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Dissociation of castor oil-induced diarrhoea and intestinal mucosal injury in rat: effect of N^G-nitro-L-arginine methyl ester

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1 Castor oil (2 ml orally) produced diarrhoea in rats 1–7 h after challenge, which was associated with gross damage to the duodenal and jejunal mucosa.

2 The injury was accompanied by release of acid phosphatase into the gut lumen, indicating cellular injury.

3 Intraperitoneal injection of the nitric oxide (NO) synthase inhibitor N^G-nitro-L-arginine methyl ester (L-NAME, 2.5–50 mg kg^{-1} twice), prevented the diarrhoea. The dose of L-NAME (50 mg kg^{-1}) completely blocked the diarrhoea but increased the release of acid phosphatase and worsened the gross damage.

4 The NO donating compound, isosorbide-5-mononitrate (IMN, 150 mg kg^{-1} twice) reversed the effects of L-NAME (50 mg kg^{-1}) on castor oil-induced diarrhoea, gross damage and acid phosphatase release.

5 The apparent dissociation of the diarrhoeal and intestinal mucosal damaging effects of castor oil suggest that NO has a protective effect on the rat duodenal and jejunal mucosa, but that NO mediates, in part, the diarrhoea effect of this laxative.

Keywords: Castor oil; ricinoleic acid; nitric oxide, N^G-nitro-L-arginine methyl ester (L-NAME); isosorbide-5-monitrate (IMN); diarrhoea; laxative; duodenum; jejunum; acid phosphatase

Introduction

Castor oil (ricinoleic acid) affects electrolyte transport and smooth muscle contractility in the intestine (Gullikson & Bass, 1984; Nell & Rummel, 1984; Binder, 1977; Izzo *et al.*, 1993b; Gaginella, 1994). The precise mechanism of action of this common laxative remains elusive, partly because of its multiple effects on the gut. It inhibits intestinal Na,K-ATPase activity (Phillips *et al.*, 1965; Gaginella & Bass, 1978; Nell & Rummel, 1984), interferes with oxidative metabolism (Nakao, 1963; Gaginella *et al.*, 1975) and has effects on adenylate cyclase or mucosal adenosine 3':5'-cyclic monophosphate (cyclic AMP) content (Gaginella *et al.*, 1978; Racusen & Binder, 1979; Simon & Kather, 1980).

Castor oil is cytotoxic to intestinal epithelial cells (Valette & Salvanet, 1936; Gaginella *et al.*, 1977a; Bretagne *et al.*, 1981), and causes histological abnormalities with enhanced mucosal permeability (Cline *et al.*, 1976; Gaginella *et al.*, 1977b; Saunders *et al.*, 1977; 1978). These effects may be related to the release of eicosanoids (Beubler & Juan, 1979; Capasso *et al.*, 1986) and platelet activating factor (PAF) by the intestinal mucosa (Pinto *et al.*, 1989; 1992). Whether or not these alterations are essential for the laxative effect of castor oil or are merely epiphenomena remains to be clarified.

Nitric oxide (NO) is protective against endotoxin-induced intestinal damage in the rat (Boughton-Smith *et al.*, 1990; 1993a; Laszlo *et al.*, 1994) and is also a mediator of gastric protection (Konturek *et al.*, 1993). However, N^G-nitro-L-arginine methyl ester (L-NAME), an NO synthase inhibitor, prevents castor oil-induced diarrhoea (Mascolo *et al.*, 1992; 1993; 1994). In order to determine whether castor oil-induced diarrhoea and intestinal mucosal damage are independent events we have studied the effects of L-NAME, and the NO donating compound, isosorbide-5-mononitrate (IMN) in rats treated with castor oil.

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Methods

Male Wistar rats (Morini, Italy), 160–180 g, were used after acclimatization for a week (23 ± 2°C; 60% humidity). Food was withheld 12 h before experiments but there was free access to drinking water. Castor oil (2 ml per rat) was given orally and 1–7 h later the individual rat cages were inspected (by an observer unaware of the particular treatment) for the presence of characteristic diarrhoeal droppings; their absence indicated protection from diarrhoea. Some animals were pretreated with L-NAME (2.5–50 mg kg^{-1}, i.p. Sigma, Milan) 15 min before and 3 h after castor oil and/or IMN (150 mg kg^{-1} p.o.; Astra, Milan), 45 min before and 3 h after castor oil.

