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Effects of senna and its active compounds rhein and rhein-anthrone on PAF formation by rat colon

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Abstract—A single or a prolonged oral administration of senna (60 mg kg^{-1}) to rats did not increase either colonic PAF (platelet activating factor) content or intraluminal release of acid phosphatase. A similar result was observed in the colonic tissue of rats perfused in-vitro with rhein (1–300 $\mu g\ mL^{-1}$) or rhein-anthrone (1–300 $\mu g\ mL^{-1}$). A single or prolonged administration of castor oil (2 mL) to rats increased both colonic PAF content and intraluminal release of acid phosphatase. Colonic tissue of rats perfused in-vitro with calcium ionophore A23187 (1 and 10 $\mu g\ mL^{-1}$) formed large amounts of PAF and acid phosphatase. Since PAF can mediate intestinal damage and acid phosphatase is a marker of cellular injury, we conclude that senna and its derivatives, rhein and rhein-anthrone, are well tolerated in rats.

Since Smith (1968, 1972) reported intestinal injury in man as well as in mice after oral administration of senna syrup, some authors have demonstrated myenteric plexus and colonic epithelium injuries following administration of anthraquinone laxatives (Riemann et al 1978; Goerg et al 1980; Nataf et al 1981; Verhaeren et al 1985). It has also been shown that senna extract and rhein induce pseudomelanosis coli, associated with sub-epithelial congestion of capillaries and epithelial alterations (Spiessens et al 1991). However, other authors have found no intestinal alteration either in man or animals even after prolonged treatment (see Dufour & Gaudre 1988). Thus possible intestinal damage induced by anthraquinone-containing laxatives still needs clarification.

Platelet activating factor (PAF), an endogenous phospholipid, produces gastrointestinal hyperaemia and haemorrhage when administered to animals (Wallace & Whittle 1986). Endotoxin, a substance that causes hypotension and diarrhoea, also causes gastrointestinal damage associated with increased formation of PAF (Whittle et al 1987). Another secretagogue that produces diarrhoea and intestinal injury is castor oil and recently it has been shown that castor oil purgation is associated with the increased formation of PAF along the intestine (Pinto et al 1989, 1992). Therefore, PAF formation throughout the digestive tract is considered an indirect sign of tissue damage. Another marker of changes in the mucosa of small and large intestine is represented by an increase of acid phosphatase in the lumenal fluid (Wallace & Whittle 1986; Whittle et al 1987) and we have recently demonstrated that castor oil also increases intraluminal release of acid phosphatase (Pinto et al 1989, 1992). In the present study we have explored whether senna and its derivatives, rhein and rhein-anthrone, lead to changes in the levels of PAF formed by rat colon, and if these changes are related to intraluminal release of acid phosphatase.

Materials and methods

Experiments were carried out in male Wistar (Morini) rats weighing 150–170 g at the start of the study. Animals were deprived of food overnight, but allowed free access to water. For in-vivo experiments, senna (60 mg kg^{-1}) was given orally and after 2–8 h the animals were killed by cervical dislocation. Long-term senna administration (60 mg kg^{-1} day^{-1} for 1–12 weeks) was also conducted and 6 h after the last treatment, the animals were killed as above. Control animals received vehicle only (0·5 mL of 5% arabic gum suspension). After an oral dose of 60 mg kg^{-1} senna, severe diarrhoea developed in all the animals treated, approximately 4 h after its administration. This persisted until 8 h after treatment and continued in a less severe form up to 24 h. In rats treated for 7–90 days, diarrhoea persisted throughout the entire treatment period. Throughout the treatment period the general conditions of rats were good. Food consumption which was normal after a 7 day treatment period, was only marginally reduced (8–12% below that in the control group) after 30–90 days treatment. In the course of the treatment period, the body weight gain of the animals was 15% (30 days treatment)–25% (90 days treatment) below that of the control group ($P < 0.05$). No difference was observed after a 7-day treatment. For PAF isolation and identification, segments of colon (200 mg wet weight) were excised, weighed, minced with scissors and processed as previously described (Pinto et al 1989). The PAF activity was measured using a scintillation proximity radioimmunoassay (Amersham, SPRIA TRK 990). The sensitivity of the assay was 20 pg. The intra- and inter-assay coefficients of variations were 4·7–6·7 and 2·9–8·3%, respectively. The percent cross reactions of the antisera used were: PAF 100; lysoPAF <0·01; GPC 0·05; 1-hexadecanoyl-2-lyso GPC 0·06; 1-octadecanoyl-2-acetyl GPC 0·05; 1-hexadecanoyl-2-lyso GPC 0·01; phosphatidylcholine <0·04; lysophosphatidylcholine <0·02; arachidonic acid <0·01. The amount of radiolabelled PAF bound to the fluomicrospheres was determined by direct counting in the vial using a Packard scintillation counter 2200 C (window 0 to 999, 4 min). Standard PAF added to the incubation mixture containing minced mucosa, followed by incubation, extraction and TLC, gave a mean recovery of 80·7±4·6%. Intraluminal release of acid phosphatase was measured by the method of Wallace & Whittle (1986) and enzymatic activity was measured as described by Ammendola et al (1975). For in-vitro experiments animals were killed and colon was removed and rinsed with Tyrode solution at room temperature (21°C). A segment 8–10 cm long was cannulated at both ends as previously described (Autore et al 1990) and perfused with Tyrode solution (1 mL min^{-1} Watson-Marlow peristaltic pump) with and without rhein (1–300 $\mu g\ mL^{-1}$), rhein-anthrone (1–300 $\mu g\ mL^{-1}$) or calcium ionophore A23187 (1–10 $\mu g\ mL^{-1}$). Both the perfusate and the bath fluid were kept at 37°C and equilibrated with O_2-CO_2 (95:5). After 30 min, colon was processed for determination of PAF and acid phosphatase. Drugs used were: dried senna pod extract (*Cassia angustifolia*) containing 45% sennoside B (Indena; the dose of senna was calculated according to the sennoside B content); rhein and rhein-anthrone (gifts from Madaus, Germany); calcium ionophore purchased from Sigma. In some experiments a single and a long-term (7 days) castor oil administration (2 mL per rat) was also conducted and 3 h after the last treatment the animals were killed and colonic tissue processed as above. Oral dose of 2 mL

