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Synergistic Effects of Anthraquinones on the Purgative Activity of Rhein Anthrone in Mice

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Abstract

This study was performed to determine whether intracaecally administered rhein anthrone and anthraquinones such as aloe-emodin, chrysophanol, emodin or rhein synergistically enhance the purgative action as has been observed for rhein anthrone and aloe-emodin anthrone.

These anthraquinones were less potent than rhein anthrone. An equimolar mixture of aloe-emodin and rhein anthrone had synergistic potentiating effects because the ED50 value (50% purgative dose) of the combination was smaller than that calculated additively from the ED50 values of aloe-emodin and rhein anthrone. An equimolar mixture of other anthraquinones and rhein anthrone tended to potentiate the purgative action.

These results confirmed that rhein anthrone and aloe-emodin synergistically exert a purgative action as has been observed for rhein anthrone and aloe-emodin anthrone.

Sennosides A, B, C and D are the main purgative constituents of senna and rhubarb (Fairbairn & Saleh 1951; Oshio et al 1978). They are metabolized into the final intraluminally active substances, aloe-emodin anthrone or rhein anthrone, in the large intestine, which synergistically exert purgative effects on mice (Sasaki et al 1979; Yamauchi et al 1992).

We have also found that a mixture of sennosides A and C exerts a synergistic effect; when combined in the molar ratio 7 : 3 there is a marked synergistic effect of (approx.) 1·8-fold (Kisa et al 1981). This results from synergistic stimulation of transit and water secretion in the large intestine (Yagi et al 1997).

These crude drugs also contain oxyanthraquinone glycosides, such as glycosides of aloe-emodin, crysophanol, emodin, physcion and rhein, which are thought to exert synergistic effects on purgative activity after being metabolized into the respective anthraquinones, and which contribute to the total activities of the crude drugs, although separately they have weak activities compared with sennosides.

Oshio et al (1978) and Harima et al (1994) showed that the purgative activities of the crude drugs could be determined approximately by analysis of sennoside content; the correlation between activity and the sennoside content is not very strong, suggesting that other factors are involved.

To investigate the synergistic purgative action between constituents in senna and rhubarb, we set out to determine whether intracaecally administered rhein anthrone and individual anthraquinones could synergistically enhance the purgative action, as had been observed for rhein anthrone and aloe-emodin anthrone. This assumption was based on the knowledge that these anthraquinones are metabolically produced from their glycosides in the large intestine.

Materials and Methods

Rhein and rhein anthrone were prepared as described previously (Yagi et al 1988). Aloe-emodin and chrysophanol were isolated from rhubarb by the method of Matsuoka (1961) with slight modification. Emodin was purchased from Funakoshi (Tokyo, Japan). Chemicals were suspended (5 mL kg^{-1}) in a 1% aqueous solution of Tween 80 (Sigma, St Louis, MO) immediately before administration, and an equimolar mixture of rhein anthrone and an anthraquinone was prepared by mixing equal volumes of both suspensions.

Animals

Female albino mice of Jcl : ICR strain (CLEA Japan, Tokyo, Japan), 22–32 g, were kept at room

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temperature (22–25°C) and allowed free access to a diet of MF pellets (Oriental Yeast, Tokyo, Japan) and tap water.

Methods

The operation was carried out as described elsewhere (Yagi et al 1988). The animals were used in the experiments on the third day after the operation. The purgative test (n = 9–11 per dose) was performed using a method described by Yamauchi et al (1992). To express relative purgative potency, the 50% purgative dose (ED50, i.e. the dose which causes diarrhoea in 50% of the mice) was determined by the Probit method. The expected ED50 based on the additive effect of each chemical was calculated according to equation 1:

$$0{\cdot}5/ED50_1 + 0{\cdot}5/ED50_2 = 1/(\text{additive ED50}) \quad (1)$$

where $ED50_1$ and $ED50_2$ are the individual ED50 values of chemicals 1 and 2. $P < 0{\cdot}05$ was regarded as indicative of statistically significant synergy (Tallarida & Raffa 1996).

Anthrones in the intestine were detected as according to Yamauchi et al (1992).

Results

Aloe-emodin, chrysophanol, emodin, rhein or equimolar mixtures of rhein anthrone and each anthraquinone were injected directly into the caecum and their purgative activities were monitored. Table 1 shows the intracaecal purgative ED50 values of the anthraquinones and the equimolar mixtures of rhein anthrone and each anthraquinone. These anthraquinones were less potent than rhein anthrone, and their intracaecal purgative ED50 values, except for that of rhein anthraquinone, were nearly equal. The ED50 values of equimolar mixtures of rhein anthrone and each anthraquinone were much smaller than those of the individual anthraquinones. The only ED50 value significantly ($P < 0{\cdot}05$) smaller than the calculated ED50 value was that of an equimolar mixture of rhein anthrone and aloe-emodin.

