J. Pharm. Pharmacol. 1997, 49: 22–25
Received February 26, 1995
Accepted April 18, 1996

The Synergistic Purgative Action of Aloe-emodin Anthrone and Rhein Anthrone in Mice: Synergism in Large Intestinal Propulsion and Water Secretion

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Abstract

This study aimed to explore the mechanism involved in the synergistic purgative action of aloe-emodin anthrone and rhein anthrone, the active metabolites of sennoside C.

Aloe-emodin anthrone and rhein anthrone, and their equimolar mixture, induced excretion of an approximately equal number of faeces by intracaecal administration at a dose of 23·2 μmol kg^{-1} in mice (= 1·0 standard dose). The number of wet faeces induced by aloe-emodin anthrone was less than those of rhein anthrone and the mixture. At the same dose, rhein anthrone and the mixture significantly stimulated large intestinal propulsion, though aloe-emodin anthrone had little stimulatory effect. Aloe-emodin anthrone and rhein anthrone decreased net water absorption but could not reverse it to the net secretion at 1/2 dose. The mixture significantly decreased net water absorption and reversed it to the net secretion at this dose. These anthrones did not stimulate mucus secretion in the colon at 1/2 dose.

We concluded that the synergistic purgative effect of aloe-emodin anthrone and rhein anthrone in mice results from synergistic stimulation of large intestinal transit and large intestinal water secretion.

Sennoside C (aloe emodin-rhein dianthrone diglucoside) is one of the purgative constituents of senna or rhubarb which is metabolized into its active metabolites mainly by intraluminal bacterial action (Yamauchi et al 1992). One mole of the glucoside is metabolized to give 1 mol each of aloe-emodin anthrone and rhein anthrone. From study of the purgative activities of 1,8-dihydroxy anthracene derivatives, Fairbairn & Moss (1970) showed that the relative purgative activities of the aloe-emodin series are less than those of the rhein series. We also found aloe-emodin dianthrone diglucoside to be less active in mice than sennoside A (rhein dianthrone diglucoside) (Nakajima et al 1985). Sennoside C was expected to be less active than sennoside A because of its partial existence as the aloe-emodin-based form. However, orally and intracaecally administered sennoside C has the same purgative activity in mice as sennoside A (Oshio et al 1972; Kisa et al 1981).

We have been interested in the reason that the purgative activity of sennoside C equals that of sennoside A. Our research has shown that aloe-emodin anthrone and rhein anthrone synergistically exert their purgative effects on mice (Yamauchi et al 1992). The present study was undertaken to elucidate the mechanism involved in this synergism with regard to large intestinal propulsion and colonic fluid transport in mice.

Materials and Methods

Materials and chemicals

Aloe-emodin anthrone was prepared from barbaloin by the procedure of Hay & Haynes (1956) and rhein anthrone was prepared as described previously (Yagi et al 1988). Both anthrones were suspended in 1% Tween 80 (Sigma) aqueous solution immediately before administration (5 mL kg^{-1}), and an equimolar mixture of both anthrones was prepared by mixing equal volumes of both anthrone suspensions.

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Animals

Female albino mice of Jcl: ICR strain (CLEA Japan Inc., Tokyo, Japan), 22–32 g, were kept at an ambient temperature of 22–25°C and allowed free access to a diet of MF pellets (Oriental Yeast Co. Ltd. Tokyo, Japan) and tap water.

Intracaecal cannulation

The operation was carried out as described in a previous work (Yagi et al 1988). A soft polyethylene tube was inserted about 1 cm into a caecum and ligated at the site. The other end of the tube was drawn out of the back of the animal's neck. The animals were used in the experiments on the third day after the operation.

Purgative test

The purgative test was carried out as described in a previous work (Yamauchi et al 1993).

Measurement of large intestinal transit

The large intestinal transit was measured as described previously (Yagi et al 1991) with slight modification. The time to the first excretion of the colour maker was measured.

Net water flux and electrolyte transport in the colon

Net water and electrolyte transport were estimated as described previously (Yagi et al 1990). Net water and electrolyte transport were expressed mL (g tissue)$^{-1}$ and μmol (g tissue)$^{-1}$, respectively. A negative value denoted net absorption and a positive value gave net secretion.

