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Metabolic Activation of Sennoside C in Mice: Synergistic Action of Anthrones

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Abstract—Sennosides A and C directly injected into the caecum of mice showed equal purgative activity. Intracaecal administration reduced time to onset of diarrhoea induced by sennoside C from about 3 h after oral administration to about 24 min. At 2·3 h after oral administration of sennoside C, nearly equimolar amounts of aloe-emodin anthrone and rhein anthrone were detected in the large intestine of mice. The purgative effect of oral sennoside C could be reduced by pretreating mice with chloramphenicol. This was observed as a decreased formation of total anthrones in the large intestine. Both anthrones and an equimolar mixture of both anthrones directly injected into the caecum exerted a purgative effect, although the activity was lower for aloe-emodin anthrone. The intracaecal ED50 values were 54·5 (24·1–89·6), 11·4 (5·0–15·7) and 11·2 (6·1–14·6) μmol kg^{-1} for aloe-emodin anthrone, rhein anthrone and an equimolar mixture of both anthrones, respectively. We concluded that aloe-emodin anthrone and rhein anthrone, formed mainly by intraluminal bacterial action, are the true active metabolites of sennoside C in mice and that both anthrones synergistically exert their purgative effects on mice.

Sennoside C, aloe-emodin-rhein dianthrone diglucoside, is a minor constituent of senna or rhubarb, which has the same purgative activity in mice as sennoside A (rhein dianthrone diglucoside), a major constituent of these preparations (Oshio et al 1972; Kisa et al 1981). We have established that sennoside A is intrinsically inactive in the glycoside form in mice and that the ultimate intraluminal active metabolite is rhein anthrone, which is liberated in the large intestine by bacterial action from the glucoside (Sasaki et al 1979).

Sennoside C has a dianthrone diglucoside structure like sennoside A and thus is expected to exert its purgative action after being metabolized into the anthrone forms, namely aloe-emodin anthrone and rhein anthrone. Study of the purgative activities of several 1,8-dihydroxy anthracene derivatives in mice by Fairbairn & Moss (1970) has shown that the relative purgative activities of the aloe-emodin series are much less than those of the rhein series. We also reported that aloe-emodin dianthrone diglucoside was less active in mice than sennoside A (Nakajima et al 1985). Therefore, sennoside C which is assumed to release 1 mol each of aloe-emodin anthrone and rhein anthrone from 1 mol of the glucoside, should be less active than sennoside A which releases 2 mol of rhein anthrone. However, both sennosides have the same purgative activity in mice.

The present study was undertaken to confirm the metabolic activation of sennoside C and to elucidate the reason for its purgative activity equalling that of sennoside A in spite of its partial existence as the aloe-emodin-based form.

Materials and Methods

Materials and chemicals

Sennosides A and C were isolated from senna leaves by the method of Miyamoto et al (1968) and further purified by silica gel column partition chromatography according to the method of Schmid & Angliker (1965). Sennoside C was additionally purified by preparative TLC on silica gel with n-propyl alcohol–ethyl acetate–water (4:4:3) as the solvent for development and 70% methanol as the extractant. Aloe-emodin anthrone was prepared from barbaloin by the procedure of Hay & Haynes (1956) and rhein anthrone from rhein as described by Yagi et al (1988).

Both sennosides were dissolved in 2% sodium bicarbonate solution and both anthrones were suspended in 1% Tween 80 (Sigma) aqueous solution immediately before administration. Chloramphenicol (Sankyo Co. Ltd, Tokyo, Japan) was suspended in 3% gum arabic solution and given orally five times over three days at a dose of 100 mg kg^{-1} each time. *p*-Nitrosodimethylaniline was synthesized by the method of Bennett & Bell (1943), purified by the method of Struyf & Verhaeren (1975) and used as 0·1% pyridine solution.

Animals

Female albino mice of Jcl:ICR strain (CLEA Japan Inc., Tokyo, Japan), 22–37 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 during the experiments.

Intracaecal cannula

The operation was carried out as described in the previous work (Yagi et al 1988). The animals were used in the experiments on the third day after the operation.

