Drug Biotransformation Interactions in Man VI: Acetaminophen and Ascorbic Acid

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Abstract □ Oral administration of 3 g of ascorbic acid 1.5 hr after an oral dose of 1 g of acetaminophen caused a rapid and pronounced decrease in the excretion rate of acetaminophen sulfate in five healthy adult volunteers. There was a statistically significant increase in the fractions of the dose of acetaminophen excreted as such and as acetaminophen glucuronide but a decrease in the fraction excreted as acetaminophen sulfate. The apparent biological half-life of acetaminophen increased from 2.3 ± 0.2 (mean ± *SD*) to 3.1 ± 0.5 hr. Concomitant administration of sodium sulfate prevented these effects. Ascorbic acid, which itself is metabolized in part to the sulfate, inhibits the conjugation of acetaminophen with sulfate by competing for available sulfate in the body.

Keyphrases □ Drug biotransformation interactions—effect of ascorbic acid on half-life and excretion rate of acetaminophen, humans □ Interactions, drug biotransformation—effect of ascorbic acid on half-life and excretion rate of acetaminophen, humans □ Biotransformation—effect of ascorbic acid on half-life and excretion rate of acetaminophen, humans □ Acetaminophen—half-life and excretion rate, effect of ascorbic acid, humans □ Ascorbic acid—effect on half-life and excretion rate of acetaminophen, humans

Conjugation with sulfate is an important biotransformation pathway for phenolic drugs (1). Humans have a limited capacity for sulfate formation, so drugs subject to this metabolic process tend to exhibit nonlinear (saturable) elimination kinetics (2, 3) and competitive inhibitory effects. For example, acetaminophen and salicylamide interact in humans by mutual inhibition of their respective conjugation with sulfate (3). This inhibitory effect can be prevented by concomitant administration of sulfate or a sulfate donor such as L-cysteine (3, 4).

It was found recently that ascorbic acid (vitamin C) is converted partly to ascorbic acid sulfate in humans (5). Since this vitamin is often taken in relatively large doses, it is desirable to explore the possible effect of ascorbic acid on the conjugation of phenolic drugs with sulfate. In a preliminary study, ascorbic acid caused a decrease in the fraction of an oral dose of salicylamide excreted as salicylamide sulfate (6). This interaction probably occurred mainly during absorption, since salicylamide is extensively metabolized in the first pass, probably largely in the intestinal wall (7).

Unlike salicylamide, acetaminophen is not subject to a pronounced first-pass effect (8, 9). It can be used, therefore, to determine if ascorbic acid can inhibit systemically the conjugation of a drug with sulfate. The investigation described here was designed to determine the effect of ascorbic acid on the biotransformation of acetaminophen under postabsorptive conditions in order to assess the effect of the vitamin on the elimination kinetics of acetaminophen and to elucidate the mechanism of the interaction.

EXPERIMENTAL

Five healthy male volunteers, 27–40 years old and capable by education and experience to give their informed consent (research assistants and associates), were instructed not to take any drugs or vitamin preparations for 1 week before and during the study. They fasted overnight, emptied their bladders in the morning, and took 1 g of acetaminophen[1] followed by 50 ml of water.

Urine was collected every 0.5 hr for 4 hr, every hour for the next 4 hr, and then at convenient intervals for a total of 24 hr. Water (100 ml) was ingested after each urine collection to assure an adequate flow rate. Food intake was permitted after the first 4 hr. Three grams of ascorbic acid in 200 ml of water, or 200 ml of water only, was administered at 1.5 hr. The experiments were carried out in random order at least 1 week apart.

Two subjects repeated the acetaminophen–ascorbic acid experiment twice, once with sodium sulfate (1 g in 100 ml of water at 0.5, 1.5, and 2.5 hr) and once with L-cysteine (2 g in 100 ml of water at 0.5, 1.5, and 2.5 hr).

The urine samples were analyzed colorimetrically for acetaminophen, acetaminophen glucuronide, and acetaminophen sulfate after selective extraction and enzymatic hydrolysis, as described previously (3, 10).

