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Stimulation of Gastric and Colonic Mucosal Eicosanoid Synthesis by Plantain Banana

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Abstract—Extracts of plantain banana (*Musa sapientum* Linn *var. paradisiaca*) were studied on the accumulation of eicosanoids in incubates of human gastric and colonic mucosa. The ethanolic extract caused a concentration-dependent increase in the eicosanoid accumulation but the water extract was ineffective. Since all the eicosanoids studied tended to increase, banana may act by increasing the availability of arachidonate. In control tissues the accumulation of PGE and TXB_2 in the incubates decreased with time while that of 6-keto-$PGF_{1\alpha}$ increased (colon only, studied).

The powder of dried plantain banana pulp inhibits gastric and duodenal ulcer formation in different models of experimental ulcers in rats, mice and guinea-pigs (Sanyal et al 1964, 1965; Elliott & Heward 1976; Goel 1983; Best et al 1984; Goel et al 1985a, 1986). This is due to stimulation of defensive mucosal resistance factors, and not to an effect on acid or pepsin (Goel et al 1985a, b). The banana pulp increases the amount of gastric mucosal carbohydrates, [^{3}H]thymidine incorporation into mucosal DNA (Best et al 1984; Goel et al 1986), and the content of mucus in the gastric juice, but it decreases the gastric juice DNA and protein (Mukhopadhyaya et al 1987). Extracts of banana powder reduced the formation of rat gastric ulcers by aspirin or pylorus ligation (Goel 1983; Best et al 1984). Double-blind studies in four centres found that about 70% of endoscopically proved duodenal ulcers healed after 12 weeks of treatment with banana powder compared to about 16% with placebo (Mukhopadhyaya et al 1987). The active ingredient is thought to be a flavonoid (D. A. Lewis, personal communication).

There is strong evidence that prostaglandins (PGs) are involved in gastric mucosal functions, and that non-steroidal anti-inflammatory drugs (NSAIDs) damage this tissue by inhibiting PG synthesis (Scratcherd 1987). PGE compounds have mucosal-protective and ulcer-healing activity (Watkinson & Akbar 1987). The aim of the present work was to study the effect of different extracts of plantain banana on eicosanoid formation by human gastric mucosa, and to compare the findings with human colonic mucosa, a tissue in which eicosanoids may also play a role in function and pathology.

Materials and Methods

Banana powder

Powder was prepared from plantain banana (*Musa sapientum* Linn var. *paradisiaca*, family Scitaminaceae) that was picked in Varanasi, India during December, dried in shade at 26–32°C, and powdered as described previously (Goel et al 1985a, 1986). The anti-ulcerogenic effect of the powder was confirmed in rats by the 60% reduction of the gastric ulcer score induced by aspirin ($n=8$, $P<0.05$) and the 50% reduction in the incidence of cysteamine-induced duodenal ulcers ($n=10$, $P<0.05$) (Goel et al 1986).

Banana extracts

An aqueous extract of banana was prepared by homogenizing banana powder in water (50 mg mL^{-1} suspension, Silverson homogenizer). This was refrigerated at 4°C for 48 h, centrifuged (1500 *g*, 10 min) and filtered through glass wool. Half of the filtrate was lyophilized at −60°C under reduced pressure using an Edwards freeze dryer, yielding a brownish powder that weighed 11·4% of the dried banana powder. This lyophilized powder and the unlyophilized portion were diluted in water to give stock concentrations of 50, 500 and 5000 $\mu g\ mL^{-1}$, and stored at −20°C for up to 40 days. For incubation they were diluted 10-fold in phosphate buffered saline pH 7·4 (PBS), to give final concentrations of 5, 50 and 500 $\mu g\ mL^{-1}$.

An ethanol extract of banana was prepared by homogenizing the dried banana powder in ethanol (1:10 w/v). The homogenate was refrigerated at 4°C for 3 days, the ethanol being decanted daily and replaced. The supernatant was filtered through glass wool, centrifuged (1500 *g*, 10 min), and dried under vacuum at 35°C using a GyroVap, to give a yield of about 0·9% of the dry powder. After reconstitution in ethanol, concentrations of 1, 10 and 100 $\mu g\ mL^{-1}$ in 1% ethanol were used for incubation.

