Studies of Aloe. IV. Mechanism of Cathartic Effect. (3)

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Charcoal transport, as an indicator of the degree of peristalsis, and water content in the large intestine after the intracæcal administration of barbaloin, were measured simultaneously in the same rat. Charcoal transport was significantly accelerated at both 3.5 and 6.5h after the administration of barbaloin. At 6.5h, diarrhea instead of normal faeces was observed. Moreover, at 1h before the acceleration of charcoal transport, a marked increase in the relative water content of the large intestine was observed. It appears that the increase in water content of the large intestine induced by barbaloin precedes the stimulation of peristalsis, attended by diarrhea. Therefore, it is suggested that the increase in water content is a more important factor than the stimulation of peristalsis in the diarrhea induced by barbaloin.

Keywords barbaloin; aloe; aloe-emodin-9-anthrone; large intestine water content; large-intestinal transport; cathartic effect mechanism

In previous papers,¹,² we have reported that barbaloin, the main laxative component of aloe, is decomposed in the rat large intestine to aloe-emodin-9-anthrone (AE-anthrone). AE-anthrone causes an increase in the water content of the large intestine by inhibiting electrolyte absorption and stimulating electrolyte secretion. Moreover, it was shown that AE-anthrone inhibits rat colonic Na⁺,K⁺-adenosine triphosphatase and increases paracellular permeability across the colonic mucosa, resulting in an increase in large-intestinal water content. Although both an increased water content and stimulation of peristalsis in the large intestine are important causative factors of diarrhea, it has never been clarified which factor is mainly responsible for the cathartic effects, especially in the case of barbaloin. If the increase in large-intestinal water content by barbaloin is found after the acceleration of large intestinal-transport, we would be able to explain the mechanism of barbaloin catharsis by the effects of AE-anthrone. In order to elucidate this relationship, peristalsis and water content in the large intestine after barbaloin administration should be measured simultaneously. Therefore, we carried out the simultaneous determination of charcoal transport, as an indicator of the degree of peristalsis, and the water content of the rat large intestine after intracæcal administration of barbaloin.

MATERIALS AND METHODS

Chemicals Barbaloin used in this study was the same as that described previously.¹ All other chemicals were of the highest grade commercially available.

Animals Male Wistar rats (150—200 g) were purchased from Shizuoka Laboratory Animal Center, Hamamatsu, Japan. The rats had free access to a commercial rat chow and water during the experiments.

A cathartic response to barbaloin (31.1 mg/10 ml/kg) in rats was induced according to the method described previously.³ Then, rats with definite diarrhea were selected and used.

Measurement of Charcoal Transport in Rat Large Intestine A surgical operation for caecal intubation was carried out in the rats as reported previously.¹ The intubated rats were used for the experiment from the third day after the operation. Administration of charcoal meal (5% charcoal in 5% gum arabic solution, 5 ml/kg) via the caecal tube was done at given times after the administration of barbaloin (31.1 mg/5 ml/kg, in 5% gum arabic solution) via the same tube. Then, the rats were killed by exposure to ether after 1.5h, and the colons were excised. The transport ratio (%) of charcoal meal in the large intestine was then determined.

We have already found that the first diarrhea was observed at 6—8h after the oral administration of barbaloin, as barbaloin needs much time to be activated.¹ Therefore, we continued our experiments in this paper until the time when diarrhea should be observed.

Although Ueda et al.⁴ used fasted animals for the measurement of charcoal transport, we used non-fasted rats. Since the transport ratio varied widely when fasted rats were used, fasting may perturb intestinal movement.

Measurement of Water Content in the Large Intestine The rat colon and caecum isolated from the same rat were then weighed immediately (wet weight: A) and dried in an air oven at 140°C until a constant weight was obtained (dry weight: B). The water content of the large intestine was expressed as the relative water content (A—B)/B, as reported previously.¹

RESULTS

Effect of Barbaloin on Charcoal Transport in Rat Large Intestine The charcoal transport ratio in the rat large intestine was measured at 2, 2.5, 3.5, 4.5, 5.5 and 6.5h after the intracæcal administration of barbaloin. The results are shown in Fig. 1.

The charcoal transport ratio in the large intestine was increased significantly at both 3.5 and 6.5h after the intracæcal administration of barbaloin, and diarrhea was observed after 6.5h.

Effect of Barbaloin on Water Content of Large Intestine The water content of the large intestine, determined at the same time as the charcoal transport ratio, is shown in Table I. In the caecum, a significant increase in the relative water content was observed at both 2.5 and 5.5h after the

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Fig. 1. Effect of Intracaeally Administered BarbaloIn on Charcoal Transport in the Large Intestine of Male Rats with Caeecal Intubation

Administration of charcoal meal via the caecal tube was done at 0.5, 1, 2, 3, 4 and 5 h after the administration of barbaloIn into the same tube. Next, the rats were killed after 1.5 h and the percentage distance travelled by the charcoal meal in the large intestine was determined (□, control; □, barbaloIn). Each bar represents the mean ± S.D. of 19–35 experiments. a) Time after intracaeal administration of barbaloIn. b) (Length of intestine containing charcoal/total length of large intestine) × 100. c) p < 0.05 as compared with the controls. d) p < 0.01 as compared with the controls.