RESULTS

To determine a possible effect of ascorbic acid and its metabolites on the assay of acetaminophen and its conjugates, urine obtained after acetaminophen administration was assayed as such and after addition of urine obtained after ascorbic acid administration. There was no interference in the assay of acetaminophen and its major metabolites.

Ascorbic acid caused a significant decrease in the fraction of acetaminophen excreted as the sulfate and a significant increase in the fractions excreted as acetaminophen and acetaminophen glucuronide, but it had no effect on the recovery of total acetaminophen (*i.e.*, the sum of acetaminophen, its glucuronide, and its sulfate) (Table I).

A rapid and pronounced decrease in the excretion rate of acetaminophen sulfate occurred after ascorbic acid administration (Figs. 1 and 2, left panels). This effect lasted for about 4 hr on the average. Concomitant administration of sodium sulfate prevented this inhibition, while administration of L-cysteine had no apparent effect (Figs. 1 and 2, right panels). Sodium sulfate, but not L-cysteine, prevented the change in the metabolic fate of acetaminophen produced by ascorbic acid (Table II).

Ascorbic acid had no significant effect on the pH or flow rate of urine. The control and ascorbic acid experiment values (mean ± *SD*) from 1.5 to 5.5 hr (the period when the effect of ascorbic acid on acetaminophen biotransformation was most apparent) were 5.90 ± 0.46 and 5.81 ± 0.66 pH units and 174 ± 152 and 220 ± 153 ml/hr, respectively.

Examples of the excretion rate profiles of acetaminophen and acetaminophen glucuronide were reported previously (3, 10), and similar results were observed in this investigation. Figure 3 shows the excretion rate profiles for acetaminophen in Subject H during concomitant administration of sodium sulfate or L-cysteine. The excretion rate profile in these and other experiments had a terminal, apparently exponential phase, which may be characterized by an apparent half-life value[2]. The half-life of acetaminophen determined by this method was significantly increased by concomitant administration of ascorbic acid (Table III). Sodium sulfate, but not L-cysteine, prevented this effect.

There was a strong ($r = -0.95$) and statistically significant ($p < 0.01$) negative correlation between the change in the half-life of acetaminophen and the change in the fraction of acetaminophen

[1] Tylenol Elixir, McNeil Laboratories, Fort Washington, Pa.

[2] The elimination kinetics of acetaminophen are obviously not truly first order following administration of a competitive inhibitor of one biotransformation pathway, but this is not apparent in the excretion rate profile of acetaminophen. The term "apparent half-life" is used because of these considerations.

Table I—Effect of Ascorbic Acid on the Metabolic Fate of Acetaminophen in Humans

Subject	Excreted in Urine, % of Dose							
	Acetaminophen		Acetaminophen Glucuronide		Acetaminophen Sulfate		Total Acetaminophen	
	Control	With Ascorbic Acid	Control	With Ascorbic Acid	Control	With Ascorbic Acid	Control	With Ascorbic Acid
H	4.1	5.2	47.7	57.8	29.4	19.6	81.2	82.6
L	4.3	6.3	34.0	44.9	41.8	20.8	80.1	72.0
O	5.4	6.6	40.9	47.6	36.1	26.0	82.4	80.2
S	5.0	6.2	54.7	62.2	26.7	24.8	86.4	93.2
Y	3.9	5.0	53.5	62.0	32.3	23.6	89.7	90.6
Mean	4.5	5.9	46.2	54.9	33.3	23.0	84.0	83.7
SD	0.6	0.7	8.7	8.2	5.9	2.7	8.4	8.4
Statistical difference from control[a]		$p < 0.005$		$p < 0.001$		$p < 0.05$		N.S.

[a] Paired *t*-test.

