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KINETICS OF COMPETITIVE INHIBITION OF SALICYLIC ACID CONJUGATION WITH GLYCINE IN MAN

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Abstract—Man has a very limited capacity for the synthesis of salicylurate from salicylate and glycine. Since the appearance of salicylate metabolites in the urine is rate-limited by formation rather than by renal excretion, it has been possible to demonstrate in intact man that salicylurate formation is describable by Michaelis-Menten kinetics. Salicylurate formation rates as a function of salicylate levels in the body have now been determined at the same time that relatively constant body levels of benzoate were maintained by administration of sodium benzoate at frequent intervals. The results of these experiments, which show that benzoate inhibits competitively the formation of salicylurate, constitute apparently the first demonstration of competitive inhibition of drug metabolism by classical enzyme-kinetic methods in intact man.

SALICYLATE is eliminated in man mainly by renal excretion of free salicylate and by formation of salicylic glucuronides and salicyluric acid.[1] All these processes proceed by apparent first-order kinetics when small, subtherapeutic doses are administered; the renal excretion of the metabolites is rate-limited by the formation process.[2] However, man has only a limited capacity for salicylurate formation and synthesizes this metabolite at an essentially constant rate (i.e. by apparent zero-order kinetics) when body levels of salicylate are in the usual therapeutic range.[2] It has been possible recently to show that salicylurate formation is describable by Michaelis-Menten kinetics in man[3] and in rats.[4] Several other aromatic carboxylic acids (for example benzoic acid, *p*-aminobenzoic acid, and *p*-aminosalicylic acid) are also metabolized in man partly by conjugation with glycine.[1] Dry *et al.*[5] have found that salicylate blood levels in man are increased by co-administration of *p*-aminobenzoic acid. Salassa *et al.*[6] have shown that this is due to the practically complete inhibition of salicylurate formation. Kinetic analysis[2] of the data reported by Salassa *et al.* indicates that the elimination kinetics of salicylate in the presence of *p*-aminobenzoic acid, under their experimental conditions, are quantitatively consistent with the rate constants for salicylate excretion and salicylic glucuronide formation determined subsequently in this laboratory. The biochemical significance and practical implications, with respect to drug interaction in man, of competitive inhibition of drug metabolism motivated us to determine whether competitive inhibition of salicylurate formation by benzoate can be demonstrated in intact man by classical enzyme-kinetic methods.

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METHODS

Single doses of 300 mg sodium salicylate, in aqueous solution, were administered orally to two healthy young males. The drug was given in the morning on an empty stomach and food was withheld for 2 hr thereafter. Urine was collected quantitatively every hour for at least 12 hr after drug administration, and at less frequent intervals thereafter. Other experimental details, analytical methods, and procedures for the evaluation of kinetic constants have been described in previous reports.[2, 3, 7] Sodium benzoate was administered orally, dissolved in water, in doses of 590 mg (equivalent to 500 mg benzoic acid) every half-hour, starting 2 hr before and terminating 8·5 to 10 hr after salicylate ingestion. The frequent administration of benzoate was necessary in order to maintain relatively constant body levels of this drug, which is eliminated extremely rapidly in low doses.[8]

RESULTS AND DISCUSSION

Figure 1 shows the elimination of salicylate in Subject A in the absence of and during administration of sodium benzoate. Salicylate is eliminated much more slowly during benzoate administration, but elimination proceeds as rapidly as in the control experiment when benzoate administration is terminated. The rapid change in kinetics after the last dose of benzoate is due to the rapid elimination of the latter. Sufficiently low doses of both drugs were used intentionally in order not to saturate the glycine conjugation process completely. That this was accomplished is evident from the exponential decline of salicylate body levels (Fig. 1), and is demonstrated more directly by the lack of linearity of the cumulative salicylurate excretion curves shown in Fig. 2.