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per rat castor oil for 1 week had no discernible effect on the general condition, food consumption or body weight gain of rats.

Results

Table 1 shows that small amounts of PAF were formed by control tissue and that this production was not increased 2–8 h after a single oral administration of senna (60 mg kg^{-1}). A similar result was observed in the colonic tissue from rats treated with senna for 7–90 days (Table 2). In the same way colonic tissue from senna-treated rats did not produce any increase of acid phosphatase (Tables 1, 2). Formation of PAF by segments of rat colon (control 270·4 ± 23·7 pg (g tissue)$^{-1}$, n = 4) was instead increased 3 h after castor oil administration (2 mL per rat). PAF formation was greater in colonic tissue from rats treated for 7 days (431·5 ± 35·8 pg (g tissue)$^{-1}$, n = 5; $P < 0.05$) than in tissue from rats treated once (351·4 ± 25·7, n = 4; $P < 0.2$). Castor oil also increased the intraluminal release of acid phosphatase (control 301·4 ± 75·7, n = 4; single treatment 507·5 ± 119·4, n = 4; $P < 0.2$; repeated treatment 954·3 ± 181·4; n = 5; $P < 0.05$). Table 3 shows that colon of rats perfused in-vitro for 30 min formed very small amounts of PAF and the presence of rhein-anthrone (1–100 $\mu g\ mL^{-1}$) did not modify this. Only a very high concentration of rhein-anthrone (200 $\mu g\ mL^{-1}$) had a slight (14%) although not significant effect; the response to 300 $\mu g\ mL^{-1}$ showed no further increase over the effect of 200 $\mu g\ mL^{-1}$. Rhein (1–300 $\mu g\ mL^{-1}$) had an effect similar to that of rhein-anthrone, whereas calcium ionophore (1–10 $\mu g\ mL^{-1}$) caused a concentration related increase of PAF (49·2–83·9%; $P < 0.05$–0.01) in the colonic tissue perfused in-vitro. The intraluminal release of acid phosphatase was not modified by senna derivatives whereas it was significantly increased (43·1–91·7%; $P < 0.05$–0.01) by calcium ionophore A23187 (Table 3).

Discussion

The ability of non-stimulated rat colon to produce PAF (Whittle et al 1987; Pinto et al 1989) is confirmed in the present study. This non-stimulated production of PAF, characterized by RIA, may be due to the manipulation of the tissue, but may also be attributed to a species difference; human colonic tissue does not release detectable amounts of PAF in normal conditions (Rachmilewitz et al 1990; Capasso et al 1991).

Nevertheless, senna, at a dose (60 mg kg^{-1}) that elicited diarrhoea in all the animals treated (Mascolo et al 1988; present results) was not able to increase the amount of intestinal PAF 2–8 h after drug challenge.

Long-term senna administration (60 mg kg^{-1} day^{-1} for 1–12 weeks) failed to induce any variation in colonic PAF content although the dose used was higher than the therapeutic dose in man. Senna activity depends on the release, by bacterial enzymes, of active metabolites, rhein and rhein-anthrone, in the gut lumen (Leng-Peschlow 1988; de Witte & Lemli 1988).