Table II—Effect of Sodium Sulfate and L-Cysteine on the Biotransformation Interaction between Acetaminophen and Ascorbic Acid[a]

Subject	Sulfate Donor	Excreted in Urine, % of Dose			
		Acetaminophen	Acetaminophen Glucuronide	Acetaminophen Sulfate	Total Acetaminophen
H	None	5.2	57.8	19.6	82.6
H	L-Cysteine[b]	5.8	56.7	20.5	83.0
H	Sodium sulfate[c]	4.5	53.8	32.7	91.0
Y	None	5.0	62.0	23.6	90.6
Y	L-Cysteine[b]	6.3	59.1	23.8	89.2
Y	Sodium sulfate[c]	4.5	52.1	31.8	88.4

[a] One gram of acetaminophen followed by 3 g of ascorbic acid 1.5 hr later. [b] Two grams at 0.5, 1.5, and 2.5 hr after acetaminophen administration. [c] One gram at 0.5, 1.5, and 2.5 hr after acetaminophen administration.

sulfate produced by ascorbic acid (Fig. 4). Conversely, there was a positive correlation between the change in the half-life of acetaminophen and the change in the fractions of unmetabolized acetaminophen ($r = 0.86$, $p < 0.05$) and acetaminophen glucuronide ($r = 0.88$, $p < 0.05$) caused by ascorbic acid administration (Fig. 4).

DISCUSSION

This study shows that ascorbic acid can inhibit the systemic conjugation of acetaminophen with sulfate by competing for available sulfate in the body. The results of this study and of previous investi-

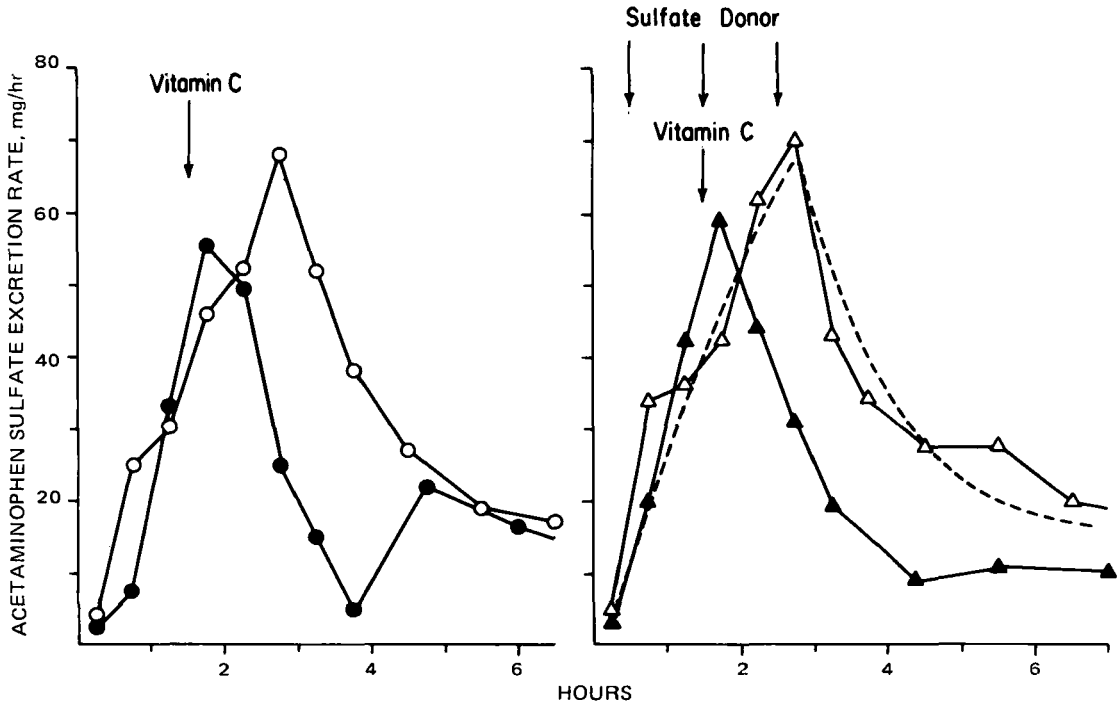

Figure 1—*Urinary excretion rate of acetaminophen sulfate after oral administration of 1 g of acetaminophen, Subject H. Key: ○ and - - -, control; ●, with 3 g of ascorbic acid at 1.5 hr; △, with 3 g of ascorbic acid at 1.5 hr and 1 g of sodium sulfate at 0.5, 1.5, and 2.5 hr; and ▲, with 3 g of ascorbic acid at 1.5 hr and 2 g of L-cysteine at 0.5, 1.5, and 2.5 hr.*

