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Research Section

SERUM METHANOL CONCENTRATIONS IN RATS AND IN MEN AFTER A SINGLE DOSE OF ASPARTAME

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Abstract—Serum methanol concentrations were measured in rats and in humans given oral aspartame. The dose given to rats was the FDA's projected 99th percentile daily intake for humans, assuming aspartame were to replace all sucrose sweeteners in the diet (34 mg/kg). Four male adult volunteers each received 500 mg, equivalent to 6–8.7 mg/kg, which is approximately the FDA's estimate of mean daily human consumption. Both treatments caused a rise in serum methanol. In rats the mean peak value was 3.1 mg/litre 1 hr after administration; serum methanol returned to endogenous values 4 hr after treatment. In the men, the mean rise over endogenous values was 1.06 mg/litre after 45 min. Two hours after treatment, serum methanol had returned to basal levels. The temporary serum methanol increase showed peak values within the range of individual basal levels.

INTRODUCTION

Methanol is released during aspartame digestion and the serum time course of release in humans after ingestion of abuse doses of the sweetener has been described (Stegink, Brummel, Filer & Baker, 1983; Stegink, Brummel, McMartin *et al.* 1981).

If aspartame were used to replace all sucrose sweeteners in the diet, its average daily ingestion would be 7.5–8.5 mg/kg (Stegink, Filer & Baker, 1977), with the upper 99th percentile equivalent to 34 mg/kg (FDA, 1981). These projected ingestion levels are respectively equivalent to 0.75–0.85 mg/kg and 3.4 mg/kg of methanol, which amounts to 10% of the aspartame molecule.

The sensitivity of conventional analytical techniques (Baker, Alenty & Zack, 1969; Drozd & Novak, 1977; Sims, 1976; Termonia, De Meyer, Wybauw & Jacobs, 1982) was too low for studies of methanol formation after aspartame treatment at these doses (Frey, 1976; Stegink *et al.* 1981 & 1983; Visek, 1984). We have described an analytical procedure that detects as little as 0.012 mg methanol/litre in aqueous solutions (Davoli, Cappellini, Airoldi & Fanelli, 1985) and, using this method, we studied methanol release at these levels.

We now report serum methanol concentrations in rats given 34 mg aspartame/kg and in healthy human volunteers after consumption of 500 mg aspartame, equivalent to a dose 6–8.7 mg/kg for 58–80 kg body weight.

EXPERIMENTAL

Reagents. Aspartame was obtained from Pierrel SpA (Milan, Italy), and [$^2H_4$]methanol, 99.8% pure, from Fluka AG (Buchs, Switzerland).

Animal studies. Male CD-COBS rats (Charles River, Italy) weighing 200 ± 10 g (mean ± SD) were used. Aspartame was diluted in water and administered by gavage after an overnight fast. Animals were divided into seven different groups, with six rats in the control group and four in each of the others. In a previous experiment it was found that endogenous methanol levels fluctuate so it was decided to have a control group larger than the treatment groups. Blood was collected at 0, 0.5, 1, 2, 3, 4 and 6 hr after dosing.

Human studies. Four healthy adult male volunteers were studied. They were required to fast for 8 hr, to refrain from drinking any alcoholic beverages for 24 hr and from drinking fruit juices and eating fruit or vegetables for 18 hr before the experiment. During the study, water was supplied *ad lib.* but no food was allowed.

Aspartame (500 mg) was suspended in 100 ml of tap-water and taken orally by the volunteers. Blood (*c.* 2 ml) was collected 30 min before aspartame ingestion, and at 0, 30, 45, 60, 90, 120 and 180 min after ingestion from each volunteer through a catheter inserted in an arm vein.

Methanol analysis. Extreme precautions were undertaken to prevent external contamination by methanol during the work-up procedure. Full details of these are given elsewhere (Davoli *et al.* 1985).

The analytical technique is rapid and simple and combines gas chromatography with selected-ion monitoring. The use of tetra-deuterated methanol as the internal standard gives the method high accuracy and reproducibility.

Serum (0.1 ml) was diluted with water in 7.5 ml crimp-seal vials. The final volume of the aqueous phase was 0.5 ml. Subsequently, tetra-deuterated methanol was added to the samples, which were placed in a heated bath held at 65°C. The time allowed for gas/liquid equilibration was 30 min. The head space gas (300 μl) was sampled with a 1-ml gas-tight Hamilton syringe kept at the same

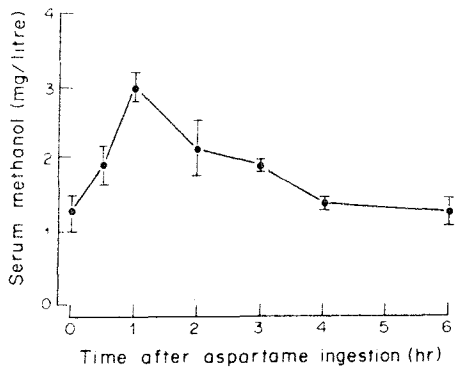

Fig. 1. Mean serum methanol concentrations (± SEM) in rats given 34 mg aspartame/kg orally. Values are for four rats except at 0 hr, when six rats were used.

temperature in order to avoid water condensation on the syringe barrel.

Gas chromatography–mass spectrometry (GC–MS) was carried out with a Finnigan 4000 mass spectrometer with a programmable multiple-ion monitor (model 1015-80) and a Finnigan 9610 gas chromatograph. GC–MS conditions were as follows: fused silica capillary column 26 m CP WAX 57 CB, i.d. 0.22 mm; oven temperature 50 C isothermal, injector temperature 150 C; carrier gas (helium) head pressure 4 lb/in^2, split flow 10 ml/min. Specific ions: methanol m/z 31, tetra-deuterated methanol m/z 33. The elution time, under the experimental conditions described, was 3 min for both methanol and the internal standard, tetra-deuterated methanol.

