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# Modification of drug biotransformation by vitamin C in man

VITAMIN C (ascorbic acid) is taken by many individuals in very large doses and there has been some concern about possible adverse effects of such regimens. It has been found that vitamin C is metabolised in part to ascorbic acid sulphate in man[1]. Sulphate formation is also an important pathway for the biotransformation of phenolic drugs[2]. This pathway is, however, of limited capacity in man[3] (and in many species of animals) and is therefore subject to saturation and competitive inhibition. Such mutual inhibition by two or more substrates is caused by the limited availability of sulphate and can be overcome by concomitant administration of sulphate or a sulphate donor[3]. Studies in man have demonstrated competitive inhibition of sulphate formation between acetaminophen and salicylamide, two widely used analgesics and antipyretics, and the prevention of this inhibition by concomitant administration of the sulphate donor L-cysteine[4]. We now report the results of a study which shows that concomitant administration of vitamin C and salicylamide causes a decrease in the conversion of the latter to salicylamide sulphate.

The results of the study are summarised in Table 1. Recovery of total salicylamide (that is, the sum of all urinary excretion products of the drug) was essentially quantitative (95% of the dose) and was not affected by vitamin C. The fraction of the dose excreted as salicylamide sulphate was significantly decreased and that excreted as salicylamide glucuronide was increased by concomitant administration of vitamin C. Eight of the ten subjects showed this decrease in salicylamide sulphate output; the effect was particularly pronounced in subjects C, H, I and L. There was a corresponding, statistically significant change in the average maximum excretion rates of the metabolites: 0.562 and 0.513 mg salicylamide equivalent per min for the sulphate in the control and vitamin C experiments, respectively ($P < 0.02$ by paired $t$ test), and 0.715 and 0.824 mg salicylamide equivalent per min for the glucuronide in the control and vitamin C experiments, respectively ($P < 0.005$ by paired $t$ test). The vitamin had no statistically significant effect on the $p$H and flow rate of urine.

Previous studies have shown that the extent to which salicylamide is converted to salicylamide sulphate in man is decreased with increasing dose, increased by administration of a sulphate donor and increased by decreasing the rate of absorption of the drug[3]. Vitamin C may decrease the rate of absorption of salicylamide because the vitamin inhibits gastric emptying in man[5]. Thus, the vitamin may cause a decrease in salicylamide sulphate formation by one mechanism and an increase by another mechanism, so that a typical dose–response relationship for the effect of vitamin C on sulphate formation should not be expected. It is likely that there is an optimum dose of vitamin C for maximum reduction of salicylamide sulphate formation, a dose at which the inhibitory effect of the vitamin on salicylamide absorption is relatively less pronounced than its effect on sulphate formation. This optimum dose probably differs between individuals and may differ also as a function of dosage form (solution, tablets, and so on) and drug (that is, drugs other than salicylamide which are also subject to sulphate formation). It is also likely that individuals differ in their capacity for vitamin C absorption (the vitamin is absorbed by a specialised saturable process[6]) and in the rate at which endogenous sulphate is available for conjugation with a substrate such as salicylamide. All of these variables may account for the interindividual variations in the effect of vitamin C on the formation of salicylamide sulphate as found in this study.

A number of drugs are extensively conjugated with sulphate (and thereby inactivated) in the first pass through the intestinal tissues and liver following oral administration. One such drug is isoprenaline (isoproterenol) which is used widely for the treatment of asthma and certain cardiac diseases. The recommended

**Table 1** Effect of vitamin C* on the biotransformation of salicylamide† in man

| Subject | Age (yr) | Total recovery in urine (% dose) | | Salicylamide sulphate (% dose) | | Salicylamide glucuronide (% dose) | |
|---|---|---|---|---|---|---|---|
| | | Control | Vitamin C | Control | Vitamin C | Control | Vitamin C |
| A | 25 | 93.4 | 101.1 | 31.6 | 25.9 | 54.4 | 59.8 |
| C | 25 | 91.8 | 92.7 | 35.0 | 25.7 | 52.1 | 60.1 |
| D | 25 | 94.7 | 95.7 | 29.3 | 29.9 | 55.9 | 58.8 |
| G | 47 | 91.2 | 92.7 | 46.5 | 41.5 | 38.5 | 46.5 |
| H | 26 | 97.9 | 96.2 | 29.7 | 19.7 | 58.8 | 69.6 |
| I | 27 | 94.5 | 94.8 | 39.9 | 28.9 | 46.3 | 53.7 |
| L | 39 | 98.0 | 98.1 | 35.8 | 25.4 | 53.7 | 64.5 |
| O | 32 | 95.3 | 94.4 | 48.9 | 42.9 | 40.0 | 43.8 |
| S | 32 | 96.6 | 92.7 | 35.7 | 31.1 | 57.2 | 59.2 |
| Y | 29 | 96.1 | 96.3 | 35.7 | 38.7 | 56.3 | 53.6 |
| Mean | | 94.9 | 95.5 | 36.8 | 31.0 | 51.3 | 57.0 |
| S.d. | | 2.3 | 2.7 | 6.6 | 7.7 | 7.2 | 7.8 |
| Statistical significance of difference from control‡ | | NS | | $P < 0.005$ | | $P < 0.005$ | |