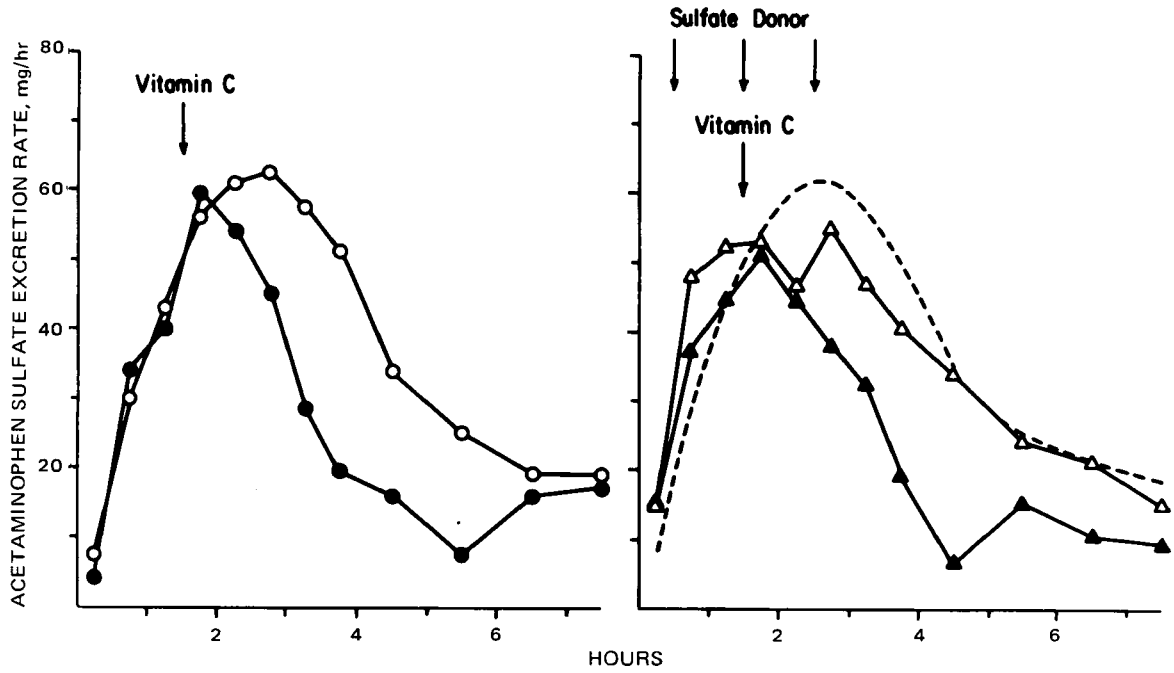

Figure 2—*Same experiment as in Fig. 1, Subject Y.*

gations in humans (3) and animals (4) lead to the conclusion that the availability of sulfate, rather than its activation to 3′-phosphoadenosine 5′-phosphosulfate or the subsequent transfer of activated sulfate to the acceptor molecule, is the rate-limiting step in phenolic sulfate formation. The limited availability of sulfate in the body causes the sulfate conjugation process to be easily saturated and subject to competitive inhibition.

Ascorbic acid is itself partly metabolized to the sulfate and will compete with phenolic drugs such as acetaminophen and salicylamide for available sulfate. It is not unreasonable to expect similar interactions between ascorbic acid and steroids or other compounds whose conjugation with sulfate is mediated by different sulfokinases. Of particular concern is the possibility of an interaction between ascorbic acid and drugs subject to pronounced first-pass biotransformation such as isoproterenol (6). Competitive inhibition of sulfate formation can increase the systemic availability of such a drug and, thereby, enhance its pharmacological effect (11). The wide use of large doses of ascorbic acid makes it necessary to be concerned about the possibility and consequences of such interactions.