Human gastric and colonic mucosa

Gastric and colonic tissues were taken from surgical specimens removed for benign or malignant disease, at least 5 cm from any macroscopically detected lesion. The mucosa and submucosa were carefully removed together from the underlying muscle, while the tissue was bathed in PBS.

Mucosal incubation. Each tissue sample was cut into pieces 3–5 mm^2, and washed with PBS which was then drained off.

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Carefully weighed samples of 100 ± 5 mg were pre-incubated (1 mL PBS, 0°C, 30 min), in the absence or presence of drugs (indomethacin 0·1, 1, 10 μg mL^{-1}; aqueous banana extract (unlyophilized) 5, 50, 500 μg mL^{-1}; freeze-dried and reconstituted banana aqueous extract 5, 50, 500 μg mL^{-1}; ethanol-extracted banana 1, 10, or 100 μg mL^{-1}).

This pre-incubation fluid, containing any prostanoids released normally or by trauma, was discarded and replaced by fresh incubation fluid (± drugs). After further incubation at 37°C for 30 min, the fluid which contained the released prostanoids was removed and stored at −20°C for up to 10 days before assay. With colonic mucosa there was a further 60 min incubation with fresh fluid (± drugs) at 37°C, which was then removed and stored at −20°C until assayed as above.

Radioimmunoassays. The method is based on that of Jaffe & Behrman (1974), using suitable dilutions of antisera and tritiated standards. Assay sensitivities were 10 pg, and the intra- and inter-assay coefficients of variation were 5–9% and 10–11% depending on the prostanoid measured. All assays were in duplicate.

Tritiated prostanoids were purchased from Amersham Radiochemical Centre. The percent cross-reactions of the antisera used were: *PGE antibody* (ICN Biomedicals Ltd) PGE_2 100; PGE_1 240; $PGF_{1\alpha}$ 0·35; $PGF_{2\alpha}$ 0·5; 15-keto-PGE_2 0·1; PGD_2 0·04; 6, 15-diketo-$PGF_{1\alpha}$ <0·04; 15-keto-$PGF_{1\alpha}$ <0·04; 13, 14-dihydro-6-keto-$PGF_{1\alpha}$ <0·04; 6-keto-$PGF_{1\alpha}$ 0·6; TXB_2 <0·04; *6-keto-$PGF_{1\alpha}$ antibody* (Wellcome) 6-keto-$PGF_{1\alpha}$ 100, $PGF_{2\alpha}$ 0·84; PGE_2 0·1; TXB_2 0·02; *TXB_2 antibody* (Wellcome) TXB_2 100, $PGF_{2\alpha}$ 0·11; PGE_2 0·008; 6-keto-$PGF_{1\alpha}$ 0·01.

Tritiated leukotriene B_4 was purchased from Amersham Radiochemical Centre. The percent cross-reactions of the antiserum used were: LTB_4 100; 20-OH-LTB_4 0·4; 6-trans-LTB_4 0·4; LTC_4 <0·05; LTD_4 <0·05; TXB_2 <0·05; $PGF_{2\alpha}$ <0·05; 6-keto-$PGF_{1\alpha}$ <0·05.

Tritiated Leukotriene C_4 was purchased from New England Nuclear. The percent cross-reactions of the antiserum, kindly provided by Dr J. Zakrzewski, were: LTC_4 100: LTD_4 29·4; LTE_4 0·7; LTB_4 0·05; PGE <0·05; arachidonic acid <0·05.

Results are expressed as means ± s.e.m., and analysed statistically by Student's *t*-test for paired data (2-tailed tests).

Results

Gastric mucosa

The amounts of prostanoids accumulating in incubates of gastric mucosal pieces, measured by RIA (ng g^{-1} wet weight tissue 30 min^{-1}) were PGE 118·2 ± 21·2, TXB_2 87·5 ± 12·6 and 6-keto-$PGF_{1\alpha}$ 59·9 ± 13·9 (n = 5). Indomethacin 0·1-10 μg mL^{-1} concentration-relatedly inhibited the accumulation of these prostanoids (PGE 40–82% $P<0·05$ to <0·01; TXB_2 14–87%, $P<0·1$ to 0·01; 6-keto-$PGF_{1\alpha}$ 49–93%, $P<0·05$ to <0·01). In contrast, ethanol 1% had little or no effect on their accumulation the mean changes being PGE 26% $P<0·3$; TXB_2 14% $P<0·05$; 6-keto-$PGF_{1\alpha}$ −6% $P<0·2$.

