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Time Course of PAF Formation by Gastrointestinal Tissue in Rats After Castor Oil Challenge

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Abstract—When castor oil was administered by gavage to rats, the duodenum and jejunum, but not the stomach, produced large amounts of platelet activating factor 3–7 h after oil challenge with a peak at 3 h. Intraluminal release of acid phosphatase was also markedly increased in the duodenum and jejunum of castor oil-treated rats, especially 3–5 h after oil challenge. No increase was observed in the stomach. There was a correlation between elevated release of acid phosphatase and intestinal hyperaemia.

Castor oil obtained from seeds of *Ricinus communis* (Euphorbiaceae), has been used as a mild purgative since ancient times. The active constituent in the oil is ricinoleic acid, a C18 aliphatic monohydroxy fatty acid. It is liberated in the small bowel by lipolysis, but on account of its 12-hydroxyl group it is poorly absorbed (Ammon & Phillips 1974). This has led to the suggestion that the intestinal epithelium may be damaged by this agent (Gaginella et al 1977) and although morphological studies have supported this suggestion (Reynell & Spray 1958; Cline et al 1976; Gaginella & Bass 1978; Bretagne et al 1981), the mechanism underlying the intestinal damage induced by castor oil is not yet established. We have recently shown that castor oil, at a dose that produced diarrhoea in animals, increased the amount of intestinal platelet activating factor (PAF) (Pinto et al 1989). This stimulated formation was markedly greater in the duodenum than in the jejunum and less in the ileum and colon. The elevated intestinal formation of PAF following castor oil was accompanied by intraluminal release of acid phosphatase and hyperaemia, suggesting a role for PAF as a mediator of intestinal damage induced by castor oil. We now describe the time course of PAF formation and release of acid phosphatase by gastrointestinal tissue following castor oil administration.

Materials and Methods

Male Wistar rats (Morini, Italy), 130–140 g, were fasted overnight but allowed free access to water. Castor oil (2 mL per rat) was administered orally and 0·5–9 h later the animals were killed by cervical dislocation. Macroscopically visible gastrointestinal damage was scored on a scale (0 normal, to 3 severe hyperaemia) by an observer unaware of the treatment. Intraluminal release of acid phosphatase was measured as described by Ammendola et al (1975).

Isolation and identification of PAF was carried out by a modification of the method described by Calignano et al (1988). In brief, segments of intestinal tissues (200 mg wet wt) were excised, weighed, minced with scissors and then suspended (20 min) in 2·0 mL of 0·25% bovine serum albumin (BSA, Sigma, Italy) in 154 mM NaCl (0°C). After vortexing for 20 s, the mixture was added to cold acetone (2 mL; 0°C) and after centrifugation (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, 60 μL), applied to thin layer chromatography plates and developed in chloroform/methanol/water (65:35:6) together with authentic standard PAF (Sigma). Zones corresponding to PAF were located by UV light and were re-extracted. The dried organic phase was resuspended in Tris buffer (25 mM, pH 8·0) containing 0·25% BSA, and the PAF activity bioassayed by aggregation of rabbit washed platelets (Whittle et al 1987).

The aggregating activity of the samples was confirmed as PAF since it was completely inhibited by incubating the platelet suspension for 1 min with 1·0 μg of CV 3988 [(*RS*)-2-methoxy-3-(octadecylcarbamoxyloxy)propyl-2-(3-thiazolio) ethylphosphate, Takeda Chemicals], a selective PAF receptor antagonist. 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; glycerophosphonylcholine (GPC) 0·05; 1-hexadecanoyl-2-acethyl GPC 0·06; 1-octadecanoyl-2-acethyl 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 2200C (window 0 to 999, 4 min).

After adding standard PAF to the incubation mixture containing minced mucosa, incubation, extraction and TLC, the above procedures gave a mean recovery of 78·5 ± 5·3% (bioassay) and 80·7 ± 4·6% (SPRIA).

Results

The formation of PAF by duodenum, which in control tissue was 108·7 ± 10 pg (g tissue)$^{-1}$, was increased substantially ($P < 0·05$) 1 h after castor oil administration (Table 1). A further significant ($P < 0·01$–$0·001$) increase in PAF forma-

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Table 1. PAF content, determined by bioassay (platelet aggregation), in rat gastrointestinal tissue after oral administration of castor oil (2 mL/rat).

Time after castor oil (h)	PAF content (pg (g wet tissue)$^{-1}$)		
	Stomach	Duodenum	Jejunum
0 (control)	63·0±7·8	108·7±10·0	97·3±9·7
0·5	60·3±3·1	130·5±13·8	135·0±15·3
1	70·4±10·1	205·5±15·1*	181·0±34·1*
3	64·1±13·1	809·7±89·4***	603·5±61·4***
5	71·0±10·9	689·4±45·1***	493·5±30·4**
7	59·0±31·1	234·1±32·1**	199·0±20·9*
9	not done	149·5±21·7	121·0±16·7

Results are expressed as mean±s.e. of 10 experiments and analysed by Student's t-test for unpaired data; *$P<0·05$; **$P<0·01$; ***$P<0·001$.

tion occurred 3–7 h after the oil challenge with a peak at 3 h. No significant increase was observed during the first 0·5 h and beyond the 7th h after oil administration. There was a similar tendency in jejunum, but this was less evident than in duodenum. No difference was observed in gastric tissue of control and castor oil-treated rats. The formation of PAF was further resolved using SPRIA (Table 2). Only very small amounts of PAF were formed by control tissue, while a significant ($P<0·05$–$0·01$) increase in PAF formation occurred in intestinal tissue 3–7 h after castor oil administration. No difference was observed in gastric tissue of control and castor oil-treated rat.

