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Dissolution and absorption of caffeine from guarana

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Abstract—The rate of release of caffeine from capsules of guarana was compared with that from capsules containing an equivalent amount of caffeine using the British Pharmacopoeia dissolution test apparatus. Determinations were carried out in media of pH 2 and 6·8 and caffeine concentrations in the dissolution fluid were determined by HPLC. No significant differences in release rates were found between the two preparations at either pH. The rate of absorption of caffeine across rat intestine using the everted gut was also compared for a guarana suspension and a solution containing an equivalent amount of caffeine. Experiments were carried out using fluids of pH 4·0 and 7·4. No significant differences in absorption between the two preparations were observed. These results show that the release and uptake of caffeine from guarana is the same as for preparations containing free caffeine.

Guarana is a preparation made by certain Amerindian groups in northern Brazil from the seed kernels of *Paullinia cupana* H.B.K. var. *sorbilis* (Mart.) Ducke (Sapindaceae). The pounded seeds are moulded into cylinders and dried to produce sticks. A refreshing drink can be made from these sticks by scraping off some of the stick and boiling it in water (Henman 1982). Guarana is used extensively as a flavouring for soft drinks and in the last two years has become popular in Great Britain as a stimulant drink, promoted through health food stores.

Caffeine was shown to be present in guarana early last century (von Martius 1826; Berthemot & Dechastelus 1840) and is thought to be the constituent responsible for guarana's stimulant activity. There is evidence that the caffeine in guarana exists both in the free form and also as a complex with tannins (Nierenstein 1922; Bempong et al 1991). It is claimed that the active ingredient in the preparation made by traditional methods is a tetramethyl xanthine, as opposed to the trimethyl xanthine, caffeine (Hildreth 1989), but no evidence for the existence of this type of compound has been found (Bempong et al 1991).

It has been suggested that the stimulant effect of guarana is probably more gradual and sustained than that given by an equivalent dose of caffeine (Henman 1982). This has been proposed on the grounds that the other constituents present in the seeds, especially the caffeine-tannin complex and the saponins, affect the dissolution rate of caffeine from guarana in the gastrointestinal tract, and also its absorption through the gut wall.

The work reported in this paper investigates the dissolution and intestinal absorption of caffeine in guarana and compares these with values for free caffeine.

Materials and methods

Materials. Gelatin capsules, size 0, were filled with 500 mg of a mixture of lactose and caffeine to give a caffeine content per capsule of 17·9 mg. These were equivalent to commercial capsules of guarana (Rio Trading Co.) which were found to contain 17·9 mg free caffeine when analysed by HPLC (Bempong et al 1992).

Buffer solutions were made up as follows: for dissolution studies, buffer pH 2·0: KCl 37·82 g, HCl 13·5 M, 9·2 mL and H_2O to 10·0 L; buffer pH 6·8: Na_2HPO_4 288 g, KH_2PO_4 114·5 g and H_2O to 10·0 L. For absorption studies, Krebs–Henseleit phosphate buffer (KHPB) pH 7·4 (mM): NaCl 154, 400 mL; KCl 168, 16 mL; $MgSO_4$ 178, 4 mL; phosphate buffer pH 7·4 (Na_2HPO_4 28·58 g, KH_2PO_4 5·36 g, H_2O to 1·0 L) 84 mL, water 12 mL. KHPB pH 4·0: as for KHPB pH 7·4 but using phosphate buffer pH 4·0 (Na_2HPO_4 5·04 g, KH_2PO_4 3·01 g, H_2O to 1·0 L). The pH of each buffer was checked with a pH meter.

HPLC. A 10 cm reverse-phase column of ODS Hypersil 5 μm was used. The mobile phase was 0·1 M aqueous sodium acetate:acetic acid:methanol 55:20:25 supplied through a Perkin-Elmer series 410 pump and adjusted to give a flow rate of 1 mL min^{-1} at 1500 p.s.i. Injection was via a 10 μL loop. The detector was a Perkin-Elmer LC-55 spectrophotometer operating at 260 nm linked to a Perkin-Elmer LC1-100 computing integrator.