Ethanolic banana extract caused a concentration-dependent (1-100 μg mL^{-1}) increase in the accumulation of PGE (by 12 to 67%), TXB_2 (−1 to 16%) and 6-keto-$PGF_{1\alpha}$ (15 to 30%). At 100 μg mL^{-1} the *P* values were <0·05, <0·05 and <0·4, respectively (Fig. 1). Indomethacin, 0·1, 1 and 10 μg mL^{-1} inhibited these increases in prostanoid synthesis by 54·8 ± 28·1 to 97·6 ± 1·3% (P 0·3 to <0·05, n = 3)

The amounts of leukotrienes accumulating in control gastric mucosal incubates, measured by RIA (ng g^{-1} wet weight 30 min^{-1}), were LTB_4 1·69 ± 0·39 and LTC_4 2·36 ± 0·57. Ethanol 1% did not alter the amounts, but the LTB_4 and LTC_4 accumulation was higher in 4/5 experiments with ethanolic banana extract 100 μg mL^{-1} ($P<0·2$ for both; Fig. 1). There was no interaction between any of the antisera and the ethanolic extract 1, 10 and 100 μg mL^{-1}.

Colonic mucosa

The amounts of prostanoids accumulating in the control incubates of human colonic mucosal pieces (ng g^{-1} wet weight tissue 30 min^{-1}) in the first 30 min were: PGE 48·9 ± 4·7, TXB_2 27·8 ± 8·0 and 6-keto-$PGF_{1\alpha}$ 40·2 ± 7·9 (n = 6).

Indomethacin in PBS 0·1–10 μg mL^{-1} usually caused a concentration-related reduction of prostanoid accumulation (PGE 31–60% inhibition $P<0·2$ to <0·01; TXB_2 59–95%, $P<0·1$ to <0·05; 6-keto-$PGF_{1\alpha}$ 45–86%, $P<0·1$ to <0·01). This inhibition was unaffected by adding 1% ethanol to the PBS (PGE 37–60% inhibition; TXB_2 61–95%; 6-keto-$PGF_{1\alpha}$ 32–79%).

In the second (60 min) incubation the amounts of prostanoids accumulating in the fluid (ng g^{-1} wet tissue 60 min^{-1}) were: PGE 42·3 ± 7·8, TXB_2 24·8 ± 4·2 and 6-keto-$PGF_{1\alpha}$ 133·5 ± 37·7 (n = 6). Indomethacin again caused a concentration-dependent inhibition, similar to that in the

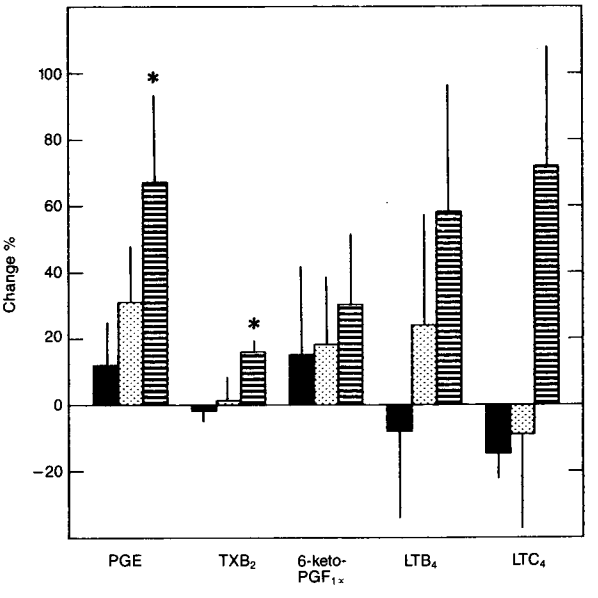

FIG. 1. Ethanolic banana extract caused a concentration-dependent increase in the accumulation of endogenous eicosanoids in incubates of human gastric mucosal pieces (30 min incubation). Values are expressed as mean % change ± s.e.m., n = 5. *$P<0·05$ compared to respective ethanol controls (Student's *t*-test for paired data). Indomethacin (0·1–10 μg mL^{-1}) concentration-dependently inhibited the accumulation of prostanoids, compared with respective controls. The inhibitions by indomethicin were PGE 40–82%, $P<0·05$ to <0·01; TXB_2 14–87%, $P<0·1$ to <0·01; 6-keto-$PGF_{1\alpha}$ 49–93%, $P<0·05$ to <0·02. Key: solid column 1, dotted columns 10, hatched columns 100 μg L^{-1} extract.

first incubation. There was little or no effect of ethanol 1% on the prostanoid accumulation. The ethanolic banana extract (1–100 $\mu g\ mL^{-1}$) concentration-relatedly increased the amount of PGE by 5–94% in the first incubation ($P<0.02$ with 100 $\mu g\ mL^{-1}$, Fig. 2), and by 5–89% in the second incubation ($P<0.05$ with 100 $\mu g\ mL^{-1}$).