Purgative test

The mice were isolated in wire-bottomed cages raised 2·5 cm above blotting paper in a stainless-steel tray. The test solutions were administered orally at 10 mL kg^{-1} or injected through an intracaecal cannula 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 8 h for diarrhoea (excretion of wet or shapeless faeces with staining on the

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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 ±s.e.m. Diarrhoea was scored according to the method of Yagi et al (1991) as follows: 0 = no faeces or normal faeces; 1 = moist faeces with faint staining on the under surface of blotting paper; 2 = soft faeces with staining on the blotting paper; 3 = shapeless sludged faeces; 4 = shapeless mucoid faeces. To express relative purgative potency, the 50% purgative dose (ED50) was determined by the Probit method. Preliminary tests showed that 5 mL kg^{-1} of 2% sodium bicarbonate aqueous solution or 1% Tween 80 aqueous solution injected into the mouse caecum had no purgative effect.

The dose unit, μmol kg^{-1}, was adopted to compare the activities of the compounds.

Detection and determination of anthrones

Aloe-emodin anthrone and rhein anthrone formed in the intestine were detected as anthrone-*p*-nitrosodimethylaniline condensation products with TLC by the method of Sasaki et al (1979).

Individual anthrone was determined by means of HPLC. The chloroform extract of anthrone-*p*-nitrosodimethylaniline condensation products prepared by the method of Sasaki et al (1979) was washed with water, dried over anhydrous sodium sulphate and evaporated to dryness under reduced pressure. The residue was dissolved in pyridine and a sample was injected onto the HPLC column. HPLC was performed using a Shimadzu LC-3A, a stainless-steel column (150 × 4·6 mm i.d.) packed with Zorbax ODS and a UV spectrophotometric detector, Shimadzu SPD-1 (at 640 nm). The peak area was counted using a data processor, Shimadzu Chromatopac C-R1A. A mixture of acetonitrile, methanol and 0·5% triethylamine-phosphate buffer solution (pH 3·5) (70:60:25) was used as the mobile phase and the flow rate was 0·6 mL min^{-1} at room temperature (21°C).

The amount of total anthrones was determined spectrophotometrically as the amount of rhein anthrone by the method of Sasaki et al (1979).

The dose unit, μmol/mouse, was adopted to estimate the quantity of anthrone produced from a fixed quantity of sennoside C, regardless of the mouse body weight.

Statistical evaluation

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

Results

Purgative activity of sennoside C intracaecally administered to mice

Sennoside C was active on intracaecal administration and displayed activity nearly equal to sennoside A in incidence and time to onset of diarrhoea at an equimolar dose (Table 1).

Detection and determination of anthrones in the intraluminal contents of mouse

Table 2 shows that aloe-emodin anthrone and rhein anthrone were formed in the large intestine at nearly equimolar amounts at 2·3 h after oral administration of sennoside C (1·16 μmol/mouse). However, neither anthrone could be detected in the small intestine by TLC.

Effect of pretreatment with chloramphenicol on the purgative activity of sennoside C and total anthrone formation in the mouse large intestine

The oral ED50 values of sennosides A and C in the mice pretreated with chloramphenicol were roughly three times higher than those in the untreated mice (Table 3). When mice were pretreated in the same way, total anthrone content in the large intestine at 2·5 h after oral administration of sennoside C (1·16 μmol/mouse) was one-third that of the control (Table 4).

Table 1. Purgative activity of sennoside C administered intracaecally to mice.

	Intracaecal dose (μmol kg^{-1})	Incidence of diarrhoea	Time to onset of diarrhoea (min) mean ± s.e.m.
Sennoside C	46·4*	10/10	23·7 ± 1·4 (n = 10)
Sennoside A		9/9	24·4 ± 2·4 (n = 9)

Incidence of diarrhoea was expressed as a ratio of the number of diarrhoeal animals to the total number of test animals.

* The dose corresponds to 39·4 mg kg^{-1} of sennoside C and 40·0 mg kg^{-1} of sennoside A.

Table 2. Amounts of anthrones formed in the large intestine of mice 2·3 h after oral administration of sennoside C (1·16 μmol/mouse*).