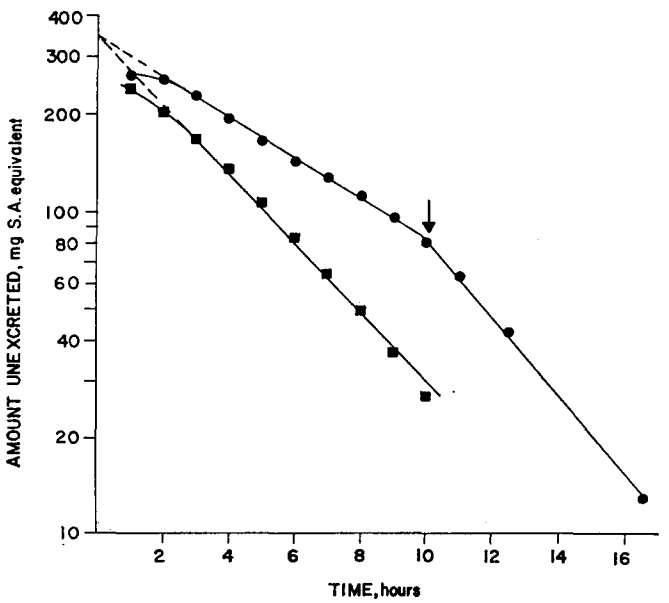

FIG. 1. Effect of benzoate on the elimination of salicylate by a healthy 23-year-old man (Subject A) after oral administration of 300 mg sodium salicylate. The unexcreted amount is expressed in terms of salicylic acid (S.A.). ■ Control experiment; ● 590 mg sodium benzoate was administered every half-hour, starting 2 hr before and terminating 10 hr after salicylate administration. Vertical arrow indicates the time when the last dose of sodium benzoate was given.

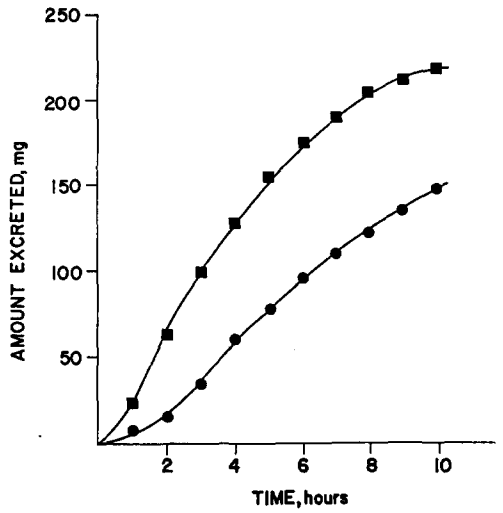

FIG. 2. Cumulative excretion of salicyluric acid as a function of time after oral administration of 300 mg sodium salicylate; Subject A. ■ Control experiment; ● 590 mg sodium benzoate was administered every half-hour, starting 2 hr before salicylate administration.

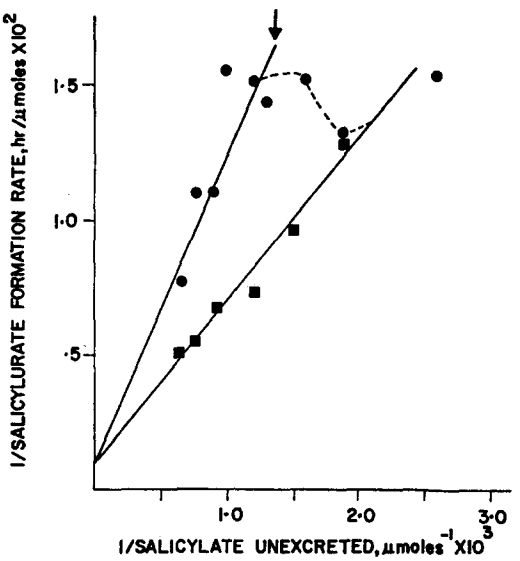

FIG. 3. Lineweaver-Burk plot of the reciprocal of the salicylurate excretion rate vs. the reciprocal of the amount of salicylate in the body at various times after oral administration of 300 mg sodium salicylate; Subject A. ■ Control experiment; ● 590 mg sodium benzoate was administered every half hour, starting 2 hr before and terminating 10 hr after salicylate administration. All solid circles to the left of the vertical arrow represent data collected while the subject took sodium benzoate every half-hour; the points to the right of the arrow are data obtained after the last dose of benzoate. This figure shows fewer points than Fig. 1 because only post-absorption and equilibration data could be used.

Since the appearance of salicylate metabolites in the urine is rate-limited by the formation process rather than by excretion, salicylurate formation rate and the amount of salicylate in the body at any time can be determined, within reasonable limits of accuracy, from urinary excretion data. Figure 3 is a Lineweaver-Burk plot[9] of the reciprocal of the salicyluric acid formation rate at various times after salicylate administration versus the reciprocal of the amount of salicylate in the body. It can be seen that the data obtained during benzoate administration show the characteristics of competitive inhibition;[9] the experimental values obtained after the last dose of benzoate soon fall on the line fitted to the data of the control experiment. The time course of events can be noted from Fig. 3 by following the points along the upper line from the left to right, and then along the stippled curve to the lower line.