Perfusion of rat colon in-vitro with either rhein or rhein-anthrone did not increase colonic PAF levels when the concentrations used were in the same range as the human intraluminal levels (1–10 μg). Senna and senna derivatives, commonly classified as irritant or stimulant laxatives, are presumed to cause intestinal damage and recently it has been demonstrated that anthranoid laxatives induce subepithelial congestion of capillaries and epithelial alterations (Spiessens et al 1991).

These findings are in disagreement with those of other authors who found no mucosal abnormalities (Douthwaite & Goulding 1957; Dufour & Gaudre 1988; Rudolph & Mengs 1988) and no alterations in parameters in the intestinal physiology after short or long-term treatment with senna preparations (Mahon & Palmade 1974; Dubecq & Palmade 1974). However it is interesting to note that animal studies, where lesions in the intestinal tissue are reported, are conducted with very high doses of drug.

It has been recently demonstrated that intestinal damage is followed by PAF formation throughout the digestive tract and by intraluminal release of acid phosphatase (Wallace & Whittle 1986; Whittle et al 1987; Pinto et al 1989, 1992; Mascolo et al 1990). Acid phosphatase release is now considered a marker of cellular injury and PAF a mediator of gastrointestinal damage. Since our data show that neither of these parameters is altered by

Table 1. Formation of platelet activating factor (PAF) and the intraluminal release of acid phosphatase by rat colon after a single oral dose of senna (60 mg kg^{-1}).

Time after senna (h)	PAF (pg (g tissue)$^{-1}$)	Acid phosphatase (μg (g tissue)$^{-1}$)
0 (Control)	278·7 ± 31·7	304·3 ± 98·3
2	261·7 ± 23·4	301·7 ± 111·4
4	300·5 ± 21·7	328·3 ± 110·7
6	309·1 ± 30·4	316·9 ± 121·4
8	277·4 ± 27·8	298·7 ± 113·4

Results are expressed as mean ± s.e. of 6 experiments.

Table 2. Formation of platelet activating factor (PAF) and the intraluminal release of acid phosphatase after prolonged daily treatment with senna (60 mg kg^{-1}).

Time after senna[a] (days)	PAF (pg (g tissue)$^{-1}$)	Acid phosphatase (μg (g tissue)$^{-1}$)
0 (Control)	288·4 ± 27·4	301·4 ± 101·3
7	306·3 ± 30·8	309·3 ± 90·8
30	281·4 ± 35·6	315·7 ± 110·1
90	303·8 ± 34·1	316·7 ± 115·4

Results are expressed as mean ± s.e. of 5–7 experiments. [a] Tissue was collected 6 h after the last treatment.

Table 3. Formation of platelet activating factor (PAF) and the intraluminal release of acid phosphatase by rat colon perfused in-vitro.

Treatment ($\mu g\ mL^{-1}$)	PAF (pg (g tissue)$^{-1}$)	Acid phosphatase (μg (g tissue)$^{-1}$)
None	108·1 ± 15·7	301·5 ± 101·3
Rhein		
1	99·7 ± 13·8	304·7 ± 99·5
10	101·5 ± 10·7	311·8 ± 95·7
50	107·1 ± 13·9	293·3 ± 111·7
100	105·7 ± 16·1	ND
200	121·3 ± 15·3	307·5 ± 116·8
300	115·5 ± 10·7	ND
Rhein-anthrone		
1	104·5 ± 11·4	321·7 ± 115·7
10	100·7 ± 12·3	311·1 ± 108·5
50	99·6 ± 15·3	305·7 ± 101·8
100	107·3 ± 13·1	ND
200	119·4 ± 10·7	320·6 ± 114·4
300	116·1 ± 15·3	ND
Calcium ionophore A23187		
1	171·3 ± 13·1*	561·4 ± 106·3*
10	198·7 ± 29·1**	678·1 ± 161·4*

Results are expressed as mean ± s.e. of duplicate determinations in each of experiments (3–7) and analysed by Student's t-test for unpaired data. ND = not determined. *$P < 0.05$, **$P < 0.01$.

senna or senna metabolites we conclude that anthranoid laxatives are well tolerated in rats.

It has recently been shown that calcium ionophore A23187 damages isolated gastric mucosal cells and stimulates the release of lysosomal enzymes (Tepperman et al 1991). It has also been demonstrated that calcium ionophore A23187 releases PAF production from human intestinal tissue (Rachmilewitz et al 1990). Our data demonstrated a similar effect in rat colon. However, the mechanism and source of this increased production of PAF are not yet understood.

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