J. Pharm. Pharmacol. 1988, 40: 27–30
Received March 30, 1987

Involvement of Prostaglandin E-like Material in the Purgative Action of Rhein Anthrone, the Intraluminal Active Metabolite of Sennosides A and B in Mice

TERUYO YAGI, YOSHIE MIYAWAKI, TOMOYO NISHIKAWA, KAZUKO YAMAUCHI AND SHIGEAKI KUWANO

Faculty of Pharmaceutical Sciences, Mukogawa Women's University, 11–68 Koshien Ryuban-cho, Nishinomiya, Hyogo 663, Japan

Abstract—Intracaecal administration of rhein anthrone, the intraluminally active metabolite of sennosides A and B, to mice quickly induced severe diarrhoea. Pretreatment with the prostaglandin (PG) biosynthesis inhibitor, indomethacin, and PGE_2 antagonist, SC-19220, prevented the onset of diarrhoea induced by rhein anthrone, but the PGE_2 antagonist polyphoretin phosphate (PPP) showed only a weak inhibitory effect. Rhein anthrone stimulated the production of PGE-like material only in the colon and its large intestinal propulsive activity was depressed by indomethacin and SC-19220, but not by PPP which suggests that the release of PGE-like material has some role in its purgative action.

Previous work from this laboratory (Sasaki et al 1979) established that sennoside A, the active principle of senna or rhubarb, is intrinsically inactive in the glycoside form and that not rhein but rhein anthrone, which is formed from sennoside A in the large intestine mainly by bacterial action, is the intraluminal active metabolite in mice. The study also showed that rhein anthrone quickly causes severe diarrhoea when administered directly into the mouse caecum. Diarrhoea is thought to result from changes in intestinal motility and the luminal fluid content. Hardcastle & Wilkins (1970) found that rhein anthrone introduced into the human transverse colon stimulated peristalsis, and Leng-Peschlow (1986) showed that rhein anthrone administered intracaecally accelerated large intestine transit in rats. Lemmens & Borja (1976) reported that rhein anthrone inhibited colonic water and sodium absorption in rats.

On the other hand, sennosides A and B (Beubler & Juan 1979), senna pod extract (Beubler & Kollar 1985), senna tablets (Cohen 1982) and senna (Capasso et al 1986) have been shown to act in the rat colon by stimulating the biosynthesis of prostaglandin (PG) E-like material. Beubler & Kollar (1985) and Leng-Peschlow (1986) have shown that indomethacin can decrease the secretory effect of the senna pod extract and partly inhibit the acceleration of large intestine transit induced by sennosides. However, the involvement of PG formation in the purgative action of rhein anthrone, the active metabolite of sennosides A and B, has not yet been proved. We set out to find whether the purgative action of rhein anthrone in-situ is mediated by PG biosynthesis in mice.

Materials and Methods

Materials and drugs

Rhein was prepared from crude sennosides available commercially by oxidation with 15% hydrogen peroxide and then by hydrolysis with 10% hydrochloric acid. Rhein anthrone was prepared by the reduction of rhein with stannous chloride in acidic medium according to the procedure of Auterhoff & Scherff (1960). Elemental analysis and spectroscopic data for this substance agreed with the assigned structure, and the purity was ascertained by thin layer chromatography (Lemli & Cuveele 1974).

The following drugs were generous gifts: 1-acetyl-2-(8-chloro-10,11-dihydrodibenz[b,f][1,4]oxazepine-10-carbonyl)hydrazine (SC-19220, Searle Research & Development Division of G. D. Searle & Co., Skokie, USA), polyphloretin phosphate (PPP, Aktiebolaget Leo, Helsingborg, Sweden), methysergide hydrogen maleate (Sandoz Ltd, Basle, Switzerland) and (±)-propranolol hydrochloride (ICI-Pharma Ltd, Osaka, Japan). The following drugs were purchased: prostaglandin E_2 (Funakoshi Pharmaceutical Co., Ltd, Tokyo, Japan), indomethacin (Wako Pure Chemical Industries, Ltd, Osaka, Japan), phenoxybenzamine hydrochloride (Nakarai Chemicals, Ltd, Kyoto, Japan), pyrilamine maleate (Sigma Chemical Co., St. Louis, USA) and hyoscine hydrobromide (E. Merck, Darmstadt, Germany).

