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# Increased ex-vivo colonic generation of PAF induced by diphenylmethane stimulant laxatives in rats, mice, guinea-pigs and rabbits

A. A. IZZO, N. MASCOLO, G. AUTORE, G. DI CARLO, F. CAPASSO, *Department of Experimental Pharmacology, University of Naples 'Federico II', via Domenico Montesano 49, 80131 Naples, Italy*

**Abstract**—The effects of in-vivo treatment with bisacodyl, phenolphthalein, picosulphate, sulphosuccinate, mannitol and lactulose laxatives were examined on the ex-vivo formation of platelet-activating factor (PAF) by duodenum and colon of rat, mouse, guinea-pig and rabbit. Bisacodyl (10 mg $kg^{-1}$), phenolphthalein (20 mg $kg^{-1}$) and picosulphate (10 mg $kg^{-1}$), but not sulphosuccinate (40 mg $kg^{-1}$), mannitol (50 mg $kg^{-1}$) or lactulose (50 mg $kg^{-1}$), at doses that all caused laxation, markedly increased PAF in the colon ($P < 0.01$) but not in the duodenum. Intraluminal release of acid phosphatase was also significantly increased in the colon of rats treated with bisacodyl, phenolphthalein and picosulphate, but not in colons of animals treated with sulphosuccinate, mannitol or lactulose. The data show that enhanced generation of PAF is associated with the colonic damage induced by diphenylmethane laxatives, but do not show whether this is a cause or a consequence of the pathophysiological changes.

Platelet-activating factor (PAF) is an endogenous phospholipid that produces extensive gastrointestinal hyperaemia and haemorrhage (Wallace & Whittle 1986). Administration of castor oil causes diarrhoea and intestinal damage that is associated with the increased formation of PAF along the intestine (Pinto et al 1989, 1992). In addition, observations of the intestinal action of ricinoleic acid, the intraluminal active metabolite of castor oil, have demonstrated a significant increase in PAF formation by human colonic mucosa incubated in-vitro (Capasso et al 1992). The elevated intestinal formation of PAF following castor oil was also accompanied by intraluminal release of acid phosphatase and hyperaemia suggesting a role for PAF in intestinal damage induced by castor oil. Castor oil and ricinoleic acid are commonly classified as stimulant or irritant laxatives. This group also includes phenolphthalein, bisacodyl, picosulphate, sulphosuccinate and anthraquinone-containing laxatives (Brunton 1990; Leng-Peschlow 1992). However, it has been recently demonstrated that neither senna nor the senna derivatives rhein and rhein-anthrone are able to increase intestinal PAF content (Mascolo et al 1992).

In this communication we present evidence that, like castor oil, other stimulant laxatives cause changes in the levels of PAF formed by intestinal tissue. In addition we show that some of these laxatives also stimulate intraluminal release of acid phosphatase.

Correspondence: F. Capasso, Department of Experimental Pharmacology, University of Naples 'Federico II', via Domenico Montesano 49, 80131 Naples, Italy.

## Materials and methods

Male Wistar rats, 140–150 g, were deprived of food overnight but had free access to water. In some experiments male Swiss mice, 22–25 g, male albino guinea-pigs, 250–260 g, and male New Zealand rabbits, 1·9–2 kg were also used. Laxatives (phenolphthalein 20 mg $kg^{-1}$, bisacodyl 10 mg $kg^{-1}$, sulphosuccinate 40 mg $kg^{-1}$, picosulphate 10 mg $kg^{-1}$, mannitol 50 mg $kg^{-1}$, lactulose 50 mg $kg^{-1}$ (all from Sigma, Milano, Italy)) were administered intragastrically with the aid of a stomach tube and

4–6 h later the animals in which diarrhoea was evident were killed by cervical dislocation (rabbits were killed by bleeding).

Intraluminal release of acid phosphatase was measured as described by Ammendola et al (1975).