A calibration curve was constructed by injection of solutions containing 5·93 mM acetanilide and concentrations of caffeine ranging from 0·5 to 10·3 mM. Peak areas were calculated electronically and caffeine:acetanilide peak area ratio was plotted against caffeine concentration to construct the curves. The data were used to calculate the calibration equation by linear regression.

The calibration curves were used to calculate the amounts of caffeine in the samples examined by the method described below.

Dissolution studies. Apparatus conforming to the specifications of the 1988 British Pharmacopoeia was used for the dissolution tests. The temperature of the dissolution medium was maintained between 36·5 and 37·5°C by means of a thermostatically-controlled waterbath. The paddles were rotated at 100 rev min^{-1}.

After constant temperature was reached, five capsules were introduced into each dissolution vessel. The paddles were then started and 1·0 mL samples of the dissolution fluid were withdrawn by means of a syringe at 1, 5, 10, 15, 30, 60, 90 and 120 min. The 1·0 mL sample was mixed with 1·0 mL 5·93 mM acetanilide as an internal standard and 5 μL of the mixture was injected onto the HPLC column. The apparatus allowed three different samples of guarana capsules and three different samples of caffeine capsules to be analysed simultaneously for caffeine by HPLC. The mean and standard deviation for each time interval measurement were calculated for both guarana and pure caffeine capsules. The amount of caffeine measured was expressed as a percentage of the caffeine present in the capsules at the beginning of the experiment.

Determinations were carried out using dissolution buffer at pH 2·0, (stomach pH), and at pH 6·8 (ileum pH).

Absorption studies using the everted gut technique. The method used followed that described by Levy & Reuning (1964). Intestine was obtained from female Wistar rats killed by cranial dislocation. A long piece of intestine was rinsed with glucose-saline solution (NaCl 9·0 g, glucose 1·0 g and H_2O to 1·0 L), everted and both ends tied with a thread ligature. The everted gut was left at room temperature (21°C) in glucose-saline solution until needed. Lengths of about 5 cm were cut and tied at one end by a ligature. A second ligature was tied loosely around the open end of the sac and the blunt needle of a syringe was inserted

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through the space. The ligature was then tightened around the needle. Krebs-Henseleit buffer solution (0·7 mL) was injected onto the sac formed by the piece of gut, the needle was withdrawn and the ligature tightened further. Each sac was immersed in separate flasks, each containing 10·0 mL of buffer with either 2·57 mM caffeine or a suspension of guarana powder added, which had previously been analysed by HPLC. Six flasks were used for each sample. The flasks were shaken at 37°C. After 60 min, three sacs from each treatment were removed and all their serosal fluid taken out with a syringe and analysed for caffeine by HPLC by mixing with an equal volume of 0·08% w/v acetanilide to serve as an internal standard. Three more sacs for each treatment were withdrawn after 90 min and analysed in the same way.

The experiment was carried out using buffers of pH 4·0 and 7·4 to simulate the environment of the stomach and intestine, respectively.

The mean and the standard deviation of the caffeine concentration in the serosal fluid for each sample was calculated and expressed as a percentage of the original amount of caffeine present in the buffer solution in which the sacs were suspended.

Results

Calibration curve. The calibration curve for the HPLC analysis gave a correlation coefficient of 0·999. A null hypothesis test that there was no linear relationship between area ratios and concentration was rejected since a very low P value ($< 0{\cdot}0001$) was obtained when the t-test was applied. The calibration curve could therefore be used to determine amounts of caffeine in samples of dissolution fluid and serosal fluid.

Dissolution studies. The rate of release of caffeine from the guarana and caffeine capsules at pH 2·0 and 6·8 is shown in Figs 1 and 2, respectively. All the caffeine was released from the caffeine-lactose capsules in 90 min but a small amount of caffeine was not released from the guarana capsules during that time. No significant difference was found between the two sets of data using the paired Student's t-test.

Absorption studies. The results from the absorption studies are shown in Table 1. Analysis using the paired Student's t-test showed that there was no significant difference between the sets of data for caffeine uptake between the guarana suspension and the caffeine solution.