Rhein anthrone was dissolved in 2% sodium bicarbonate solution immediately before administration. PGE_2 was dissolved in 0·4 mL of ethanol and 0·18 mL of 0·1% sodium bicarbonate, then diluted to 5 mL with water. Indomethacin and SC-19220 were suspended in a small amount of Tween 80 and then diluted with water. All other drugs were dissolved in distilled water. Doses of salts are expressed in terms of their free bases.

Animals

Female albino mice of the Jcl : ICR strain (CLEA, Japan, Inc., Tokyo) 26–32 g, were kept at an ambient temperature of 22 to 25 °C and allowed free access to a diet of MF pellets (Oriental Yeast Co., Ltd, Tokyo, Japan) and tap water during the experiments. For bioassay of PGE-like material, male Sprague-Dawley rats (180–300 g) were purchased from CLEA, Japan, Inc.

Intracaecal cannula

An intracaecal cannula was inserted by the modified method of Ueda et al (1969). Mice were anaesthetized with

Correspondence to: S. Kuwano, Faculty of Pharmaceutical Sciences, Mukogawa Womens University, 11–68 Koshien Ryuban-cho, Nishinomiya, Hyogo 663, Japan.

sodium pentobarbitone, and, after laparotomy, the caecum was exposed and slightly incised, and a soft polyethylene tube (about 1 mm in outer diameter and about 6 cm in length) inserted about 1 cm and ligated at the site. The other end of the tube was conducted subcutaneously and drawn out about 2 cm at the back of the animal's neck. The incision was closed, and the polyethylene tube was ligated at the exit on the dorsum. On the third day after the operation, the animals were used in the experiments.

Purgative test

The mice were isolated in wire-bottomed cages raised 2·5 cm above blotting paper in a stainless steel tray. A rhein anthrone or a PGE_2 solution was injected at 5 mL kg^{-1} followed by 2 mL kg^{-1} of water to complete the injection into the caecum. The mice were observed over 6 h for diarrhoea (excretion of wet or unformed faeces with staining on the blotting paper). The purgative activity was usually expressed as the ratio (incidence of diarrhoea) of the number of diarrhoeal animals to the total number of test animals, or expressed as the mean of the time to onset of diarrhoea ± standard error of the mean. Preliminary tests showed that 5 mL kg^{-1} of 2% sodium bicarbonate aqueous solution injected into the mouse caecum had no purgative effect.

Bioassay of prostaglandin E-like material

When diarrhoea was evident, or at 30 min after administration of the rhein anthrone solution or 2% sodium bicarbonate solution as the control, the mice were killed by exposure to ether. The large intestinal tissue was removed, separated into caecum and colon, rinsed in saline containing 0·01% indomethacin and immediately immersed in 10 mL of 70% ethanol in a weighed vial. Subsequent reweighing gave the sample weight. The sample tissue was homogenized in 70% ethanol and centrifuged at 7000 g for 5 min at 5 °C. The supernatant was shaken with 20 mL of light petroleum (b.p. 40–60 °C), acidified to approximately pH 3·0 with 2% formic acid, and extracted twice with 10 mL of chloroform. The chloroform extract was washed with 20 mL of water, dried over anhydrous sodium sulphate and evaporated to dryness under nitrogen. The residue was dissolved in Krebs-Henseleit solution and bioassayed against PGE_2 on rat gastric fundus strips (Vane 1957) in the presence of hyoscine, phenoxybenzamine, pyrilamine (each 0·1 μg mL^{-1}), propranolol (2 μg mL^{-1}) and methysergide (10 ng mL^{-1}) according to the modified method of Yagasaki et al (1980). The amounts of PGE-like material were expressed as PGE_2 equivalents.

Large intestine propulsion test

This test measured the time to the first excretion of white faeces containing barium sulphate after injection of each animal with 0·1 mL of 20% barium sulphate aqueous suspension through the intracaecal cannula. Also, the incidence of barium sulphate excretion was expressed as the ratio of the number of animals which excreted barium sulphate within 6 h to the total number of test animals.

Statistical analysis

The mean values were subjected to statistical evaluation using Student's *t*-test; $P < 0.05$ was considered significant.

Results

As shown in Table 1, intracaecal administration of rhein anthrone at doses above 6·24 mg kg^{-1} corresponding to 10 mg kg^{-1} of sennosides A and B caused severe diarrhoea in most of the mice within 30 min of treatment. In comparative experiments, an intracaecal dose of 0·2 mg kg^{-1} PGE_2 also caused diarrhoea of similar severity to that induced by 6·24 mg kg^{-1} rhein anthrone.