Acid phosphatase measurements

Intraluminal release of the lysosomal enzyme, acid phosphatase was determined as previously described (Pinto *et al.*, 1989; Izzo *et al.*, 1993a). Rats were killed and 8 cm segments of duodenum and jejunum were excised. The segment was rinsed with saline to remove food and mucus. A ligature was placed at one end of the segment and isotonic saline (1 ml, 0.9% w/v) was instilled into the segment by a polyethylene cannula introduced into the other end. The segment was placed in an organ bath containing Tyrode solution (37°C) gassed with 95% O_2, 5% CO_2. The tissue was incubated for 30 min. After 15 min the fluid inside the segment was carefully removed by syringe and was replaced with 1 ml of fresh saline. Immediately after the incubation period the intraluminal solution was removed and the acid phosphatase activity measured by a spectrophotometric assay.

Damage injury assessment

Rats were killed and two segments from standardized regions of duodenum and jejunum were visibly evaluated for macroscopical damage by an observer unaware of the particular treatment. Mucosal injury was graded as follow: 0 (normal), 1 (hyperaemia), 2 (hyperaemia with evidence of haemorrhage into the lumen), 3 (severe haemorrhage into the lumen).

Statistics

The Chi-Square test was used to determine the significance between groups with diarrhoea. The visual score and the intraluminal release of acid phosphatase are expressed as mean ± s.e.mean and were compared by Student's t test. A P value less than 0.05 was considered significant.

Results

Castor oil (2 ml per rat) produced diarrhoea in 40% of animals after 1 h and copious diarrhoea in all animals 3, 5 and 7 h after challenge (Figure 1). Macroscopic damage was produced throughout the duodenum and jejunum, characterized mainly by vasocongestion (Table 1,2). The injury was mild (hyperaemia) by 1 h, severe 3 and 5 h after castor oil administration and less severe 7 h after challenge (Table 1,2). No injury was observed at 0.5 h or at 9 h after castor oil administration and the tissue appeared normal by visual examination.

L-NAME dose-dependently prevented diarrhoea (Figure 1) but markedly ($P<0.01$) enhanced macroscopic injury (Table 1), causing extensive hyperaemia and haemorrhage into the small intestinal lumen. The effect of L-NAME (50 mg kg^{-1}) on castor oil-induced damage (Table 2) and diarrhoea was abolished by orally administered IMN (150 mg kg^{-1}): diarrhoea in 40% of animals after 1 h, in 80% after 3 h and in 90% after 5–7 h (c.f. Figure 1) $n=10$, $P<0.01$ at 3–7 h. IMN (150 mg kg^{-1}) given alone did not modify the diarrhoea due to castor oil (diarrhoea in 40% of animals after 1 h, in 100% of animals after 3–7 h, $n=10$). In contrast, IMN (150 mg kg^{-1}) reduced castor oil-induced damage (mean ± s.e., $n=10$, $P<0.01$ at 3–7 h; duodenum 1 h, 0.2 ± 0.1; 3 h, 0.5 ± 0.1; 5 h, 0.6 ± 0.2; 7 h, 0.3 ± 0.1; jejunum, 1 h, 0.2 ± 0.1; 3 h, 0.4 ± 0.1; 5 h, 0.4 ± 0.2; 7 h, 0.2 ± 0.1; compare to Table 1) but was devoid of any effect in rats untreated with castor oil (normal rats). L-NAME (50 mg kg^{-1}) itself caused mild mucosal congestion after 1 h (duodenum 0.4 ± 0.1; jejunum 0.4 ± 0.1).

The macroscopic changes in the mucosa indicating injury were accompanied by significant increases ($P<0.05$–0.001) in release of the acid phosphatase (Table 2). L-NAME (50 mg kg^{-1}) enhanced this effect, especially at the 3 and 5 h time points. IMN (150 mg kg^{-1}) partially negated this additive effect of L-NAME ($P<0.05$).