Isolation and identification of PAF was carried out as previously described (Pinto et al 1992). In brief, segments of intestinal tissue (200 mg wet wt) were excised, weighed, minced with scissors and then suspended in 2·0 mL 0·25% bovine serum albumin (BSA, Sigma) in 154 mM NaCl (0°C). After vortexing for 20 s, the mixture was added to cold acetone (2 mL, −20°C) and after centrifugation at 2000 *g* for 5 min, the acetone-water phase was extracted by vortex mixing for 10 s with chloroform (2 mL). The upper aqueous phase was discarded after separation of the mixture by centrifugation for 10 min at 2000 *g*. The organic phase containing the extracted PAF was evaporated to dryness, redissolved in chloroform/methanol (1 : 1, 75 μL), applied to thin layer chromatography (TLC) plates and developed in the solvent system chloroform/methanol/water (65:35:6) together with authentic standard PAF (Sigma), visualized by exposure to iodine and assayed for PAF using a scintillation proximity radioimmunoassay (SPRIA, Amersham), as previously described (Capasso et al 1992; Pinto et al 1992). The procedure used gave a mean recovery of 81·7 ± 5% for standard PAF. Values were corrected for extraction losses.

## Results

Table 1 shows that segments of rat duodenum and colon produced large amounts of PAF. Table 1 also shows that PAF formation by colon was increased substantially ($P < 0·01$) after bisacodyl, phenolphthalein or picosulphate administration, while no significant difference was observed in duodenal tissue of control and treated rats. Sulphosuccinate caused a small, but not significant, effect while mannitol and lactulose had no effect on either tissue considered. A significant ($P < 0·01$) increase in PAF formation occurred in colonic tissue of mouse, guinea-pig and rabbit in response to administration of bisacodyl, phenolphthalein, and picosulphate (Table 2), but not in response to administration of the other three laxatives tested (data not shown). The intraluminal release of acid phosphatase was significantly elevated in colon from diphenol-treated rats but not in duodenum (Table 3). The other three laxatives tested did not cause release of acid phosphatase either in colon or in duodenum (Table 3).

Table 1. PAF content, determined by SPRIA, in rat duodenum and colon after oral administration of laxatives.

| Treatment | Dose (mg kg$^{-1}$) | PAF content (ng (g tissue)$^{-1}$) Duodenum | Colon |
|---|---|---|---|
| None | | 0·64 ± 0·15 | 0·30 ± 0·10 |
| Stimulant laxatives | | | |
| Bisacodyl | 10 | 1·00 ± 0·21 | 1·67 ± 0·18* |
| Phenolphthalein | 20 | 0·89 ± 0·16 | 1·48 ± 0·21* |
| Sulphosuccinate | 40 | 0·80 ± 0·11 | 0·49 ± 0·08 |
| Picosulphate | 10 | 0·81 ± 0·15 | 1·27 ± 0·11* |
| Osmotic laxatives | | | |
| Mannitol | 50 | 0·45 ± 0·15 | 0·34 ± 0·10 |
| Lactulose | 50 | 0·67 ± 0·13 | 0·32 ± 0·09 |

Results are expressed as mean ± s.e. of eight experiments (eight animals were treated with laxatives and intestinal segments processed separately) and analysed by Student's *t*-test. * $P < 0·01$.

Table 3. Intraluminal release of acid phosphatase by intestinal tissue of rats after oral administration of laxatives.

| Treatment | Dose (mg kg$^{-1}$) | Acid phosphatase release (μg substrate transformed (g dry wt)$^{-1}$) Duodenum | Colon |
|---|---|---|---|
| None | | 750·5 ± 141·8 | 681·3 ± 104·3 |
| Stimulant laxatives | | | |
| Bisacodyl | 10 | 819·7 ± 138·4 | 1937·1 ± 171·4* |
| Phenolphthalein | 20 | 793·4 ± 143·4 | 1993·4 ± 164·3* |
| Picosulphate | 10 | 731·8 ± 133·5 | 1741·7 ± 191·4* |
| Sulphosuccinate | 40 | 773·6 ± 141·8 | 801·4 ± 113·7 |
| Osmotic laxatives | | | |
| Mannitol | 50 | 763·4 ± 112·7 | 791·3 ± 140·7 |
| Lactulose | 50 | 791·7 ± 144·8 | 708·8 ± 131·4 |

Results are expressed as means ± s.e. of six experiments (see Table 1) and analysed by Student's *t*-test. * $P < 0·01$.