The specific interaction between acetaminophen and ascorbic acid described here is important in demonstrating a principle but is probably of little clinical significance under the usual conditions, *i.e.*, when acetaminophen is taken in single recommended doses as an analgesic or antipyretic. The somewhat slower elimination of acetaminophen when taken with ascorbic acid may result in a slightly more pronounced and protracted effect of the former, but this result

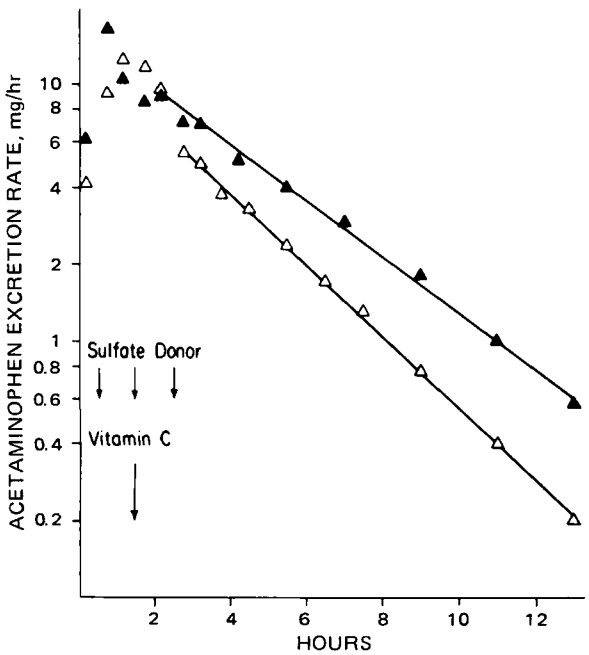

Figure 3—*Urinary excretion rate of acetaminophen after oral administration of 1 g of acetaminophen and 3 g of ascorbic acid 1.5 hr later, Subject H. Key: Δ, with sodium sulfate; and ▲, with L-cysteine.*

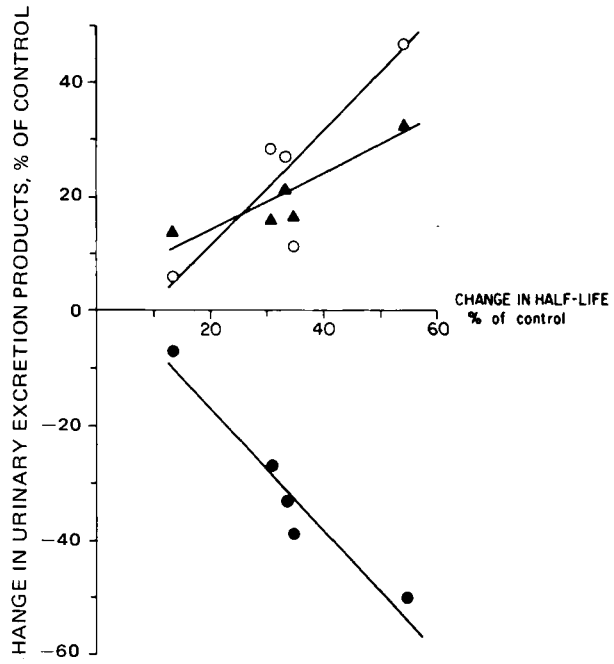

Figure 4—*Relationship between the change in the apparent half-life of acetaminophen produced by ascorbic acid and the change in the amount of excreted acetaminophen (○), acetaminophen glucuronide (▲), and acetaminophen sulfate (●) in five subjects.*

Table III—Effect of Ascorbic Acid and Sulfate Donors on the Apparent Terminal Half-Life of Acetaminophen

Subject	Apparent Half-Life, hr			
	Control	Ascorbic Acid	Ascorbic Acid and L-Cysteine	Ascorbic Acid and Sodium Sulfate
H	2.1	2.8	2.8	2.2
L	2.2	3.4	—	—
S	2.6	3.5	—	—
O	2.2	2.5	—	—
Y	2.6	3.4	3.2	2.6
Mean	2.3	3.1	3.0	2.4
SD	0.2	0.5		
Statistical difference from control[a]		$p < 0.01$		

[a] Paired *t*-test.

will be difficult to establish under clinical conditions.