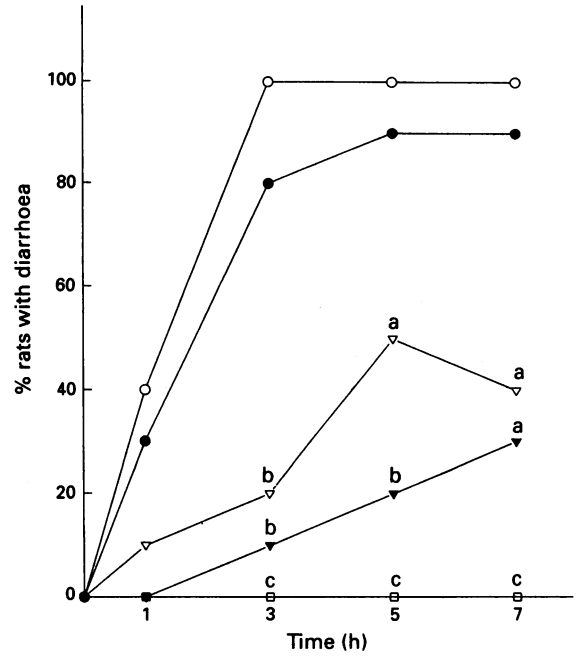

Figure 1 Effect of graded doses of N^G-nitro-L-arginine methyl ester (L-NAME) on castor oil-induced diarrhoea. Ten animals were used for each group. L-NAME was given i.p. 15 min before and 3 h after castor oil. Results were analysed by Chi-Square test. $^aP<0.05$; $^bP<0.01$; $^cP<0.001$ vs. castor oil (CO). Symbols; (○) castor oil (CO); (●) CO + L-NAME 2.5 mg kg^{-1}; (△) CO + L-NAME 10 mg kg^{-1}; (▼) CO + L-NAME 25 mg kg^{-1}; (□) CO + L-NAME 50 mg kg^{-1}.

Table 1 Effect of N^G-nitro-L-arginine methyl ester (L-NAME 2.5–50 mg kg^{-1}, i.p. twice) on macroscopic intestinal damage (scored 0–3) induced by castor oil (2 ml, p.o.).

Time (h)	*Castor oil*	*Castor oil + L-NAME* 2.5	10	25	50 mg kg^{-1}
			Duodenum		
1	0.5 ± 0.1	0.6 ± 0.2	0.7 ± 0.2	0.9 ± 0.3	1.2 ± 0.1**
3	1.4 ± 0.2	1.6 ± 0.3	1.7 ± 0.3	2.2 ± 0.3*	2.7 ± 0.3**
5	1.3 ± 0.2	1.5 ± 0.3	1.7 ± 0.3	2.0 ± 0.3*	2.5 ± 0.3**
7	1.0 ± 0.2	1.2 ± 0.2	1.3 ± 0.2	1.6 ± 0.2*	1.9 ± 0.3**
			Jejunum		
1	0.4 ± 0.1	0.5 ± 0.2	0.6 ± 0.2	0.6 ± 0.2	0.7 ± 0.1
3	1.2 ± 0.2	1.6 ± 0.3	1.9 ± 0.2	2.1 ± 0.3*	2.5 ± 0.3**
5	1.2 ± 0.2	1.4 ± 0.2	1.7 ± 0.3	2.0 ± 0.3*	2.2 ± 0.2**
7	0.7 ± 0.1	0.9 ± 0.2	1.0 ± 0.2	1.2 ± 0.2*	1.5 ± 0.2**

Results are the mean ± s.e.mean for 10 rats per experimental group.
*$P<0.05$ and $P<0.01$ vs. castor oil group

Table 2 Isosorbide 5-mononitrate (IMN 150 mg kg^{-1}, p.o. twice) reverses the effect of N^G-nitro-L-arginine methyl ester (L-NAME 50 mg kg^{-1}, i.p. twice) on castor oil (2 ml)-induced macroscopic damage (scored 0–3)

	Castor oil		*Castor oil + L-NAME*			
			No IMN	*IMN*	*No IMN*	*IMN*
Time	*Duodenum*	*Jejunum*	*Duodenum*		*Jejunum*	
1	0.5 ± 0.1	0.4 ± 0.1	1.2 ± 0.1*	0.6 ± 0.2†	0.7 ± 0.1	0.4 ± 0.1
3	1.4 ± 0.2	1.2 ± 0.2	2.7 ± 0.3*	1.3 ± 0.2†	2.5 ± 0.3*	1.1 ± 0.3†
5	1.3 ± 0.2	1.2 ± 0.2	2.5 ± 0.3*	1.2 ± 0.3†	2.2 ± 0.2*	1.0 ± 0.2†
7	1.0 ± 0.2	0.7 ± 0.1	1.9 ± 0.3*	0.9 ± 0.2†	1.5 ± 0.2*	0.7 ± 0.1†

Results are the mean ± s.e.mean for 10 rats per experimental group.
*$P<0.01$ vs. castor oil group
†$P<0.01$ vs. castor oil + L-NAME group (No IMN)

Table 3 Intraluminal release of acid phosphatase by intestinal tissue of rats treated with castor oil (2 ml/rat) and pretreated with or not with N^G-nitro-L-arginine methyl ester (L-NAME 50 mg kg^{-1} i.p., twice) and isosorbide 5-mononitrate (IMN 150 mg kg^{-1}, i.p. twice)

