] Not statistically significantly different from control value.

acid–hydrochloric acid mixture at 100° for 1 hr. Reference curves were based on absorbance data obtained with solutions of known sulfanilamide concentrations in plasma and water. Recovery of drug from spiked plasma samples from rats given 4-hydroxyantipyrine or oxyphenbutazone did not differ from the recovery from plasma of unmedicated animals.

Oxyphenbutazone–Phenytoin Experiments—Oxyphenbutazone[6], 80 mg/kg, was given intraperitoneally to 15 of 29 rats 1 hr before intravenous injection of ^{14}C-phenytoin, 10 mg/kg. A 40-mg/kg dose of oxyphenbutazone was administered 3 hr after the first dose of that drug. The control rats received only solvent instead of oxyphenbutazone. Six of the 29 rats were studied in crossover fashion.

Blood samples (~250 μl) were obtained at 10, 20, 40, 60, 80, 100, 120, 150, 180, and 210 min after phenytoin injection. Preparation of the injection solutions and analytical methodology were described previously (3, 4).

Plasma Protein Binding of Antipyrine—Plasma from unmedicated rats, with antipyrine added to yield concentrations from 7.4 to 74 μg/ml, was dialyzed at 37° for 3 hr against isotonic sodium phosphate buffer solution, pH 7.4, in dialysis cells separated by a cellophane membrane. Preliminary studies showed that dialysis equilibrium is attained in less than 3 hr under the experimental conditions.

Pharmacokinetic Analysis—Half-life values were obtained from the slope of the terminal linear portion of individual plots of the logarithm of drug concentration *versus* time, fitted to the data by the method of least squares.

RESULTS

The results of the crossover study of the effect of 4-hydroxyantipyrine on the elimination of antipyrine are summarized in Table I; a typical example of the detailed data is shown in Fig. 1. Treatment with 4-hydroxyantipyrine resulted in an almost fourfold increase in the average half-life of antipyrine.

Protein binding of antipyrine in rat plasma was 13.1 ± 1.5% (mean ± *SD*, n = 23), with no apparent concentration dependence in the 7.4–74-μg/ml range.

[6] Ciba-Geigy, Summit, N.J.

Table V—Effect of Oxyphenbutazone on the Elimination of Phenytoin in Rats

Type of Comparison	Half-Life ($t_{½\beta}$)[a], min: Control	Half-Life ($t_{½\beta}$)[a], min: With Oxyphenbutazone	Statistical Significance of Difference (p Value)
14 animals in control group, 15 in oxyphenbutazone group	57 ± 37	147 ± 93	<0.005[b]
Six animals in crossover study	47 ± 24	109 ± 97	<0.05[c]

[a] Mean ± *SD*. [b] Student t test. [c] Sign test for paired data.

Crossover experiments with and without 4-hydroxyantipyrine showed that this compound had no apparent effect on the elimination kinetics of sulfanilamide (Table II and Fig. 2). The same lack of effect was found when the plasma half-life of sulfanilamide was determined with and without oxyphenbutazone (Table III and Fig. 3). Neither 4-hydroxyantipyrine nor oxyphenbutazone had any apparent effect on the metabolic fate of sulfanilamide, as reflected by the composition of urinary excretion products (Table IV).

The results of the study of the effect of oxyphenbutazone on the elimination of phenytoin are summarized in Table V; detailed data obtained in crossover experiments on one animal are shown in Fig. 4 as a typical example. Administration of oxyphenbutazone with phenytoin caused an approximately 2.5-fold average increase in the plasma half-life of the latter. There was a strong (r = 0.95) and statistically significant (p < 0.005) positive correlation between the individual half-life values of phenytoin in control and oxyphenbutazone experiments in the group of six rats used for crossover studies.

DISCUSSION

The results of this investigation demonstrate directly *in vivo* that the hydroxylated metabolite of one drug can inhibit the elimination of another drug metabolized by an oxidative pathway. On the other hand, neither of the two hydroxylated metabolites with demonstrated inhibitory activity on oxidative biotransformation had any effect on the elimination of sulfanilamide, which is removed from the body mainly by acetylation and glucuronidation (*i.e.*, nonoxidative processes) and renal excretion (13). These results are consistent with the *in vitro* observations of others, which