Table 1. Purgative activity of rhein anthrone administered intracaecally. Occurrence of diarrhoea was observed for 6 h after rhein anthrone administration. Incidence of diarrhoea was expressed as the ratio of the number of diarrhoeal animals to the total number of test animals.

Dose (mg kg^{-1})	Incidence of diarrhoea	Time to onset of diarrhoea (min) mean ± s.e.m.
1·56	0/9	—
3·12	4/10	32·6 ± 6·1
6·24	9/10	19·6 ± 4·1
12·48	9/9	12·2 ± 1·3

Effects of indomethacin and prostaglandin antagonists on rhein anthrone-induced diarrhoea

Indomethacin administered by various routes before agent administration markedly inhibited rhein anthrone-induced diarrhoea, but not that caused by PGE_2 administered intracaecally. The effects of the two PG antagonists on the rhein anthrone-induced diarrhoea were compared with their effects on intracaecal PGE_2-induced diarrhoea. In general, SC-19220 interfered strongly with the onset of diarrhoea caused by both rhein anthrone and PGE_2. In contrast, PPP only showed weak inhibitory effects and delayed the onset of diarrhoea only when administered orally 1 h or intraperitoneally 30 min before rhein anthrone (Table 2).

Effect of rhein anthrone on the production of prostaglandin E-like material in the mouse large intestine

Table 3 shows that rhein anthrone administered intracaecally significantly increased the production of PGE-like material in the colon but insignificantly in the caecum. As anticipated, pretreatment with indomethacin greatly reduced the formation of PGE-like material induced by rhein anthrone in the colon. Indomethacin given alone to control mice decreased the levels of PGE-like material in all large intestinal regions (Table 3).

Effects of indomethacin, SC-19220 and PPP on the large intestinal propulsive activity of rhein anthrone

When barium sulphate aqueous suspension was injected into the caecum through the cannula immediately after the

Table 2. Effects of indomethacin, SC-19220 and PPP on diarrhoea induced by rhein anthrone or PGE_2.

		Rhein anthrone		PGE_2	
Pretreatment	Route	Incidence of diarrhoea	Time to onset of diarrhoea (min) mean ± s.e.m.	Incidence of diarrhoea	Time to onset of diarrhoea (min) mean ± s.em.
None		9/10	21·1 ± 2·6	8/9	19·6 ± 4·1
Indomethacin	p.o.	1/10	134	8/10	44·4 ± 15·5
	i.p.	0/10	—	7/10	21·7 ± 6·4
	i.c.	4/10	20·5 ± 3·6	10/10	26·5 ± 6·5
SC-19220	p.o.	0/10	—	0/10	—
	i.p.	1/10	11	1/10	40
	i.c.	5/10	27·8 ± 5·5	8/10	18·3 ± 7·3
PPP	p.o.	8/10	73·3* ± 24·2	9/10	16·4 ± 1·7
	i.p.	6/10	37·7** ± 3·4	8/10	24·0 ± 6·5
	i.c.	6/9	20·8 ± 6·9	10/10	18·1 ± 5·6

Rhein anthrone (6·24 mg kg^{-1}) or PGE_2 (0·2 mg kg^{-1}) was administered intracaecally (i.c.).

Indomethacin (3 mg kg^{-1}) was administered 90 min (p.o.), 15 min (i.p.) or 10 min (i.c.) before rhein anthrone or prostaglandin E_2 administration.

SC-19220 (100 mg kg^{-1}) was administered 30 min (p.o.), 15 min (i.p.) or 10 min (i.c.) before rhein anthrone or prostaglandin E_2 administration.

PPP (100 mg kg^{-1}) was administered 60 min (p.o.), 30 min (i.p.) or 10 min (i.c.) before rhein anthrone or prostaglandin E_2 administration.

*$P < 0.05$ and **$P < 0.01$ compared with treatment with rhein anthrone alone.

Table 3. Prostaglandin E-like material recovered from mouse large intestine by the administration of rhein anthrone alone or after pretreatment with indomethacin.

	PGE-like material (ng PGE_2 equivalent g^{-1} tissue)			
	Control (2% $NaHCO_3$)		Rhein anthrone	
	Caecum	Colon	Caecum	Colon
Without indomethacin	65·9 ± 7·4	41·3 ± 5·3	88·6 ± 8·2	90·2* ± 10·8
With indomethacin	37·7** ± 3·3	20·2** ± 2·7	63·3*** ± 6·6	26·8**** ± 1·9

Rhein anthrone (6·24 mg kg^{-1}) or 2% $NaHCO_3$ solution was administered intracaecally.