As described previously, castor oil produced macroscopic damage throughout duodenum and jejunum, which was characterized by extensive hyperaemia (Pinto et al 1989). In the present study, castor oil produced moderate damage at 1 h (damage score: duodenum 0·5±0·2, n=10, $P<0·2$; jejunum 0·4±0·2, n=8, $P<0·1$) which was more severe after 3 h (duodenum 1·4±0·3, n=10, $P<0·01$; jejunum 1·2±0·3, n=9, $P<0·01$) and 5 h (duodenum 1·3±0·3, n=8, $P<0·01$; jejunum 1·0±0·2, n=10, $P<0·01$). After 7 h, damage was still appreciable (duodenum 1·0±0·2, n=10, $P<0·05$; jejunum 0·7±0·2, n=9, $P<0·05$). No damage was observed in either duodenum and jejunum beyond the 7th h and during the first 0·5 h after castor oil challenge. The gastric mucosa of castor oil-treated rats was completely intact as in control animals.

The intraluminal release of acid phosphatase was significantly elevated in duodenum and jejunum from castor oil-treated rats but not in the stomach (Table 3). The maximum release of acid phosphatase was observed 3 h after oil

Table 2. PAF content, determined by SPRIA (scintillation proximity radioimmunoassay), in rat gastrointestinal tissue after oral administration of castor oil (2 mL/rat).

Time after castor oil (h)	PAF content (pg (g wet tissue)$^{-1}$)		
	Stomach	Duodenum	Jejunum
0 (control)	81·0±10·4	450·4±53·1	393·7±43·1
1	87·4±10·4	1530·7±37·4*	822·3±65·1
3	79·3±7·90	3630·5±100·7***	2340·4±200·4***
5	69·7±10·1	2702·6±89·9***	1950·3±98·3**
7	68·7±10·5	879·0±61·1	513·4±57·1

Results are expressed as mean±s.e. of duplicate determinations in each experiment (3–5) and analysed by Student's t-test for unpaired data; *$P<0·05$; **$P<0·01$, ***$P<0·001$.

Table 3. Intraluminal release of acid phosphatase by gastrointestinal tissue of rats after oral administration of castor oil (2 mL/rat).

Time after castor oil (h)	AP release (μg substrate transformed (g dry wt)$^{-1}$)		
	Stomach	Duodenum	Jejunum
0 (control)	127·3±7·9	871·7±171·8	801·5±113·9
0·5	131·4±10·0	1141·6±203·5	981·3±109·1
1	115·7±9·1	1441·5±194·7*	1200·5±264·1
3	124·5±8·5	2791·4±391·5**	2449·7±304·5**
5	127·3±9·4	2649·5±401·7**	2388·7±209·1**
7	130·0±9·6	1749·0±301·4*	1581·4±206·6*
9	119·3±7·9	1049·8±209·0	931·7±216·4

Results are expressed as mean±s.e. of 10 experiments and analysed by Student's t-test for unpaired data. *$P<0·05$; **$P<0·01$.

administration, and was greater in the duodenum than in the jejunum.

Discussion

The ability of non-stimulated rat intestine to produce PAF (Whittle et al 1987; Pinto et al 1989) has been confirmed in the present study. This non-stimulated production of PAF, characterized by bioassay (platelet aggregation) is, however, lower compared with the amounts found by SPRIA. Apart from these differences which, probably reflect a different experimental protocol, the SPRIA technique seems reliable for the measurement of PAF in tissue (Sugatani et al 1990). Castor oil, at a dose that elicited diarrhoea in the animals treated, stimulated PAF release from rat duodenum and jejunum but not from stomach. This stimulated formation was markedly greater in the duodenum than in jejunum, throughout the time course considered, and was greater 3–5 h after oil challenge, coincident with the most pronounced diarrhoea (Pinto et al 1989). Castor oil purgation depends on the rapid release, poor assimilation and therefore increasing concentration of free ricinoleic acid in the small intestine. As a consequence, the stimulated intestinal formation of PAF appears to be due to irritant ricinoleic acid released in the small bowel and not to castor oil as such. This is further supported by the observation that in the stomach, where castor oil is not yet hydrolysed to ricinoleic acid, there is no stimulated production of PAF.

The nature of this increase of production of PAF following castor oil administration remains, however, a matter for conjecture. PAF is an extremely active product of cell phosphatide catabolism and its production has been demonstrated in different cells (Parente & Flower 1985; Vargaftig et al 1981; Alonso et al 1982; Lee et al 1982) and tissues (Eliakim et al 1988; Rachmilewitz et al 1990) in response to various stimuli. PAF is synthesized by chondroitin sulphate E-containing mast cells as well as by several other types of cells (Mencia-Huerta et al 1983) present in intestinal mucosa (Eliakim et al 1988). There may be a contribution from infiltrated platelets, monocytes, macrophages, mast cells, eosinophil, neutrophil and basophil leucocytes as well as endothelial and smooth muscle cells.

The elevated intestinal formation of PAF following castor oil was accompanied by hyperaemia which was more severe in the duodenum than in the jejunum, confirming that this tissue was the most susceptible to castor oil-induced damage

(Pinto et al 1989). No hyperaemic areas were observed in the stomach of rats treated with castor oil; similar results were obtained with intraluminal release of acid phosphatase, an indicator of cellular damage (Wallace & Whittle 1986). Oral administration of castor oil caused extensive damage in the small intestine, as judged by acid phosphatase production, while the stomach was spared of such damage. These results, together with the evidence that PAF is formed in castor oil-induced diarrhoea suggest a role for PAF in mediating castor oil damage.

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