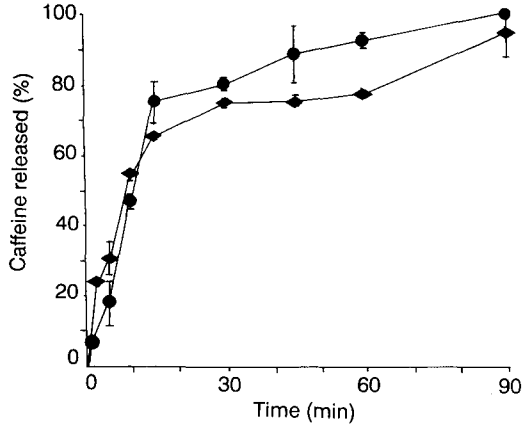

FIG. 1. Release of caffeine from capsules using the British Pharmacopoeia dissolution apparatus, medium pH 2·0. Caffeine capsules, ●; guarana capsules, ◆.

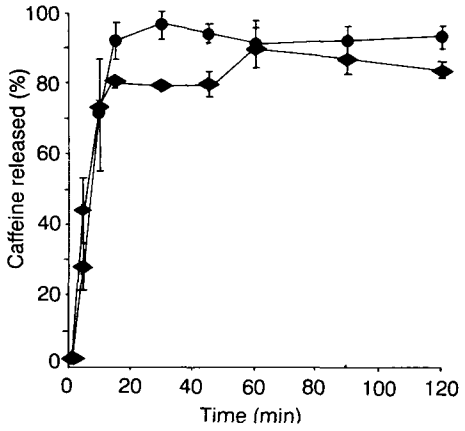

FIG. 2. Release of caffeine from capsules using the British Pharmacopoeia dissolution apparatus, medium pH 6·8. Caffeine capsules, ●; guarana capsules, ◆.

Table 1. Amount of caffeine absorbed across the gut wall using the everted gut technique.

Sample (time taken min)	Mean (n = 3) caffeine in 0·7 mL serosal fluid ($mg \times 10^{-2}$)	Mean percentage caffeine absorbed (s.d.)
pH 4·0		
Caffeine (60)	13·02	26·04 (6·1)
Caffeine (90)	14·33	28·66 (1·9)
Guarana (60)	11·97	23·9 (1·9)
Guarana (90)	12·81	25·62 (8·0)
pH 7·4		
Caffeine (60)	12·96	25·92 (7·8)
Caffeine (90)	14·45	28·90 (15·3)
Guarana (60)	12·66	25·48 (2·3)
Guarana (90)	13·69	27·38 (13·4)

Caffeine concentration in 10 mL immersion fluid is 2·5 mM.

Discussion

There was no significant difference in the time taken for < 70% caffeine to be released from guarana capsules and capsules containing an equivalent amount of caffeine (Figs 1, 2). The dissolution of caffeine follows a first-order release process up to this point but subsequently the rate of release is much slower.

The fatty substances present in the guarana do not appear to impede release, as has been suggested as a justification for claims that guarana has a prolonged stimulant effect (Henman 1982). The complete dissolution of caffeine from a capsule takes approximately 60 min, implying that its absorption in the stomach would be slower than the same amount taken as a beverage. However, an experiment to compare the absorption of caffeine from capsules and from a solution was not carried out.

The amount of caffeine absorbed across the intestinal wall over a 60 or 90 min period was not significantly different between the guarana and caffeine capsules at pH 4·0 or 7·4 (Table 1). It has been suggested that the presence of saponins in the seeds from which guarana is made affect the absorption of caffeine in similar ways—such as solubilization—to those shown for a variety of other compounds (Reuning & Levy 1968a, b) and prolong the stimulant effect. The results presented here indicate that this is probably not the case.

Scientifically-unsubstantiated claims that guarana gives a more prolonged stimulant effect cannot, therefore, be explained in terms of caffeine release or uptake.