Indomethacin (3·0 mg kg^{-1}) was given orally 90 min before rhein anthrone or 2% $NaHCO_3$ soln administration.

Each value is the mean ± s.e.m. of 9 experiments.

*$P < 0.01$ compared with the control group.

**$P < 0.01$ compared with the control group not pretreated with indomethacin.

***$P < 0.05$ and ****$P < 0.001$ compared with the rhein anthrone group not pretreated with indomethacin.

administration of rhein anthrone or 2% sodium bicarbonate solution, rhein anthrone accelerated the excretion through the large intestine. Indomethacin and SC-19220 pre-administered orally prevented not only normal excretion but also suppressed the excretion stimulated by rhein anthrone. In contrast, PPP alone had no significant effect on normal and stimulated large intestinal propulsion (Table 4).

Discussion

A previous study (Sasaki et al 1979) showed that among sennoside A and its intraluminal metabolites, rhein anthrone produced diarrhoea most rapidly and potently, but oxidized rhein had only weak purgative effect when

Table 4. Effects of indomethacin, SC-19220 and PPP on normal and rhein anthrone-induced stimulatory excretions of barium sulphate administered intracaecally to mouse.

Administration				Student's *t*-test		
First	Second	Incidence of $BaSO_4$ excretion	Time (min) onset of excretion of $BaSO_4$ mean ± s.e.m.			
—	2% $NaHCO_3$	9/10	73·3 ± 13·2	control	control	
—	Rhein anthrone	9/9	20·8 ± 2·1	$P<0.01$		control
Indomethacin	2% $NaHCO_3$	9/10	145·9 ± 5·4	control	$P<0.001$	
Indomethacin	Rhein anthrone	5/10	109·6 ± 10·5	$P<0.01$		$P<0.001$
SC-19220	2% $NaHCO_3$	3/10	313·0 ± 10·0	control	$P<0.001$	
SC-19220	Rhein anthrone	4/10	266·0 ± 70·2	n.s.		$P<0.001$
PPP	2% $NaHCO_3$	10/10	67·8 ± 20·2	control	n.s.	
PPP	Rhein anthrone	10/10	36·1 ± 9·4	n.s.		n.s.

Rhein anthrone (6·24 mg kg^{-1}) or 2% $NaHCO_3$ solution was administered intracaecally.

A 20% $BaSO_4$ aqueous suspension, 0·1 mL, was injected into the caecum immediately after rhein anthrone or 2% $NaHCO_3$ solution administration.

Incidence of $BaSO_4$ excretion was expressed as the ratio of the number of animals which had excreted $BaSO_4$ within 6 h to the total number of test animals.

Indomethacin (3 mg kg^{-1}), SC-19220 (100 mg kg^{-1}) or PPP (100 mg kg^{-1}) was given orally 90 min, 30 min or 60 min respectively, before rhein anthrone or 2% $NaHCO_3$ solution administration.

n.s., not significant.

administered directly into the mouse caecum. Also, examination of the intestines after oral administration of sennoside A revealed a larger amount of rhein anthrone in the large intestine at the action site of sennoside A. Pretreatment of mice with chloramphenicol suppressed the purgative effect of sennoside A and concurrently reduced the formation of rhein anthrone in the large intestine. These observations led to the conclusion that rhein anthrone, which is formed mainly by intraluminal bacterial action, is the active metabolite of sennoside A in mice. Dreessen et al (1981) also reported that, in rats, rhein anthrone was the active metabolite of sennosides A and B, and that rhein was an artifact and less important. Earlier studies from their laboratory (Lemmens & Borja 1976; Lemmens 1979) had suggested that rhein and rhein anthrone were equally the most active compounds metabolized from sennosides A and B partly by the action of bacterial enzymes. The mode of purgation by rhein anthrone in-situ should be explored further to elucidate the purgative mechanism of sennosides A and B administered orally.

