Biotransformation of Salicylic Acid to Its Acyl and Phenolic Glucuronides in Man

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Abstract □ Computer simulations were used to determine suitable experimental conditions for distinguishing between a recently developed pharmacokinetic model for salicylate elimination (involving saturable salicyl phenolic glucuronide formation) and a previously proposed model (based on the assumption that the syntheses of salicyl phenolic and acyl glucuronides are apparent first-order processes). Experimental studies in man designed on the basis of these simulations yielded results which are in agreement with the new pharmacokinetic model and which represent further evidence that man has a limited capacity to form salicyl phenolic glucuronide.

Keyphrases □ Salicylic acid biotransformation in man—capacity-limited formation of salicyl phenolic glucuronide, compared to computer simulations □ Computer simulation of salicylic acid biotransformation—compared to experimental studies in man □ Biotransformation of salicylic acid—capacity-limited salicyl phenolic glucuronide formation, man

The prospective use of computer simulations for designing experiments to test pharmacokinetic models is one of the most useful yet least used research strategies in pharmacokinetics. It has been said, perhaps somewhat facetiously, that even the most complicated plasma concentration or urinary excretion curve can be described mathematically if a sufficient number of constants are used. Such mathematical description may, however, be entirely meaningless and of no predictive value. Verification of pharmacokinetic models by experiments especially designed for that purpose is, therefore, desirable if not mandatory. Computer simulations of experiments involving different variables (dose, infusion *versus* rapid injection, single *versus* repeated doses, *etc.*) and based on the pharmacokinetic model to be tested as well as on one or more alternative models can be a powerful means for identifying those experiments most suitable for distinguishing between different pharmacokinetic models. This approach was used to verify a recently developed model for salicylate elimination in man (1).

Previous studies in human subjects showed that salicylate is eliminated by urinary excretion and by formation of salicyluric acid, salicyl phenolic glucuronide, salicyl acyl glucuronide, and gentisic acid (2). It was noted previously that man has a rather limited capacity for salicyluric acid formation in the therapeutic dose range of salicylate (3, 4). More recently, experimental evidence was obtained which indicates that such limited capacity is also evident in the formation of salicyl phenolic glucuronide (1). It was found that, after oral administration of 3 g. salicylic acid to four healthy male volunteers, the formation of salicyluric acid and salicyl phenolic glucuronide followed Michaelis–Menten kinetics and not apparent first-order kinetics, while the other processes involved in salicylate elimination were describable by apparent first-order kinetics under the experimental conditions (1). In view of the significant clinical implications of the saturation phenomena (5), it was deemed important to verify that salicyl phenolic glucuronide formation is indeed capacity limited in man. The computer simulations used to determine the most informative and practical experiments for this purpose and the results of these experiments are reported here.

Table I—Rate Constants for Salicylate Elimination in Man[a]

Constant[b]	Value[c]
V_{SU}	60.3 mg./hr.
K_M^{SU}	338. mg.
V_{SPG}	32.3 mg./hr.
K_M^{SPG}	629. mg.
k_{SA}	0.0075 hr.$^{-1}$
k_{SAG}	0.0071 hr.$^{-1}$
k_{GA}	0.0023 hr.$^{-1}$

[a] Based on data for four healthy male adults; from *Reference 1*. [b] *SU*, salicyluric acid; *SPG*, salicyl phenolic glucuronide; *SA*, salicylic acid; *SAG*, salicyl acyl glucuronide; and *GA*, gentisic acid. [c] Expressed in terms of salicylic acid.

Table II—Predicted Effect of Dose on Metabolic Fate of Salicylic Acid in Man Based on the Pharmacokinetic Constants Listed in Table I

Dose, g.	Percent of Dose[a]				
	SA	*SU*	*SPG*	*SAG*	*GA*
<0.030	3.04	72.3	20.8	2.88	0.932
0.125	3.48	70.4	21.8	3.30	1.06
0.250	3.90	68.8	22.4	3.70	1.20
0.500	4.72	66.1	23.2	4.46	1.45
1.00	6.22	62.2	23.8	5.89	1.91
2.00	8.82	56.5	23.6	8.35	2.71
4.00	12.9	49.0	22.0	12.2	3.95
8.00	18.3	39.9	18.9	17.3	5.61

[a] For abbreviations, see Footnote [b] in Table I.

EXPERIMENTAL

All digital simulations were carried out with the MIMIC program on a CDC-6400 digital computer. The input consisted of the differential equations, describing apparent first-order excretion of salicylic acid and formation of salicyl acyl glucuronide and gentisic acid, and Michaelis–Menten kinetics for formation of salicyluric acid and salicyl phenolic glucuronide (1). The values of all rate constants are listed in Table I. In another set of simulations, salicyl phenolic glucuronide formation was assumed to be a first-order process.

Four healthy male volunteers, 25–41 years old, participated in the clinical study. Single oral doses of 0.19 and 3.0 g. salicylic acid as sodium salicylate were administered in aqueous solution in the morning on an empty stomach. Urine was collected at intervals for 36 and 72 hr. after the low and high dose, respectively, and assayed for salicyl acyl and phenolic glucuronides by the method of Schachter and Manis (6).