In another set of experiments the lyophilized and the unlyophilized aqueous extracts (5, 50 and 500 $\mu g\ mL^{-1}$) had little or no effect on the accumulation of prostanoids (PGE 40.9 ± 2.9, TXB_2 28.1 ± 5.9 and 6-keto-$PGF_{1\alpha}$ 38.0 ± 6.5 ng g^{-1}/30 min in the first (30 min) PBS control incubation; PGE 46.6 ± 5.7, TXB_2 33.0 ± 6.6 and 6-keto-$PGF_{1\alpha}$ 101.4 ± 22.0 ng g^{-1}/60 min in the second (60 min) PBS control incubation). Compared with the first incubation, the accumulation of PGE and TXB_2 ($g^{-1}\ min^{-1}$) in the second incubation was less ($P<0.01$ for PGE and $P<0.02$ for TXB_2), whereas that of 6-keto-$PGF_{1\alpha}$ was greater ($P<0.05$), although the total amount of accumulated prostanoids in both incubations was similar ($P<0.1$, Table 1).

Leukotriene C_4 accumulation in control incubates of colonic mucosa was 2.11 ± 0.53 ng g^{-1} wet weight/30 min in the first 30 min study. The ethanolic banana extract tended to increase leukotriene C_4 (by 29–57% $P<0.1$ at 100 $\mu g\ mL^{-1}$, Fig. 2), whereas with ethanol 1% the mean amount of LTC_4 was 20% less than the PBS control ($P<0.1$).

Discussion

Pieces of human gastric and colonic mucosa incubated in buffer accumulated eicosanoids as determined by radioimmunoassay. We use the term 'accumulation' since the amount of released material degraded during the incubation is not known. The amounts of PGE and TXB_2 declined in the second (60 min) incubation (colonic tissue only studied) whereas that of 6-keto-$PGF_{1\alpha}$ increased. Since the total prostanoid accumulation was similar, this may indicate a redirection of PGI_2 formation at the expense of PGE_2 and TXB_2. In the 30 min incubations the amounts of PGE and TXB_2 in gastric mucosal incubates were nearly three times higher than in colonic incubates, while the amounts of 6-keto-$PGF_{1\alpha}$ were similar. These findings agree with the results of Peskar et al (1986a) and Tavares et al (1987). In general, responses of the stomach and colon mucosa to the banana ethanol extract or to indomethacin were qualitatively similar. The banana ethanol extract usually caused a concentration-dependent increase of the three prostanoids measured, and a similar tendency also occurred with LTB_4 and LTC_4. The water extracts, tested only in the colon, were inactive in concentrations of 5, 50 and 500 $\mu g\ mL^{-1}$.

Table 1. Amounts of accumulated prostanoids (ng g^{-1} min^{-1}) by human colonic mucosa incubated for different times in phosphate buffer.

Prostanoids	n	1st incubation (30 min) (ng g^{-1} min^{-1})	2nd incubation (60 min) (ng g^{-1} min^{-1})	P
PGE	11	1.50 ± 0.11	0.69 ± 0.08	<0.01
TXB_2	11	0.85 ± 0.16	0.48 ± 0.08	<0.02
6-keto-$PGF_{1\alpha}$	11	1.29 ± 0.20	1.84 ± 0.36	<0.05
Total	11	3.64 ± 0.35	3.01 ± 0.37	<0.1

Results are expressed as means ± s.e.m., and analysed by Student's t-test for paired data. Accumulation of PGE and TXB_2 during the second incubation (60 min) was less than in the first period, but the amount of 6-keto-$PGF_{1\alpha}$ increased.