Ten healthy male volunteers 25–47 yr old, capable by education and experience to give their informed consent (research assistants and associates), participated in the study. They were instructed not to take any drugs or vitamins for at least one week before and during the study. They fasted overnight, emptied their bladder in the morning and took 100 ml of water or 2 g of vitamin C in 100 ml water. One hour later, they collected their urine and took 150 mg salicylamide alone or with 1 g vitamin C, both dissolved in 100 ml water. No food or drink other than water was permitted for the following 2 h. Urine was collected at 0.5, 1, 1.5 and 2 h after salicylamide administration and at convenient times thereafter for a total of 24 h, each time followed by 100 ml water to assure adequate urine flow. Five of the subjects took salicylamide with vitamin C, whereas the other five took salicylamide only. At least 1 week later, the former took salicylamide alone, whereas the latter took it with vitamin C. The urine samples were analysed for total salicylamide and its metabolites (salicylamide sulphate, salicylamide glucuronide and gentisamide) as described previously[3]. Blank values obtained from the 1 h urine sample collected before salicylamide administration were used to correct the analytical results; blank values from urine obtained after administration of the initial 2-g dose of vitamin C did not differ from those obtained without prior ingestion of the vitamin. Several urine samples obtained after administration of salicylamide alone were diluted with an equal volume of urine either from unmedicated subjects or from subjects taking vitamin C; there were no differences in analytical results, showing that the vitamin and its metabolites did not interfere with the analysis of salicylamide and its metabolites. Some urine samples obtained after ingestion of salicylamide alone and after ingestion of salicylamide with vitamin C were analysed immediately after collection and again after 10 d of storage in a refrigerator. These analyses yielded essentially identical results, thereby ruling out a possible effect of ascorbic acid or its metabolites on the stability of the salicylamide metabolites.

*2 g taken 1 h before salicylamide and 1 g taken concomitantly.

†150 mg salicylamide dissolved in 100 ml water.

‡Paired $t$ test.

oral dose of this drug is about 100–1,000 times larger than its intravenous dose[7], reflecting largely its extensive inactivation by biotransformation during absorption. It has been predicted[8] and demonstrated in dogs[9] that salicylamide can markedly inhibit this inactivation. Following introduction of isoprenaline into the intestinal lumen of anaesthetised dogs, only 3.6% of the total amount absorbed was unmetabolised drug; 68% was isoprenaline sulphate. When isoprenaline was given with salicylamide, 73.7% of the absorbed amount was unmetabolised isoprenaline and only 3.3% was in the form of the sulphate conjugate, on the average. Similar effects may occur when vitamin C is taken with drugs which ordinarily undergo extensive conjugation with sulphate during their initial passage across the intestinal mucosa and through the liver. This could result in a significant potentiation in the pharmacological activity of drugs so affected.

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# Tryptophan pyrrolase activity regulation in *Drosophila:* role of an isoacceptor tRNA unsettled

Tryptophan pyrrolase is a key enzyme in the biosynthetic pathway leading to the ommochromes[1]. In wild-type flies of *D. melanogaster* or *D. hydei*, tryptophan is converted by this enzyme into *N*-formyl kynurenine, which is hydrolysed to formic acid and kynurenine. Further metabolism of kynurenine results in the deposition of the yellow-brown pigments in the eyes of these insects. Tryptophan pyrrolase activity, however, is lacking or greatly reduced in freshly prepared homogenates of living imagines of the vermilion mutants of *D. melanogaster*[1,2,3] or *D. hydei* (Table 1). Finding a stimulation of tryptophan pyrrolase activity in homogenates of *D. melanogaster* vermilion flies by RNase $T_1$ treatment and an inhibition of this activity by wild-type tRNA preparations or by an isoaccepting tyrosine tRNA ($tRNA_2^{Tyr}$) led Jacobson[4,5] to suggest the implication of this specific isoaccepting tRNA in the regulation of tryptophan pyrrolase activity and in the mechanism of genetic suppression of the vermilion mutant. Because of the importance of these conclusions and their bearing on the general understanding of the basic molecular mechanisms of enzyme regulation and suppression in eukaryotes, we reinvestigated the entire system including the assay procedure.