Table III—Predicted Effect of Dose on Metabolic Fate of a Drug with the Pharmacokinetic Characteristics of Salicylic Acid, Except that "*SPG*" Formation is First Order

Dose, g.	Percent of Dose "*SA*"	"*SU*"	"*SPG*"[a]	"*SAG*"	"*GA*"
<0.030	3.04	72.3	20.8	2.88	0.932
0.125	3.41	69.0	23.3	3.22	1.05
0.250	3.73	66.0	25.5	3.53	1.14
0.500	4.29	60.9	29.4	4.06	1.32
1.00	5.14	53.0	35.2	4.87	1.58
2.00	6.25	43.1	42.8	5.92	1.92
4.00	7.44	32.3	50.9	7.04	2.28
8.00	8.52	22.5	58.3	8.07	2.61

[a] $k_{"SPG"} = V_{SPG}/K_M^{SPG} = 0.05$ hr.$^{-1}$.

RESULTS AND DISCUSSION

The effect of dose on the metabolic fate of salicylic acid in man was calculated over a wide dose range on the basis of two different pharmacokinetic models: (*a*) capacity-limited salicyluric acid and salicyl phenolic glucuronide formation and linear kinetics for all other parallel processes (Table II), and (*b*) capacity-limited formation of salicyluric acid only and linear kinetics for all other parallel processes (Table III). The rate constant for apparent first-order formation of the phenolic glucuronide [(k_{SPG}) for Case *b*] was obtained by dividing V_{SPG} by K_M^{SPG} based on theoretical considerations (3, 4). An examination of the data in Tables II and III revealed that an assessment of the relative amounts (fractions of the dose) of the phenolic and acyl glucuronides formed from low (<0.25 g.) and high (2–4 g.) doses of salicylic acid, respectively, would be an effective means of distinguishing between the two pharmacokinetic models. Comparison of the salicylic and/or gentisic acid fractions was less attractive on practical grounds, due to the pronounced sensitivity of the former to urine pH (7) and the small size of the latter. Accordingly, all subjects participating in this study received single test doses of 0.19 and 3.0 g. salicylic acid. Consistent with the recently developed pharmacokinetic model based on capacity-limited salicyl phenolic glucuronide formation (1), and contrary to the alternative model (in which phenolic glucuronide formation is assumed to be apparent first order), there was no significant difference in the fraction of salicyl phenolic glucuronide formed in the low and high dose experiments (Table IV). Similarly, the salicyl acyl glucuronide fraction in the two experiments was quantitatively in agreement with the calculations in Table II and quite different from those in Table III (Table V).

In examining Table II, it is of interest that the salicyluric acid fraction decreases with increasing dose (reflecting the saturation effects) while the fractions of salicylic acid, acyl glucuronide, and gentisic acid increase accordingly. The fraction of salicyl phenolic glucuronide, which would decrease ordinarily in the absence of a concurrent and competing saturable process, remains essentially constant over a wide dose range. Determinations of this fraction alone, in the absence of other data, would suggest apparent first-order formation kinetics. Furthermore, the relative increase in the fractions of salicylic acid, salicyl acyl glucuronide, and gentisic acid with increasing dose is the same; *i.e.*, all increase sixfold from a very small dose (<30 mg.) to 8 g. Also, the ratio of the fractions of these three excretion products is independent of dose and equal to the ratio of their formation (or excretion, in the case of salicylic acid) rate constants. These relationships are useful for the pharmacokinetic analysis of these and similar data.

Table IV—Formation of Salicyl Phenolic Glucuronide after Oral Administration of 0.19 and 3.0 g. Salicylic Acid

Subject	Percent of Dose Dose of 0.19 g.	Dose of 3.0 g.
A	22.4	19.3
B	18.8	21.5
C	22.9	24.5
D	16.2	15.8
Mean	20.1	20.3
Statistical significance[a]	N.S.	
Theoretical value[b]	22.1	22.9

[a] Difference between results of the high and low dose experiments; calculated by paired *t* test. [b] Based on the pharmacokinetic constants in Table I.

Table V—Formation of Salicyl Acyl Glucuronide after Oral Administration of 0.19 and 3.0 g. Salicylic Acid

Subject	Percent of Dose Dose of 0.19 g.	Dose of 3.0 g.
A	2.9	12.0
B	4.2	10.6
C	5.0	10.7
D	5.3	7.4
Mean	4.3	10.2
Statistical significance[a]	$p < 0.05$	
Theoretical value[b]	3.5	10.4

[a] Difference between results of the high and low dose experiments; calculated by paired *t* test. [b] Based on the pharmacokinetic constants in Table I.

The capacity-limited nature of salicyl phenolic glucuronide formation has important clinical implications. It explains the mutual inhibition in glucuronide formation beween salicylates and certain other drugs in man (8) and, together with the capacity-limited kinetics of salicylurate formation, accounts for the pronounced accumulation characteristics of salicylates under clinical conditions (9). The elaboration of a functional pharmacokinetic model for salicylate elimination in man permits the design of optimum dosage regimens for safe and effective salicylate therapy under various conditions.

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ACKNOWLEDGMENTS AND ADDRESSES

Received November 24, 1971, from the *Department of Pharmaceutics, School of Pharmacy, State University of New York at Buffalo, Buffalo, NY 14214*

Accepted for publication January 31, 1972.

Supported in part by Grant FD00015 from the U. S. Public Health Service.

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