## Discussion

The present results show that intestinal tissue from different animals (rat, mouse, guinea-pig, rabbit) produces substantial amounts of PAF in normal conditions. Although this non-stimulated production of PAF by control (non-laxative-treated tissues) may be due to the manipulation of the tissue, there may also be a species difference since it is greater in rat and rabbit tissue than in mouse and guinea-pig while human colonic tissue, although processed in a different way, does not release detectable amounts of PAF (Rachmilewitz et al 1990; Capasso et al 1992). PAF production seems also to display regional metabolic variations since it is greater in the small than in the large intestine (Pinto et al 1989; this study). Nevertheless, the present results

Table 2. Effect of bisacodyl, phenolphthalein and picosulphate on PAF formation by intestinal tissue from different species.

| | No treatment | Bisacodyl (10 mg kg$^{-1}$) | Phenolphthalein (20 mg kg$^{-1}$) | Picosulphate (10 mg kg$^{-1}$) |
|---|---|---|---|---|
| Mouse | | | | |
| Duodenum | 0·35 ± 0·08 | 0·39 ± 0·20 | 0·30 ± 0·10 | 0·45 ± 0·10 |
| Colon | 0·20 ± 0·07 | 1·05 ± 0·09* | 1·00 ± 0·16* | 0·87 ± 0·07* |
| Guinea-pig | | | | |
| Duodenum | 0·26 ± 0·07 | 0·30 ± 0·10 | 0·30 ± 0·07 | 0·30 ± 0·10 |
| Colon | 0·20 ± 0·09 | 1·25 ± 0·10* | 0·70 ± 0·13* | 0·77 ± 0·07* |
| Rabbit | | | | |
| Duodenum | 0·61 ± 0·10 | 0·70 ± 0·11 | 0·60 ± 0·10 | 0·53 ± 0·09 |
| Colon | 0·49 ± 0·10 | 1·70 ± 0·18* | 1·30 ± 0·13* | 1·35 ± 0·11* |

Results are expressed as mean ± s.e. of eight experiments (see Table 1) and analysed by Student's *t*-test. * $P < 0·01$.

demonstrate that bisacodyl at a dose that elicited diarrhoea in all the animals treated, increased the amount of intestinal PAF 4 h after drug challenge in the colon but not in the duodenum. A similar effect was shown for phenolphthalein which has some chemical similarity to bisacodyl (Sund 1989) and for picosulphate, although their effects were weaker. Similar results were also observed in intestinal tissue of mice, guinea-pigs and rabbits.

The present results also show that the intraluminal release of acid phosphatase was markedly increased (about 3-fold that of control) in the colon of rats treated with bisacodyl, phenolphthalein or picosulphate.

Sulphosuccinate and the osmotic laxatives mannitol and lactulose did not modify or modified weakly both PAF content and the intraluminal release of acid phosphatase in the intestinal segments considered. Enhanced generation of PAF is associated with the colonic damage induced by diphenylmethane laxatives, but it is not clear whether this is a cause or a consequence of the pathophysiological changes, although PAF seems not to be involved in the damage induced by sulphosuccinate (Saunders et al 1975; Bretagne et al 1981). Diphenols may reach the large intestine as glucuronides (bisacodyl, phenolphthalein) or unconjugated (picosulphate); in the colon the bacterial flora is indispensable for their activation to unconjugated and pharmacologically active diphenols. This could explain the localization of the damage induced by these agents which consists of desquamation of surface cells, erosive morphological changes in colonic mucosal cells and a marked alteration of the cellular infiltrate in the lamina propria (Meisel et al 1977; Saunders et al 1990).

The underlying mechanism for this increase in production of PAF following the administration of diphenol laxatives, is unclear. PAF is rapidly synthesized in response to various stimuli by numerous inflammatory cell types including monocytes, macrophages, neutrophils, eosinophils, platelets, basophils and vascular endothelial cells (Benveniste 1988; Pinckard et al 1988). As a consequence PAF is released in numerous digestive ailments associated with inflammation and ulceration. PAF is also released following administration of castor oil (Pinto et al 1989, 1992), another laxative used to induce experimental gastroenteritis (Reynell & Spray 1958), but not after senna or sennoside derivatives, laxatives which seem not to induce intestinal alterations (see Mascolo et al 1992).

It is possible that after administration of diphenylmethane laxatives, the PAF might originate from infiltrated leucocytes, intestinal cells or from activation of bacterial flora (Benveniste et al 1992). Although PAF induces damage in the gastrointestinal tract (Wallace & Whittle 1986) and stimulates secretion (Buckley & Hoult 1989), it is still unclear whether this mediator plays a role in diarrhoea induced by stimulant laxatives such as diphenols and castor oil, or whether it is simply a consequence of cell damage.

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