Tryptophan pyrrolase activity is assayed on the basis of the amount of kynurenine produced from tryptophan in homogenates of the respective insect tissues. This assay, however, is usually hampered by microbial contamination and the lack of a good technique for the direct determination of the kynurenine produced in the assay mixture. Avoiding the unreliable and troublesome diazotation reaction of Bratton and Marshall[6], we identified kynurenine by its $R_f$ value and typical light-blue fluorescence after thin-layer chromatography on cellulose powder, and quantitated kynurenine on the basis of its extinction at 360 nm. To eliminate microbial contamination, penicillin and streptomycin were included in the assay mixture.

All our attempts failed to induce tryptophan pyrrolase activity in freshly prepared homogenates of *D. melanogaster* or *D. hydei* vermilion flies by RNase $T_1$ or RNase A treatment (Table 1) following exactly the procedure published[4]. This failure cannot be attributed to either lack of RNase activity or a difference in strain: the vermilion mutant was suppressable with the *D. melanogaster* vermilion suppressor *su*(s)[2], and RNase activity stays very high for several hours in our assay system.

Tryptophan pyrrolase activity can be stimulated to almost wild-type activity levels when homogenising vermilion flies in the presence of 0.02 M EDTA, a standard ingredient in RNase assay mixtures. To prove that the substance produced in the EDTA activated vermilion mutants is kynurenine, 2-$^{14}$C-tryptophan was added to our standard tryptophan pyrrolase assay mixture. The resulting product was purified to constant specific radioactivity, and the identity of this radioactive compound with L-kynurenine was demonstrated by thin layer chromatography on cellulose powder in three different solvent systems. In the presence of EDTA, tryptophan pyrrolase activity is generally stimulated in homogenates of wild-type and vermilion mutant strains of *D. melanogaster* as well as *D. hydei*, independent of the developmental stage of the respective organisms (Table 1).

The tryptophan pyrrolase activity stimulated by EDTA in the vermilion homogenates could not be blocked by tRNA preparations of the respective wild strains. The EDTA stimulated tryptophan pyrrolase activity of *D. melanogaster* vermilion imagines was separated from the EDTA by passing the homogenate through a Sephadex G-75 column. This activity could also not be blocked by the addition of a purified tRNA preparation of wild-type *D. melanogaster* imagines (Table 1).

Table 1 shows an additivity of the normal and EDTA stimulated tryptophan pyrrolase activity, from which it may be

**Table 1**

| Organism and treatment* | Kynurenine (μg, average produced by 100 mg of fresh tissue) Without EDTA | With EDTA | EDTA-activated, tRNA added |
|---|---|---|---|
| *D. melanogaster* wild strain, imagines | 46 | 70 | — |
| *D. melanogaster* vermilion, imagines | ND | 32 | 18 |
| *D. melanogaster* vermilion, imagines, RNase $T_1$ | ND | 36 | — |
| *D. melanogaster* vermilion, imagines, RNase A | ND | 35 | — |
| *D. melanogaster* vermilion, IIIrd instar larvae | ND | 26 | — |
| *D. melanogaster* vermilion, imagines, EDTA removed | — | — | 14 |
| *D. hydei* wild strain, imagines | 26 | 36 | — |
| *D. hydei* wild strain, IIIrd instar larvae | 10 | 28 | — |
| *D. hydei* vermilion, imagines | ND | 14 | — |

*Living organisms (500 mg) were homogenised in 5.5 ml Tris-HCl (0.01 M, *p*H 7.5) supplemented with 3 mg mercaptoethanol, 4 mg ascorbate, 4.3 mg streptomycin and 2.15 mg penicillin. Following centrifugation, either 1 ml tryptophan solution (5 mg $ml^{-1}$ homogenisation medium) or 1 ml homogenisation medium was added to 1.5 ml supernatant. Incubation was for 3 h at 37 °C. Ethanol was added to a final concentration of 70% and kynurenine production was determined as in the text. Reproducibility of kynurenine determination in duplicate experiments was better than 95%. For tryptophan pyrrolase activity stimulation the homogenisation medium was made 0.02 M in EDTA. Purified tRNA of *D. melanogaster* wild strain imagines (10 μg $ml^{-1}$), RNase $T_1$ (up to 1,500 U) and RNase A (4 units) were present in the reaction mixture where indicated.

ND, not detectable.