	Anthrone formed (μmol/mouse) mean ± s.e.m.
Aloe-emodin anthrone	0·036 ± 0·013 (n = 4)
Rhein anthrone	0·039 ± 0·012 (n = 4)

* The dose corresponds to 46·4 μmol kg^{-1}. Amounts of anthrones were determined by HPLC.

Table 3. Purgative activity of sennoside C administered orally to mice pretreated with chloramphenicol.

	Oral ED50 (μmol kg^{-1}) (95% confidence limits)	
	Control	Pretreated with chloramphenicol
Sennoside C	13·1 (11·4–14·5)	42·2 (33·8–56·8)
Sennoside A	12·9 (10·6–15·1)	36·7 (17·6–45·9)

Occurrence of diarrhoea was observed for 8 h after sennoside administration. Chloramphenicol (100 mg kg^{-1}) was given orally five times for three days.

Table 4. Amounts of total anthrones formed in the large intestine of mice pretreated with chloramphenicol 2·5 h after oral administration of sennoside C (1·16 μmol/mouse*).

	Total anthrones (μmol/mouse) mean ± s.e.m.
Control	0·061 ± 0·017 (n = 10)
Pretreated with chloramphenicol	0·021 ± 0·010 (n = 8)

*The dose corresponds to 46·4 μmol kg^{-1}. Amounts of total anthrones were determined spectrophotometrically and expressed as rhein anthrone. Chloramphenicol (100 mg kg^{-1}) was given orally five times for three days.

Purgative activities of aloe-emodin anthrone and rhein anthrone injected directly into the caecum

Aloe-emodin anthrone, rhein anthrone or an equimolar mixture of both was directly injected into the caecum and the purgative activities were observed as to incidence, time to onset and mean score of diarrhoea. As shown in Table 5, rhein anthrone caused severe diarrhoea at a dose of 23·2 μmol kg^{-1} which corresponded to 11·6 μmol kg^{-1} of sennoside A, while aloe-emodin anthrone was far less active at an equimolar dose. However, an equimolar mixture of the two anthrones at the dose corresponding to 11·6 μmol kg^{-1} of sennoside C, namely 11·6 μmol kg^{-1} of aloe-emodin anthrone plus 11·6 μmol kg^{-1} of rhein anthrone, was equipotent with 23·2 μmol kg^{-1} of rhein anthrone. Furthermore, Table 6 shows that the intracaecal purgative ED50 value of the mixture was practically equal to the value of rhein anthrone, despite the higher value of the aloe-emodin anthrone.

Discussion

Sennoside C induced diarrhoea in mice 2·5 h or more after oral administration. It had the same purgative oral ED50 value as that of sennoside A, as previously reported (Kisa et al 1981). There was virtually no difference between the two sennosides in the intensity of the purgative effects estimated by observing the time to onset of diarrhoea, the number of soft or wet faeces, and the extent of staining on the blotting paper due to diarrhoea.

The activity of sennoside C was compared with that of sennoside A by direct injection into mouse caecum at an equimolar dose. The activities of both sennosides were also equal after intracaecal administration. Both sennosides exerted their purgative action more rapidly than by oral administration. The reduction time, 2–3 h, seemed to correspond to their transit time in the small intestine (unpublished data). These findings suggest that the site of action of sennoside C is the large intestine, as is the case with sennoside A.

Sennoside C, like sennoside A, was expected to be metabolized into aloe-emodin anthrone and rhein anthrone in the mouse large intestine. After oral administration of sennoside C, we tried to detect both anthrones in the intraluminal contents and determine their levels. Both were found at approximately equimolar amounts in the large intestine but not in the small intestine. The amounts of both anthrones detected 2·3 h after administration of sennoside C were as little as 3% of the oral dose of the glucoside. These amounts are assumed to have come from the glucoside which had reached the large intestine and had been metabolized there, while the rest of the glucoside was still passing through the small intestine. Once diarrhoea begins, the anthrones are excreted and cannot be estimated, so experimentally we adopted 2·3 h after administration for the determination time. When considering the intracaecal ED50 value (11·2 μmol kg^{-1}) of the mixture of both anthrones, the amount of anthrones produced until the onset time of diarrhoea at the site of action seem to be sufficient for the purgative threshold.