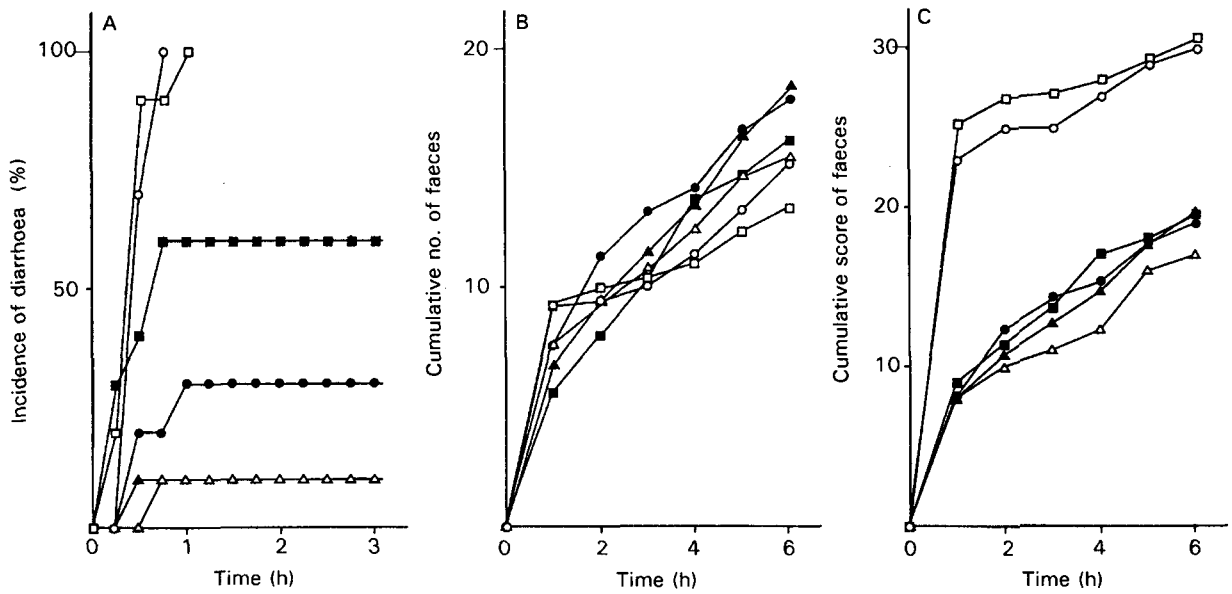

FIG. 1. Time course of purgative activities of aloe-emodin anthrone, rhein anthrone and their equimolar mixture administered intracaecally to mice at 1·0 dose (23·2 μmol kg^{-1}). A: incidence of diarrhoea, expressed as the percentage of the number of diarrhoeal animals to the total number of test animals. B: cumulative number of faeces; C: cumulative score of faeces. The cumulative number of faeces and cumulative score were the mean value of 10 mice per group. Diarrhoea was scored as follows: 1 = normal faeces, 2 = moist faeces with faint staining on the under surface of blotting paper, 3 = soft faeces with staining on the blotting paper, 4 = shapeless sludge faeces, 5 = shapeless mucoid faeces. The cumulative score was expressed as the product of score and the number of faeces per mouse. Aloe-emodin anthrone at standard dose 1·0 (△) or 1/2 dose (▲); rhein anthrone at 1·0 dose (○) or 1/2 dose (●); aloe-emodin anthrone + rhein anthrone (1:1) at 1·0 dose (□) or 1/2 dose (■).

Mucus secretion

Colonic mucus secretion was estimated by measuring the output of total protein-bound hexose (TPBH) in the colonic fluid, and expressed as mg TPBH (g tissue)$^{-1}$, according to the method of Yagi et al (1990).

Statistical evaluation

The results were expressed as mean ± s.e.m. Statistical significance was assessed using Student's *t*-test.

Results

Purgative activities of aloe-emodin anthrone, rhein anthrone and their equimolar mixture

Aloe-emodin anthrone, rhein anthrone and their equimolar mixture were directly injected into the caecum at a dose of 23·2 μmol kg^{-1} which corresponded to 10 mg kg^{-1} (11·6 μmol kg^{-1}) of sennoside A, and was assigned as the standard dose (1·0). The purgative action was observed as the incidence of diarrhoea and the number and score of faeces. As shown in Fig. 1, at the standard dose of rhein anthrone or the equimolar mixture (half standard dose of aloe-emodin anthrone plus half standard dose of rhein anthrone) caused severe diarrhoea in almost all animals within 30 min after intracaecal administration. Aloe-emodin anthrone was far less active at the equimolar dose and caused diarrhoea in only 10% of the test animals. Thus its score was lower than those of rhein anthrone and the equimolar mixture. Although aloe-emodin anthrone, rhein anthrone and the equimolar mixture induced an approximately equal number of faeces, the cumulative score of rhein anthrone and the equimolar mixture which caused severe diarrhoea were higher than that of aloe-emodin anthrone (Fig. 1).