It is interesting that sodium sulfate prevented the inhibition of acetaminophen sulfate formation by ascorbic acid while L-cysteine had no such effect. L-Cysteine is a source of inorganic sulfate (12). It was used as a sulfate donor in a previous study because it was thought that inorganic sulfate would not be well absorbed from the GI tract (3). More recently, oral administration of sodium sulfate was found to increase the amount of available sulfate in the body (13). The results of this investigation confirm that orally administered sodium sulfate is an adequate source of sulfate. Its effectiveness in preventing the inhibition of acetaminophen sulfate formation by ascorbic acid shows that the mechanism of the interaction is the competition for available sulfate.

L-Cysteine is oxidized in the body to cysteine sulfinate which, in turn, is converted to sulfinyl pyruvate; sulfinyl pyruvate then decomposes into sulfite and pyruvate. The sulfite is then oxidized to sulfate by sulfite oxidase (12). Orally administered L-cysteine reduces or prevents the mutual competitive inhibition of sulfate formation by acetaminophen and salicylamide in humans (3). The reason for its lack of effectiveness in preventing the acetaminophen–ascorbic acid interaction is not readily apparent. The amount of L-cysteine administered was the same as in the previous study and represents almost five times the dose of inorganic sulfate used, on a molar basis.

Ascorbic acid may be able to inhibit the conversion of L-cysteine to sulfate. It is also possible that the effectiveness of L-cysteine with salicylamide is related to the likelihood that the biotransformation of salicylamide occurs largely in the intestinal wall. Irrespective of these speculations, this study demonstrated that ascorbic acid can inhibit the conjugation of a drug with sulfate, a type of interaction that assumes particular significance because of the wide use of large doses of the vitamin.

REFERENCES

(1) R. T. Williams, "Detoxification Mechanisms," 2nd ed., Chapman and Hall, London, England, 1959, pp. 279–282.

(2) G. Levy and T. Matsuzawa, *J. Pharmacol. Exp. Ther.*, **156,** 285(1967).

(3) G. Levy and H. Yamada, *J. Pharm. Sci.*, **60,** 215(1971).

(4) H. Büch, C. H. Eschrick, and K. Pfleger, *Arch. Exp. Pathol. Pharmakol.*, **225,** 6(1966).

(5) E. M. Baker, D. C. Hammer, S. C. March, B. M. Tolbert, and J. E. Canham, *Science*, **173,** 826(1971).

(6) J. B. Houston and G. Levy, *Nature*, **255,** 78(1975).

(7) W. H. Barr, T. Aceto, Jr., M. Chung, and M. Shukur, *Rev. Can. Biol., Suppl.*, **32,** 31(1973).

(8) G. Levy, *J. Pharm. Sci.*, **60,** 499(1971).

(9) D. T. Lowenthal, S. Øie, J. C. Van Stone, W. A. Briggs, and G. Levy, *J. Pharmacol. Exp. Ther.*, **196,** 570(1976).

(10) G. Levy and C.-G. Regårdh, *J. Pharm. Sci.*, **60,** 608(1971).

(11) C. F. George, E. W. Blackwell, and D. S. Davies, *J. Pharm. Pharmacol.*, **26,** 265(1974).

(12) B. Sörbo, *Scand. J. Clin. Lab. Invest., Suppl. 86,* **17,** 21(1965).

(13) I. Smith and P. D. Mitchell, *Biochem. J.*, **112,** 189(1974).

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