If the anthrones are formed by bacterial action and they are the active metabolites, the lower purgative activity of sennoside C administered orally after pretreatment with chloramphenicol, which suppresses bacterial action, should correspond to the lesser formation of total anthrones in the large intestine after the same pretreatment. Actually the oral ED50 value of sennoside C in the pretreated mice was about three times higher than that of the control, and the degree of suppression was similar to that of sennoside A. The amount of total anthrones formed from sennoside C in the pretreated mice was also one-third of the control, although no statistically significant difference could be detected because of the large deviation.

In a further attempt to establish that aloe-emodin anthrone and rhein anthrone are the true active metabolites of sennoside C, we examined the purgative effects of both and

Table 5. Purgative activities of aloe-emodin anthrone, rhein anthrone and their equimolar mixture administered intracaecally to mice.

Anthrones	Intracaecal dose (μmol kg^{-1})	Incidence of diarrhoea	Time to onset of diarrhoea (min) mean ± s.e.m.	Mean score
Aloe-emodin anthrone	23·2	1/10	87 (n = 1)	0·2 (n = 10)
Rhein anthrone	23·2*	10/10	26·4 ± 1·59 (n = 10)	3·9 (n = 10)
Mixture	11·6 + 11·6**	10/10	27·7 ± 3·14 (n = 10)	4·0 (n = 10)

All anthrones were suspended in 1% Tween 80 solution. Occurrence of diarrhoea was observed for 8 h after anthrone administration. Incidence of diarrhoea was expressed as the ratio of the number of diarrhoeal animals to the total number of test animals. The scoring system was as follows: 0 = no faeces or normal faeces; 1 = moist faeces with faint staining on the under surface of blotting paper; 2 = soft faeces with staining on the blotting paper; 3 = shapeless sludged faeces; 4 = shapeless mucoid faeces.

*This dose corresponds to 10 mg kg^{-1} of sennoside A.

**The total dose corresponds to 9·84 mg kg^{-1} of sennoside C.

Table 6. Relative purgative potencies of aloe-emodin anthrone, rhein anthrone and their equimolar mixture administered intracaecally to mice.

Anthrones	Intracaecal ED50 (95% confidence limits) (μmol kg^{-1})
Aloe-emodin anthrone	54·5 (24·1–89·6)
Rhein anthrone	11·4 (5·0–15·7)
Mixture	11·2 (6·1–14·6)

All anthrones were suspended in 1% Tween 80 solution. Occurrence of diarrhoea was observed for 1 h after anthrone administration.

their equimolar mixture after direct injection into the caecum, at the corresponding molar doses, taking into account that 1 mol of sennoside C releases 1 mol each of aloe-emodin anthrone and rhein anthrone and 1 mol of sennoside A releases 2 mol of rhein anthrone. As Fairbairn & Moss (1970) have already pointed out, we found that with direct injection into the large intestine, aloe-emodin anthrone was less potent than rhein anthrone. However, an equimolar mixture of aloe-emodin anthrone (11·6 μmol kg^{-1}) and rhein anthrone (11·6 μmol kg^{-1}) was equipotent with rhein anthrone (23·2 μmol kg^{-1}) as to incidence, time to onset and the mean score of the diarrhoea. Also, for the intracaecal ED50 value, the mixture and rhein anthrone had equal purgative activity, while aloe-emodin anthrone alone had far lower activity.

These results confirmed that sennoside C is intrinsically inactive in the glycoside form, as is sennoside A, and that the true active metabolites in mice are aloe-emodin anthrone and rhein anthrone, which are formed from sennoside C in the large intestine mainly by bacterial action. The activity of sennoside C can be explained as a synergistic effect of aloe-emodin anthrone and rhein anthrone but not an additive one. The mechanism of the synergism remains to be clarified.

Previously we reported the potentiating effect of sennoside C (Kisa et al 1981) or aloe-emodin dianthrone diglucoside (Nakajima et al 1985) on purgative activity of sennoside A in mice. These effects by the glucosides based on aloe-emodin anthrone are probably due to a similar synergistic effect of both anthrones.

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