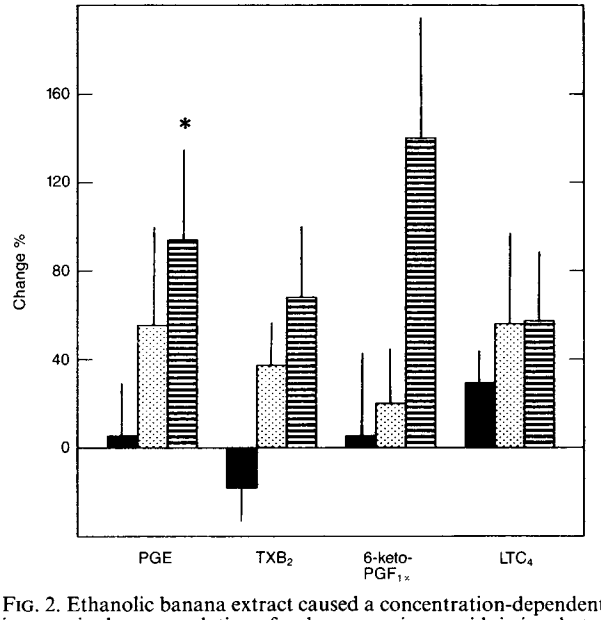

FIG. 2. Ethanolic banana extract caused a concentration-dependent increase in the accumulation of endogenous eicosanoids in incubates of human colonic mucosal pieces (30 min incubation). Values are expressed as mean % change ± s.e.m., n = g. *$P<0.02$ compared with respective ethanol controls (Student's t-test for paired data). Indomethacin (0·1–10 $\mu g\ mL^{-1}$) concentration-dependently inhibited the accumulation of prostanoids, compared to respective controls. The inhibitions were PGE 31–60%, $P<0.2$ to <0.01; TXB_2 59–95%, $P<0.1$ to <0.05; 6-keto-$PGF_{1\alpha}$ 45–86%, $P<0.1$ to <0.01. Key as Fig. 1.

Banana increases the gastric mucosal defensive factors by promoting mucin secretion, increasing the mucoprotein content of mucosa, decreasing the shedding of cells and leakage of protein in the gastric secretion in response to ulcerogens, and promoting healing as shown by the increase in [^{3}H]thymidine uptake by the gastric mucosal cells (Best et al 1984; Goel et al 1985a, b, 1986; Mukhopadhayaya et al 1987). Banana did not affect acid or pepsin secretion (Goel et al 1985a). Endogenous PGs play an important role in gastric mucosal protection and ulcer healing by their various actions on the mucosal defensive factors listed above, and on others such as bicarbonate secretion and maintenance of mucosal blood flow. The therapeutic effect of plantain banana might therefore be due at least in part to increased prostanoid formation. A tendency for the formation of both cyclooxygenase and lipoxygenase products to increase may indicate that banana augments the release of arachidonate. Diarrhoea with plantain banana powder (Goel et al unpublished) might also be explained by increased eicosanoid formation, since prostaglandins and leukotrienes increase intestinal secretion (Rampton & Hawkey 1984).

The role of endogenous leukotrienes as mediators of gastric mucosal damage is not clear. Peskar et al (1986b) found a relationship between increased LTC_4 production and the gastric damage produced in rats by ethanol; the lipoxygenase inhibitor nordihydroguaiaretic acid protected against this damage. However, Boughton-Smith & Whittle

(1988a) obtained no relationship between inhibition by BW A4C or BW A137C (*N*-(3-phenoxycinnamyl) and *N*-(4-benzyloxybenzyl) aceto-hydroxamic acid) of lipoxygenase product formation and rat gastric ulceration. PGE_2 protected against ethanol-induced damage, but it did not effect leukotriene formation (Dreyling et al 1986; Boughton-Smith & Whittle 1988b). On the other hand, the stable 16,16-dimethyl analogue of PGE_2 and carbenoxolone reduced gastric damage and leukotriene formation in rats (Peskar et al 1986b; Boughton-Smith & Whittle 1988b). It is therefore not clear what part an increase in leukotriene formation by banana might play in the gastric mucosa.

The similarity of responses of eicosanoid formation by human gastric and colonic mucosa to banana raises the question of the effect of banana on colonic disease. In ulcerative colitis PGs may have a protective role, since non-steroidal anti-inflammatory drugs can worsen the disease (Rampton & Hawkey 1984), and leukotrienes may contribute to the damage (Sharon & Stenson 1984; Wallace et al 1989).

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