	Castor oil		*Castor oil + L-NAME*			
			No IMN	*IMN*	*No IMN*	*IMN*
Time	*Duodenum*	*Jejunum*	*Duodenum*		*Jejunum*	
0	724 ± 140	680 ± 113	720 ± 120	700 ± 135	685 ± 102	695 ± 136
1	1430 ± 180[a]	1118 ± 264[a]	2730 ± 201[b,d]	1620 ± 160[a,e]	2520 ± 384[b,d]	1433 ± 173[a,e]
3	2591 ± 383[b]	2480 ± 226[b]	5024 ± 403[c,d]	2980 ± 303[b,e]	4341 ± 428[c,d]	2822 ± 293[b,e]
5	2368 ± 325[b]	2124 ± 296[b]	4820 ± 413[c,d]	794 ± 93[b,e]	4003 ± 395[c,d]	774 ± 101[b,e]
7	1629 ± 290[a]	1499 ± 273[a]	3354 ± 280[c,d]	704 ± 79[a,e]	3243 ± 287[c,d]	731 ± 84[a,e]

Results [μg substrate transformed (g dry wt)$^{-1}$)] are the mean ± s.e.mean for 10 rats per experimental group.
[a]$P<0.05$, [b]$P<0.01$ and [c]$P<0.001$ vs control (time 0) [d]$P<0.01$ vs castor oil [e]$P<0.05$ vs castor oil + L-NAME (No IMN)

Discussion

Our results show that castor oil produced concurrent diarrhoea and gross evidence of mucosal damage. The latter effect was exacerbated by the NO synthase inhibitor, L-NAME, suggesting that NO serves in a protective capacity. The fact that L-NAME blocked the diarrhoeal effect of castor oil while worsening the apparent injury to the mucosa, suggests that these two events are not related in cause and effect. The augmentation of the damaging effect to the epithelium with L-NAME is supported by the enhanced release of acid phosphatase. Furthermore, the NO-donating compound IMN, prevented the deleterious action of L-NAME, reducing the injury scores and the acid phosphatase values nearly to the level of the castor oil controls (see Tables 2,3).

The effects of castor oil on fluid transport have been previously associated with, but not proved to be due to, mucosal injury. In 1936 Valette & Salvanet noted the damaging effects of ricinoleic acid on the rat intestine, followed by a report of 'chemical gastroenteritis' in rats fed castor oil (Raynell & Spray, 1958). More recent studies have demonstrated potentially detrimental metabolic (Nakao, 1963; Gaginella *et al.*, 1975) and cytotoxic effects of ricinoleic acid on intestinal cells *in vitro* (Gaginella *et al.*, 1977b) and *in vivo* (Bretagne *et al.*, 1981). Increased intestinal epithelial permeability (sometimes with cytomorphological changes) is associated with intraluminal fluid accumulation in response to ricinoleic acid (Dobbins & Binder, 1976; Cline *et al.*, 1976; Gaginella *et al.*, 1977a; Gullikson *et al.*, 1977). We have previously demonstrated that castor oil-induced mucosal hyperaemia and haemmorhage is associated with intraluminal release of acid phosphatase and PAF, two markers of cellular injury (Pinto *et al.*, 1989; 1992).

In our study, NO generated in response to the castor oil could have several actions. We believe it contributed to the diarrhoea because of the stimulatory effect of NO-donating compounds (McNaughton, 1993; Wilson *et al.*, 1993) and NO itself (Tamai & Gaginella, 1993), and the inhibitory action of L-NAME (Mascolo *et al.*, 1993; 1994) on water and electrolyte transport. This response may be related to interference with cellular metabolism, but not be due to gross mucosal damage. Tepperman *et al.* (1993) showed that induction of NO synthase is associated with decreased viability of rat enterocytes. The diphenylmethane laxatives (phenolphthalein and bisacodyl) increase the activity of inducible NO synthase (Gaginella *et al.*, 1994), increase mucosal permeability and injure the mucosa (Saunders *et al.*, 1977), but, it is not known if L-NAME worsens the mucosal injury. When the rat jejunum and colon were exposed to endotoxin, vascular permeability and leakage of plasma proteins were increased; this was reversed by inhibition of inducible NO synthase (Boughton-Smith *et al.*, 1993a). Induction of colonic nitric oxide synthase was also detected in a trinitrobenzene colitis model (Boughton-Smith *et al.*, 1993b). Other authors have likewise concluded that NO promotes mucosal injury in chronic ileitis (Miller *et al.*, 1992).

Alternatively, there is evidence for protective effects of NO on the gastric and intestinal mucosa. The healing of chronic gastric ulcers in rats is delayed by inhibition of NO synthesis (Konturek *et al.*, 1993). Furthermore, NO synthase inhibition increases intestinal permeability, probably through activation of mucosal mast cells (Kanwar *et al.*, 1994). In models of ileitis in rodents, NO may enhance the restitutive capacity of the epithelium (Miller *et al.*, 1993). The significance of the present results lies in the apparent dual action of NO in response to castor oil, illustrating that it is possible to dissociate the laxative effect from intestinal mucosal damage.

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