Calculations were made on the basis of the d0/d4 peak height with reference to a calibration curve.

RESULTS

The time course of serum methanol concentrations in rats given a single dose of 34 mg aspartame/kg is shown in Fig. 1. Methanol levels in these rats were significantly higher than in controls ($P < 0.01$; Dunnett's test) for up to 3 hr after aspartame ingestion and returned to endogenous values by 4 hr.

As expected, endogenous serum methanol concentrations in humans showed inter-individual variability. Figure 2 shows the time course of methanol

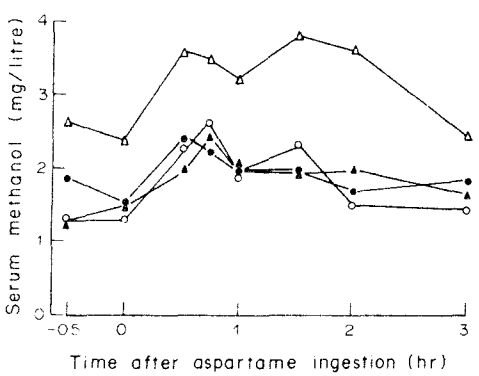

Fig. 2. Serum methanol concentrations in four adult male volunteers after ingestion of 500 mg aspartame.

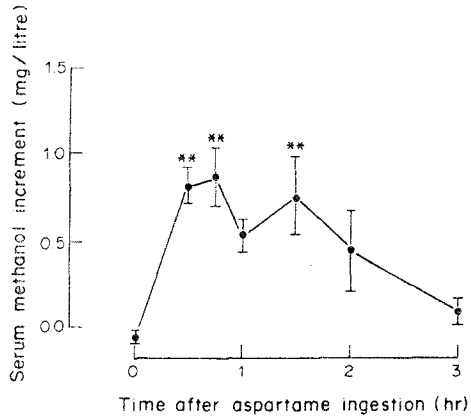

Fig. 3. Increment (over basal level) in serum methanol concentration in adult male volunteers after ingestion of 500 mg aspartame orally. Values are means ± SEM for four subjects; those marked with asterisks are significantly higher than the means levels at −0.5 and 0 hr (**$P < 0.01$; Dunnett's test). The mean of the methanol levels at −0.5 and 0 hr was taken as the basal valve for each individual curve.

serum concentrations for each subject after ingestion of 500 mg aspartame. Figure 3 shows the mean increments in serum methanol concentrations above basal values for all subjects. The mean levels at 30, 45 and 90 min after aspartame ingestion differed significantly from the basal levels ($P < 0.05$; Dunnett's test).

DISCUSSION

The findings in rats given a single dose of 34 mg aspartame/kg are close to those extrapolated by Stegink *et al.* (1983) in humans: they suggested that a dose of 34 mg/kg would give rise to a mean peak

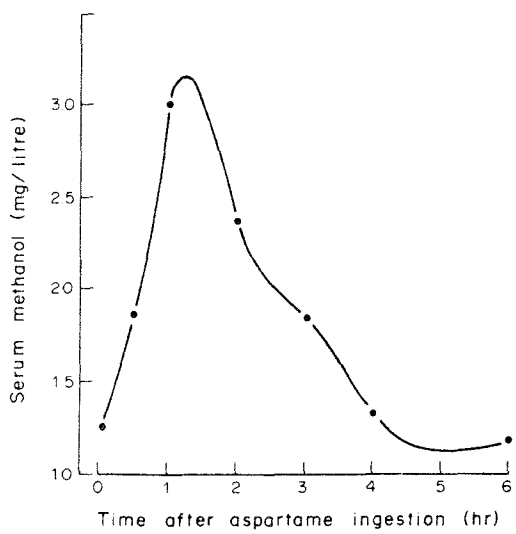

Fig. 4. Mean serum methanol concentration in rats given 34 mg aspartame kg orally. Cubic Spline interpolation of data represented in Fig. 1 obtained using Apple II computer and Curve Fitter program (Interactive Microware, Inc., State College, Pennsylvania, 1980).

blood methanol concentration of approximately 4.2 mg/litre, i.e. about 8.4 mg/litre serum. We found a mean value of 3.1 mg/litre serum at the peak time. The difference might well be explained by species differences. Also, the calculations of Stegink *et al.* (1983) were developed from data obtained after administering high doses of aspartame; methanol may be cleared more rapidly after lower aspartame doses.

The drop in methanol levels observed in humans 1 hr after ingesting 500 mg aspartame may indicate, as suggested by Stegink (1984), that hydrolysis of this sweetener to aspartate, phenylalanine and methanol occurs both in the intestinal lumen and in mucosal cells, probably explaining the bimodality of the curve. This shape is obvious with the low aspartame dose in humans, but is also visible in the case of rats given 34 mg/kg, especially if values are interpolated (Fig. 4).

The results indicate that consumption of an aspartame dose equivalent to the projected 99th percentile of total daily aspartame ingestion (34 mg/kg) and to the projected average ingestion (7.5–8.5 mg/kg) leads to a rise in serum methanol, as theoretically assumed, but that this increment is of the same order of magnitude as the variations in endogenous methanol levels. Moreover the single load of 500 mg of aspartame leads to a rise in serum methanol with peak values falling within the range of individual basal levels (Fig. 2). Obviously, when the 500 mg aspartame intake is not divided into several small doses, as normally occurs, the small increment in serum methanol would probably be undetectable and certainly not significant.

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