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PAF formation by human gastrointestinal mucosa/submucosa in-vitro: release by ricinoleic acid, and inhibition by 5-aminosalicylic acid

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Abstract—Human isolated gastrointestinal mucosa/submucosa incubated with ricinoleic acid (12·5–100 μg mL^{-1}) or the calcium ionophore A23187 (10 μg mL^{-1}) released platelet-activating factor (PAF) as determined by a scintillation proximity assay after extraction and purification. 5-Aminosalicylic acid (25–100 μg mL^{-1}) inhibited PAF release by ricinoleic acid in a concentration-dependent manner, and 50 μg mL^{-1} reduced the effect of A23187. We suggest that PAF may play a role in the laxation and mucosal damage by ricinoleic acid released from castor oil.

Platelet-activating factor (PAF), a phospholipid with several biological activities, is produced by different cell types (Parente & Flower 1985) and tissues (Rachmilewitz et al 1990) in response to various stimuli. A substantial release of PAF occurs from incubated intestine of rats with induced experimental colitis (Mascolo et al 1990) and from inflamed colon of patients with ulcerative colitis (Rachmilewitz et al 1990). PAF possesses prodiarrhoeagenic secretory effects in rat isolated intestine (Buckley & Hoult 1989). Rats treated with castor oil also have intestinal release of PAF (Pinto et al 1989), together with intestinal hyperaemia and intraluminal release of acid phosphatase (a marker of cellular damage). These findings suggest a role for PAF as a mediator of the laxation and the intestinal damage induced by castor oil. However, experiments so far have mainly been performed in animals, and their relevance to man has not been determined. The present study has examined the effect of ricinoleic acid, the active constituent in castor oil, on PAF release from human isolated gastrointestinal mucosa.

Materials and methods

Human gastrointestinal tissues (colon, ileum and stomach) were taken at least 5 cm from any macroscopically detected lesions in surgical specimens removed for benign or malignant disease. As far as we are aware, the patients had not within the previous 4–6 days consumed any drug known to affect eicosanoid synthesis. Samples were transported to the laboratory at ambient temperature within 30 min of removal and then transferred to ice-cold 154 mM NaCl (saline). The layer of mucosa/submucosa was carefully cut off from the underlying muscle while the tissue was bathed in saline, cut finely with scissors and washed with saline. Accurately weighed aliquots (200 ± 10 mg) were suspended in 2 mL of 0·25% bovine serum albumin/saline with drugs or vehicle. The drugs used were: ricinoleic acid (6·25, 12·5, 25, 50 and 100 μg mL^{-1}), calcium inonophore A23187 (10 μg mL^{-1}), and 5-aminosalicylic acid (5-ASA; 25, 50 and 100 μg mL^{-1}). Solvents were: for ricinoleic acid, 10 mg mL^{-1} ethanol; for A23187, 0·2 mg mL^{-1} DMSO; and for 5-ASA, 10 mg mL^{-1} saline.

Samples were incubated in a shaking water bath (37°C, 30 min), and PAF was then extracted and purified by thin-layer chromatography (Pinto et al 1989). Briefly, cold acetone (2 mL, −20°C) was added to the incubate, centrifuged (2000 *g*, 5 min), and the acetone/water phase was extracted with 2 mL chloroform. After evaporation to dryness, the residue was redissolved in 75 μL of chloroform/methanol (1:1), applied to thin layer chromatography plates (silica gel, Kodak) and developed in chloroform/methanol/water (65:35:6) together with authentic standard PAF (Sigma, Poole). Zones co-migrating with authentic PAF, visualized by exposure to iodine, were re-extracted, dried, and assayed for PAF using a scintillation proximity radioimmunoassay (Amersham, SPRIA, TRK 990; sensitivity 20 pg, intra- and inter-assay coefficients of variations 4·7–6·7% and 2·9–8·3%). The amount of radiolabelled PAF bound to the fluomicrospheres was determined by direct counting in the vials, using a Packard scintillation counter 2200C (window 0 to 999, 4 min).

The above procedure gave a mean recovery of 85·3 ± 5·2% for standard PAF. Percent cross-reactions of the antiserum used were PAF 100; lyso-PAF < 0·01; octadecyl-2-acetyl GPC PAF 40; 1-hexadecanoyl-2-acetyl GPC 0·06; 1-octadecanoyl-2-acetyl GPC 0·05; 1-hexadecanoyl-2-lyso GPC 0·01; phosphatidylcho-

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