After intracaecal administration of the anthraquinones, neither anthrone could be detected in the intestine.

Discussion

In a previous study we found that sennosides A and C synergistically exerted their purgative activities in mice after being metabolized into the anthrone form by intraluminal bacterial action (Sasaki et al 1979; Kisa et al 1981; Yamauchi et al 1992). Study of the purgative activity of several samples of rhubarb and senna with mice revealed that there was a weak correlation between purgative activity and sennoside content and that oxyanthraquinone glycosides seem to contribute to the total activity of the crude drug (Oshio et al 1978; Harima et al 1994). We hypothesized that there might be a synergistic effect between sennosides and these anthraquinone glyco-sides as there was for sennosides A and C.

In the current study we examined the purgative effects of intracaecally administered aloe-emodin, chrysophanol, emodin and rhein, thought to be formed in-situ from the respective glycosides, and

Table 1. Relative purgative potencies of aloe-emodin, chrysophanol, emodin, rhein and rhein anthrone and equimolar mixtures of each anthraquinone and rhein anthrone administered intracaecally.

Chemical	Intracaecal ED50 (μmol kg^{-1})*	Calculated additive ED50 (μmol kg^{-1})
Rhein anthrone	11·4 (5·0–15·7)	
Anthraquinones		
Aloe-emodin	246·3 (176·7–827·2)	
Chrysophanol	212·5 (164·7–264·9)	
Emodin	247·2 (169·0–446·7)	
Rhein	91·0 (33·8–159·9)	
Equimolar mixtures		
Rhein anthrone and aloe-emodin	15·7 (11·6–20·7)†	21·8
Rhein anthrone and chrysophanol	18·7 (14·6–24·0)	21·6
Rhein anthrone and emodin	15·6 (3·7–22·0)	21·8
Rhein anthrone and rhein	18·5 (15·7–21·4)	20·3

*The values in parentheses are the 95% confidence limits. All anthraquinones and rhein anthrone were suspended in 1% aqueous solution of Tween 80. An equimolar mixture of anthraquinone and rhein anthrone was prepared by mixing equal volumes of both suspensions. All chemicals were administered intracaecally to mice (n = 9–11 per dose). The occurrence of diarrhoea was observed for 6 h after administration. †$P < 0{\cdot}05$, significantly different from the calculated ED50.

their potentiating effects on the purgative activity of rhein anthrone. We found them to be less potent than rhein anthrone (ED50 11·4 μmol kg^{-1}). An equimolar mixture of each anthraquinone and rhein anthrone had a potentiating effect because the ED50 value was smaller than that calculated additively from the ED50 values of the individual anthraquinones and rhein anthrone. In the absence of potentiating effects between aloe-emodin and rhein anthrone, the ED50 value of their equimolar mixture would be 21·8 μmol kg^{-1} whereas the measured ED50 value was 15·7 μmol kg^{-1}—aloe-emodin and rhein anthrone synergistically exerted a potentiating effect of (approx.) 1·4-fold ($P < 0.05$). Chrysophanol, emodin and rhein also had potentiating effects on rhein anthrone of (approx.) 1·2-, 1·4- and 1·1-fold, respectively, but the effects are not statistically significant. Although ED50 values were lower for rhein than for the other anthraquinones, the ED50 value of the equimolar mixture of rhein and rhein anthrone was similar to those of the other mixtures.

An equimolar mixture of aloe-emodin anthrone and rhein anthrone synergistically exerted a purgative effect on mice which is the same as that of rhein anthrone itself, though aloe-emodin anthrone has far less potent purgative activity (Yamauchi et al 1992).

These results confirmed that rhein anthrone and aloe-emodin exerted a synergistic purgative action, as had been found for rhein anthrone and aloe-emodin anthrone. Rhein anthrone and chrysophanol, emodin or rhein tended to enhance the purgative action. Such findings suggest that these potentiating effects contribute to the total purgative activity of rhubarb and senna. When the sennoside content is high compared with that of anthraquinone glycosides, the purgative activity of the crude drug can be estimated on the basis of the sennoside content. However, if a large amount of anthraquinone glycosides is present in the crude drug, the influence of their synergistic effects can become so large that they cannot be ignored.

The mechanism of this synergistic effect remains to be clarified. However, because we did not detect aloe-emodin anthrone in the large intestine of mice within 6 h of intracaecal administration of aloe-emodin, we concluded that aloe-emodin anthrone was not formed from the anthraquinone under the anaerobic conditions of the large intestine. This means that there is probably no synergistic effect between rhein anthrone and the anthrone arising from aloe-emodin.

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