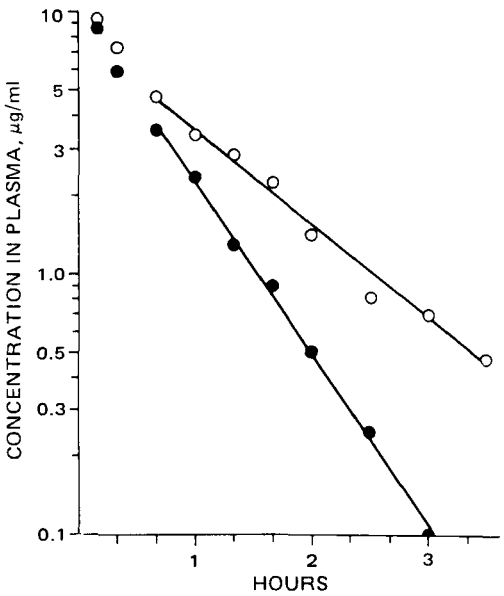

Figure 4—*Effect of oxyphenbutazone on the elimination of phenytoin, 10 mg/kg iv, in Rat 13-IV. Key:* ●, *control experiment; and* ○, *with oxyphenbutazone, 80 mg/kg, at −1 hr and 40 mg/kg at +2 hr relative to the time of phenytoin injection.*

suggested that product inhibition (including cross-product inhibition) of drug biotransformation processes may be due to an interaction of the inhibitory agents with cytochrome P-450 (8, 14). Specifically, a competition between the hydroxylated metabolite and the drug for binding sites on cytochrome P-450 was suggested (14).

The observation of product inhibition in the elimination of antipyrine is of particular interest because this drug differs from the others studied so far in that it is very hydrophilic and negligibly bound to plasma proteins. The metabolite 4-hydroxyantipyrine may be a potentially useful inhibitor of drug elimination in human drug therapy (analogous to the use of probenecid as an inhibitor of renal excretion of drugs). However, since its biological half-life in humans is very short[7], it may be most useful as an inhibitor of "first-pass" metabolism. This possibility remains to be explored.

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[7] Semilogarithmic plots of antipyrine concentration in plasma and 4-hydroxyantipyrine excretion rate *versus* time after administration of antipyrine to human subjects yield essentially identical half-life values (15), showing thereby that the biological half-life of 4-hydroxyantipyrine is much shorter than that of antipyrine.

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Solid-State Decomposition of *para*-Substituted Salicylic Acids

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Abstract □ A series of *para*-substituted salicylic acids, all crystallizing monoclinically and decomposing by decarboxylation, were shown to adhere to Bawn-type kinetics, and the decomposition rate constants were shown to adhere approximately to a Hammett-type relation. The value of the reaction parameter was −8. Both *para*-substituted salicylic and benzoic acids occur as dimers in the crystalline state. The mechanism whereby the latter decomposes is speculated to be intermolecular in the sense that the substituent from one dimer interacts with the carboxyl group of a neighboring dimer. In the same sense of the word, the substituted salicylic acids decompose by an intramolecular mechanism, *i.e.*, within the dimer unit.

Keyphrases □ Salicylic acids, *para*-substituted—decomposition in the solid state, intramolecular mechanism, Bawn kinetics, Hammett relation □ Solid-state decomposition—*para*-substituted salicylic acids, intramolecular mechanism, Bawn kinetics, Hammett relation □ Decomposition—solid state, *para*-substituted salicylic acids, intramolecular mechanism, Bawn kinetics, Hammett relation

The theory of the kinetics of solid-state decomposition, where a solid decomposes into a liquid and a gas, was developed by Bawn (1); experimental data supporting the theory were reported by Carstensen and Musa (2). In this type of reaction, there are (at least) two parallel decomposition paths: (*a*) the solid itself decomposes *via* a first-order decay route with rate constant k_s in reciprocal time units, and (*b*) the compound saturates the liquid decomposition layer and here decomposes in solution with a rate constant k_l in reciprocal time units. It is assumed that the reaction products do not participate actively in the decomposition in any other way than supplying the liquid vehicle in which part of the overall reaction takes place.

BACKGROUND

As long as there is solid present, the fraction decomposed, x, will accelerate exponentially as shown in Fig. 1; as time progresses, there will be an increasing amount of liquid phase and a decreasing amount of solid phase present. At time t_S, the last solid compound disappears and the solubility, S (moles per mole), of the compound in its decomposition product can be determined from the fraction decomposed, x_S, at time t_S. At times later than t_S, the decomposition will be a conventional first-order decomposition with the shape denoted by BC in Fig. 1, so the overall curve (ABC in Fig. 1) will be of the frequently reported sigmoid shape.

One assumption made is the first-order decomposition in the solid phase; this point has been justified both theoretically and ex-