Effects of aloe-emodin anthrone, rhein anthrone and their equimolar mixture on large intestinal propulsion

Aloe-emodin anthrone did not stimulate large intestinal transit in the dose range of 1/8 to 1·0. Rhein anthrone and the equimolar mixture significantly stimulated large intestinal transit at doses above 1/2 and 1/8 respectively (Table 1).

Effects of aloe-emodin anthrone, rhein anthrone and their equimolar mixture on net water flux and electrolyte transport in the colon

Aloe-emodin anthrone and rhein anthrone decreased net water absorption compared with that in control mice, but they could not reverse net water absorption to become net secretion. Their equimolar mixture significantly decreased net water absorption and reversed net water absorption into net water secretion at 1/2 dose (Table 2).

Aloe-emodin anthrone did not influence net Na^+ and K^+ transport. Rhein anthrone tended to decrease net Na^+ absorption at 1/2 dose. The equimolar mixture decreased net Na^+ absorption significantly and increased net K^+ secretion insignificantly at 1/2 dose (Table 3).

Mucus secretion in the colon

As shown in Table 3, aloe-emodin anthrone, rhein anthrone and the equimolar mixture tended to stimulate mucus secretion at 1/8 and 1/2 doses.

Discussion

In an earlier study, we found sennoside C, like sennoside A, was metabolized into aloe-emodin anthrone and rhein anthrone in the mouse large intestine. One mole of sennoside C releases 1 mol each of aloe-emodin anthrone and rhein

Table 1. Effects of aloe-emodin anthrone, rhein anthrone and their equimolar mixture on large intestinal transit in mice.

	Standard dose	Transit time(min)	n
Control		42·3 ± 7·1	9
Aloe-emodin anthrone	1/8	37·6 ± 5·4	10
	1/2	35·7 ± 5·6	10
	1·0	34·9 ± 12·4	9
Rhein anthrone	1/8	33·9 ± 4·4	9
	1/2	19·3 ± 3·1*	10
	1·0	20·7 ± 2·1*	10
Aloe-emodin anthrone + rhein anthrone(1:1)	1/8	18·7 ± 4·3*†	10
	1/2	20·1 ± 2·2*	9
	1·0	13·3 ± 2·0*	9

Standard dose $1{\cdot}0 = 23{\cdot}2\ \mu mol\ kg^{-1}$. All anthrones were administered intracaecally. Carmine red solution, 0·1 mL, was injected into the caecum immediately after anthrone. Transit time was expressed as the time to the first excretion of the colour marker. Values are mean ± s.e.m. $*P < 0{\cdot}01$ compared with control; $\dagger P < 0{\cdot}05$ compared with 1/8 dose of rhein anthrone.

Table 2. Effects of aloe-emodin anthrone, rhein anthrone and their equimolar mixture on net water flux in mouse ligated colon.

	Standard dose	Net water flux (mL (g tissue)$^{-1}$)	n
Control		−0·091 ± 0·072	15
Aloe-emodin anthrone	1/8	−0·002 ± 0·045	11
	1/2	−0·076 ± 0·109	13
Rhein anthrone	1/8	−0·076 ± 0·109	12
	1/2	−0·004 ± 0·065	14
Aloe-emodin anthrone + rhein anthrone (1:1)	1/8	0·065 ± 0·081	11
	1/2	0·115 ± 0·046*	14

Standard dose $1{\cdot}0 = 23{\cdot}2\ \mu mol\ kg^{-1}$. Values are mean ± s.e.m.; negative value denotes net absorption and a positive value gives net secretion. $*P < 0{\cdot}05$ compared with control.

anthrone; 1 mol of sennoside A releases 2 mol of rhein anthrone. Aloe-emodin anthrone has far less potent purgative activity ($ED50 = 54{\cdot}5\ \mu mol\ kg^{-1}$) than rhein anthrone ($ED50 = 11{\cdot}4\ \mu mol\ kg^{-1}$), however the equimolar mixture of aloe-emodin anthrone and rhein anthrone has an intracaecal ED50 ($11{\cdot}2\ \mu mol\ kg^{-1}$) equal to rhein anthrone (Yamauchi et al 1992).