TABLE 1. EFFECT OF BENZOATE ON APPARENT FIRST-ORDER RATE CONSTANTS* FOR SALICYLATE ELIMINATION IN TWO HEALTHY ADULT MALES

Experiment	Conditions	$t_{\frac{1}{2}}$ (hr)	k (hr^{-1})	k_{SU} (hr^{-1})	k_{SA} (hr^{-1})	k_{SG} (hr^{-1})
		Subject A, 23 years old				
1	Salicylate only	2·7	0·257	0·170	0·017	0·070
2	During benzoate†	4·7	0·147	0·081	0·014	0·052
	After benzoate	2·5	0·277	0·173	0·028	0·076
		Subject B, 22 years old				
1	Salicylate only	3·2	0·216	0·178	0·003	0·035
2	During benzoate‡	5·3	0·130	0·092	0·003	0·035
	After benzoate	3·1	0·223	0·182	0·004	0·036
3	During benzoate§	6·6	0·105	0·067	0·004	0.034
	After benzoate	2·8	0·247	0·178	0·017	0·052

The subjects received 300 mg sodium salicylate orally either alone or with 590 mg sodium benzoate every half-hour, starting 2 hr before and terminating 8·5 to 10 hr after salicylate administration.

* k, Overall salicylate elimination rate constant; k_{SU}, salicylurate formation rate constant; k_{SA}, salicylate excretion rate constant; k_{SG}, salicylic glucuronide formation rate constant; $t_{\frac{1}{2}}$, salicylate elimination half-life.

† For 10 hr.
‡ For 8·5 hr.
§ For 9 hr.

The pharmacokinetic constants obtained in the various experiments are listed in Table 1. Benzoate administration decreased the rate constant for salicylurate formation to about half the control value but had no significant effect on the rate constants for salicylate excretion and glucuronides formation. Fluctuations in the salicylate excretion rate constant, unrelated to benzoate administration, were due to changes in urinary pH; the latter tended to increase in the afternoon and affected mainly the rate constant for the "after benzoate" period. These results rule out the possibility that the inhibitory effect of benzoate was due to inhibition of tubular secretion. More direct evidence was obtained by administering salicyluric acid alone and with sodium benzoate; the latter had at best only a small effect on the excretion kinetics of salicylurate (Table 2). It is more likely that the small decrease in salicylurate excretion rate was due to the fact that the average urine pH in the experiment with benzoate

was about one unit lower than during the control experiment. Regardless of these considerations, the excretion rate constant for salicylurate is at least five times greater than the formation rate constant, and changes in urinary excretion rate of salicylurate after salicylate administration will, under these conditions, reflect changes in the rate constant of salicylurate formation rather than of excretion. It was possible in this experiment to show that a small fraction of salicylurate is biotransformed to an acid-labile conjugate (probably glucuronide); this has been observed previously in rabbits[10] but is difficult to demonstrate in man by administration of salicylate.

TABLE 2. ELIMINATION OF SALICYLURIC ACID GIVEN ALONE OR WITH SODIUM BENZOATE (SUBJECT A)

Experiment	Amount recovered (mg)			Elimination rate constant (hr^{-1})	Renal excretion rate constant (hr^{-1})
	Total	Free	As conjugate*		
Salicyluric acid alone	192	176	16	0·866	0·762
Salicyluric acid with benzoate†	201	169	32	0·730	0·617

* Expressed as mg salicyluric acid equivalent.

† Sodium benzoate (590 mg) every half hour, starting 2 hr before and terminating 8 hr after oral administration of 200 mg salicyluric acid.

The apparent first-order kinetics of a saturable biotransformation process such as salicylurate formation in the low dose range are due to the fact that the Michaelis-Menten equation[11] reduces to a first-order expression when the substrate concentration is appreciably smaller than the Michaelis constant.[2] The apparent first order rate constant for salicylurate formation, k_{su}, represents V_{max}/K_m, where V_{max} is the extrapolated maximal salicylurate formation rate and K_m is the apparent *in vivo* Michaelis constant. The k_{su} values obtained directly by pharmacokinetic procedures[2] were very similar to the values calculated from V_{max} and K_m values obtained from Lineweaver-Burk plots; for example, k_{su} for Subject A in the absence of benzoate was 0·17 reciprocal hr when determined by pharmacokinetic methods, and 0·16 reciprocal hr when calculated from K_m and V_{max}.

The results of these experiments are considered to be of theoretical significance because they represent apparently the first classical demonstration of competitive inhibition of drug metabolism in intact man. They are of practical importance because of recent findings which indicate that a number of commonly used drugs may be metabolized in man by processes which are saturated in the usual dose range.[12]

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