Beubler & Juan (1979), Beubler & Kollar (1985), Cohen (1982) and Capasso et al (1986) have emphasized the possibility that sennosides A and B and senna preparations act by stimulating the biosynthesis of PGE-like material. Capasso et al (1983, 1986) and Autore et al (1984) also reported that other purgative drugs such as aloin, castor oil and phenolphthalein stimulated the formation of PGE-like material in the rat colon. Since indomethacin is known as an inhibitor of PG biosynthesis, the findings that pretreatment with it suppresses rhein anthrone-induced diarrhoea but does not affect PGE_2-induced diarrhoea strongly suggest that rhein anthrone action is mediated by prostaglandin biosynthesis and release. Further support for this hypothesis may come from a series of experiments using PG antagonists. SC-19220 (Sanner 1969; Bennett & Posner 1971) and PPP (Eakins et al 1970; Bennett & Posner 1971) antagonize PGE_2 stimulation of smooth muscle preparations in-vitro. Capasso et al (1984) recently showed that PPP given 1 h before phenolphthalein prevented the purgative effect in mice in the same manner as indomethacin given for 3 days. Our present results show that pretreatment with SC-19220 exerts a strong antidiarrhoeal effect except with intracaecal pretreatment, while PPP has little effect. Since the reason for the marked difference between the effects of these antagonists is not known, studies should be done to assay the PGE-like material released in the large intestine during the purgation by rhein anthrone in mice.

Our present findings showed that rhein anthrone injected into the caecum significantly stimulated the production of PGE-like material only in the colon, and this stimulating effect could be reduced by pretreatment with indomethacin. On the other hand, as expected, PGE_2 administered intracaecally caused severe diarrhoea, though the doses needed were much larger than those of the endogeneously released PGE-like material that were needed to reach the threshold, because presumably the PGE_2 was degraded by the caecal microflora. These findings clearly indicate that the purgative action of rhein anthrone is mediated by the biosynthesis and release of the PGE-like material in the mouse colon.

Although diarrhoea may involve stimulated intestinal transit and/or secretion, our present study on the property of rhein anthrone action was confined to examining intestinal transit because of the difficulties of handling the small volume of fluid from the large intestine of mice. Rhein anthrone showed potent propulsive activity in the mouse large intestine. The finding that indomethacin and SC-19220, but not PPP, depressed the stimulated propulsion by rhein anthrone in a similar manner as purgation also suggests that PGs play some role in the enhanced propulsive effect of rhein anthrone.

Acknowledgements

We would like to express our gratitude to Professor O. Yagasaki of the Department of Veterinary Pharmacology, College of Agriculture, University of Osaka Prefecture, Sakai, Japan, for his helpful advice on the bioassay of PGE-like material. We also thank Mrs K. Hori for her technical assistance.

References

Auterhoff, H., Scherff, F. C. (1960) Arch. Pharm. 293/65: 918–925

Autore, G., Capasso, F., Mascolo, N. (1984) Br. J. Pharmacol. 81: 347–349

Bennett, A., Posner, J. (1971) Ibid. 42: 584–594

Beubler, E., Juan, H. (1979) J. Pharm. Pharmacol. 31: 681–685

Beubler, E., Kollar, G. (1985) Ibid. 37: 248–251

Capasso, F., Mascolo, N., Autore, G., Duraccio, M. R. (1983) Prostaglandins 26: 557–562

Capasso, F., Mascolo, N., Autore, G., Duraccio, M. R. (1984) J. Pharm. Pharmacol. 36: 132–133

Capasso, F., Mascolo, N., Autore, G., Romano, V. (1986) Ibid. 38: 627–629

Cohen, M. M. (1982) Prostaglandins Leukotrienes Med. 8: 389–397

Dreessen, M., Eyssen, H., Lemli, J. (1981) J. Pharm. Pharmacol. 33: 679–681

Eakins, K. E., Karim, S. M. M., Miller, J. D. (1970) Br. J. Pharmacol. 39: 556–563

Hardcastle, J. D., Wilkins, J. L. (1970) Gut 11: 1038–1042

Lemli, J., Cuveele, J. (1974) Planta Med. 26: 193–195

Lemmens, L. (1979) Pharm. Weekbl. Sci. Ed. 1: 2–9

Lemmens, L., Borja, E. (1976) J. Pharm. Pharmacol. 28: 498–501

Leng-Peschlow, E. (1986) Ibid. 38: 369–373

Sanner, J. H. (1969) Arch. Int. Pharmacodyn. Ther. 180: 46–56

Sasaki, K., Yamauchi, K., Kuwano, S. (1979) Planta Med. 37: 370–378

Ueda, M., Matsuda, S., Kawakami, M., Minesita, T., Takeda, H. (1969) Ôyôyakuri 3: 265-269

Vane, J. R. (1957) Br. J. Pharmacol. 12: 344–349

Yagasaki O., Takai, M., Yanagiya, I. (1980) Jap. J. Pharmacol. 30: 853–860