The present study showed that an approximately equal number of faeces was excreted by intracaecal administration of aloe-emodin anthrone, rhein anthrone and the equimolar mixture at the dose of $23{\cdot}2\ \mu mol\ kg^{-1}$ (= standard dose 1·0). However, the number of wet faeces induced by aloe-emodin anthrone was lower than that induced by rhein anthrone and the mixture. Thus, the cumulative score of faeces for aloe-emodin anthrone was less than that of rhein anthrone and the mixture. This shows that aloe-emodin anthrone and rhein anthrone synergistically increased the excretion of wet faeces.

At the same standard dose (1·0), rhein anthrone and the mixture significantly stimulated large intestinal propulsion, though aloe-emodin anthrone had little stimulatory effect. At a lower dose (1/8), the mixture showed a significant stimulating effect on large intestinal propulsion, but aloe-emodin anthrone and rhein anthrone did not. These results suggest that both anthrones also synergistically enhance large intestinal propulsive effects.

As for net fluid transfer, aloe-emodin anthrone and rhein anthrone separately decreased net water absorption but could not reverse it to become net secretion at 1/2 dose. On the other hand, the mixture significantly decreased net water absorption and reversed it to the net secretion at 1/2 dose. Rhein anthrone could not reverse it to net secretion even at 1·0 dose (Yagi et al 1990). These results suggests that aloe-emodin anthrone synergistically enhances the water accumulative effects of rhein anthrone. Beubler & Kollar (1985) have suggested that senna exerts its purgative action at least partially via stimulation of colonic fluid and electrolyte secretion. Here we did not find these anthrones to have any significant effects on the Na^+ and K^+ transport corresponding to their effects on fluid transfer, except for Na^+ transport at 1/2 dose of the mixture.

We also found that the mucoid diarrhoea induced by rhein anthrone in mice results mainly from PGE_2-mediated colonic

Table 3. Effects of aloe-emodin anthrone, rhein anthrone and their equimolar mixture on electrolyte transport and mucus secretion in mouse ligated colon.

	Standard dose	Electrolyte transport (μmol (g tissue)$^{-1}$)		Mucus secretion (mg TPBH (g tissue)$^{-1}$)	n
		Na^+	K^+		
Control		-55.48 ± 10.12	11.30 ± 1.44	0.83 ± 0.06	15
Aloe-emodin anthrone	1/8	-49.27 ± 9.13	8.96 ± 0.97	0.93 ± 0.09	11
	1/2	-58.94 ± 8.60	10.76 ± 0.60	0.99 ± 0.07	13
Rhein anthrone	1/8	-48.90 ± 8.34	9.31 ± 0.68	1.06 ± 0.08	12
	1/2	-27.23 ± 12.63	10.95 ± 0.90	1.09 ± 0.08	14
Aloe-emodin anthrone + rhein anthrone (1:1)	1/8	-16.87 ± 13.55	12.68 ± 1.34	1.02 ± 0.10	11
	1/2	-15.31 ± 10.76*	13.36 ± 0.80	1.02 ± 0.05	14

Standard dose $1.0 = 23.2\ \mu mol\ kg^{-1}$. Values are mean $\pm$ s.e.m. *$P < 0.05$ compared with control.

mucus synthesis and secretion (Yagi et al 1990). In the present study, these anthrones did not stimulate mucus secretion in the colon at doses lower than 1/2 dose, though rhein anthrone stimulated mucus secretion in the colon at 1·0 dose (Yagi et al 1990). It seems that aloe-emodin anthrone and rhein anthrone do not have a synergistic effect on colonic mucus secretion. Anthrones may stimulate colonic mucus secretion only at high doses.

Leng-Peschlow (1989, 1993) studied the correlation between the laxative effects of sennosides and their acceleration effects on colonic transit or net fluid secretion. They clearly showed that the excretion of soft faeces closely followed the time course of transit acceleration and was less linked to net water transport. In our study, as the colonic fluid transport was examined with a ligated colon system over a 60-min interval, our data do not reflect the net amounts of colonic fluid at the moment the diarrhoea occurred. Consequently, we can not definitely state whether or not the secretory effect leads mainly to a laxative effect. However, the mixture did significantly stimulate both large intestinal propulsion and colonic net water secretion.

We concluded that the synergistic purgative effect of aloe-emodin anthrone and rhein anthrone in mice results from synergistic stimulation of large intestinal transit and large intestinal water secretion.

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