TABLE I. Effect of Intracaeally Administered BarbaloIn on the Water Content of the Large Intestine of Male Rats with Caeecal Intubation

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Compounds</th>
<th>Water content (g)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Caecum</td>
<td>Colon</td>
</tr>
<tr>
<td>2.0</td>
<td>Control</td>
<td>1.804 ± 0.146</td>
</tr>
<tr>
<td></td>
<td>BarbaloIn</td>
<td>1.843 ± 0.233</td>
</tr>
<tr>
<td>2.5</td>
<td>Control</td>
<td>1.683 ± 0.118</td>
</tr>
<tr>
<td></td>
<td>BarbaloIn</td>
<td>1.866 ± 0.244a</td>
</tr>
<tr>
<td>3.5</td>
<td>Control</td>
<td>1.725 ± 0.129</td>
</tr>
<tr>
<td></td>
<td>BarbaloIn</td>
<td>1.790 ± 0.189</td>
</tr>
<tr>
<td>4.5</td>
<td>Control</td>
<td>1.689 ± 0.111</td>
</tr>
<tr>
<td></td>
<td>BarbaloIn</td>
<td>1.752 ± 0.173</td>
</tr>
<tr>
<td>5.5</td>
<td>Control</td>
<td>1.698 ± 0.105</td>
</tr>
<tr>
<td></td>
<td>BarbaloIn</td>
<td>1.928 ± 0.176d</td>
</tr>
<tr>
<td>6.5</td>
<td>Control</td>
<td>1.806 ± 0.172</td>
</tr>
<tr>
<td></td>
<td>BarbaloIn</td>
<td>1.880 ± 0.149</td>
</tr>
</tbody>
</table>

Each value represents the mean ± S.D. of 16–33 experiments. a) Time after intracaeal administration of barbaloIn. b) relative water content = (wet weight - dry weight)/dry weight. c) p < 0.05 as compared with the corresponding control. d) p < 0.01 as compared with the corresponding control.

intracaeal administration of barbaloIn. In the colon, the relative water content increased significantly from 5.5 h on. The water content of the caecum after the intracaeal administration of barbaloIn reflected the water content in the whole large intestine.

DISCUSSION

In order to elucidate the relationship between charcoal transport and water content, based on the absorption and secretion of electrolytes, we determined the charcoal transport and water content simultaneously in the rat large intestine after intracaeal administration of barbaloIn. In order to understand this relationship more clearly, the results in Fig. 1 and Table I are illustrated together in Fig. 2.

The values of the charcoal transport ratio and water content obtained after the intracaeal administration of barbaloIn were expressed for convenience in terms of the ratio relative to each corresponding control value. During the experimental period after the intracaeal administration of barbaloIn, the ratio of the relative water content was always greater than 1.0, indicating the wetness of the contents. At 2.5 h after, an initial and marked increase in water content was observed in the caecum. At 3.5 h after, large-intestinal charcoal transport was accelerated significantly (p < 0.05), and then the water content of the caecum was reduced, but no significant increase in the water content of the colon was observed. Therefore, the increment of water content in the caecum is thought to be extinguished not only by movement of the caecal content to the colon, but also by the absorption of water in the caecum and colon. Consequently, diarrhea is not always induced by an increase in water content in the caecum. At 5.5 h after barbaloIn administration, the water content of the caecum showed a marked increase, and the water content of the colon also increased significantly. Perhaps the marked increase in the water content of the whole large intestine was induced by the secretion of water which overcame the absorption of water, and as a result, the content of the whole large intestine swelled. Furthermore, 1 h later (at 6.5 h), the water content of the colon increased beyond the level at 5.5 h after the administration, and large-intestinal transport was accelerated. At 6.5 h, the water content of the colon is very

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high, and diarrhea is caused by such a condition. It thus appears that the acceleration of large-intestinal charcoal transport observed 3.5 h after administration is not related directly to diarrhea, but the acceleration at 6.5 h after administration is connected to the diarrhea. The increase in water content at 2.5 h after the administration was less than that at 5.5 h. Perhaps these increments in water content corresponded with the amount of AE-anthron produced from barbaloin. The first stimulation of peristalsis might be related to the release of normal faeces from the colon, so a condition for the movement of moisturized caecal content to the colon might be achieved.

Leng-Peschlow\textsuperscript{39} investigated the quantity and consistency of faecal output, large-intestine transit time and colonic net fluid absorption in rats after the oral administration of sennoside \( A + B \), and reported that the laxative effect of the sennosides consisted of changes in colon motility as well as colonic fluid absorption, although motility seemed to be an earlier and more sensitive parameter than net absorption.

In contrast, in the case of barbaloin, it seems that the increase in water content induced by barbaloin is a more important factor than the stimulation of peristalsis.

Previously,\textsuperscript{2) we reported that the increase in water content of the large intestine caused by barbaloin was the result of an inhibition of electrolyte absorption and stimulation of electrolyte secretion by AE-anthron, which was formed from barbaloin in the large intestine. Therefore, it is possible to consider that the AE-anthron produced from barbaloin in the large intestine caused the diarrhea. However, we have still not estimated the amount of AE-anthron in the large intestine after the administration of barbaloin, although its presence has been confirmed.\textsuperscript{1}) Previously, we performed the same kind of experiment as that reported here, except that barbaloin was administered orally, instead of intracaeically, to rats. However, we were unable to elucidate the relationship between water content and charcoal transport in the large intestine following oral administration, since the increase in water content and the acceleration of charcoal transport were observed at the same time. Consequently, in order to elucidate the relationship between the cathartic effect of barbaloin and the amount of AE-anthron formed from it, a further experiment involving intracaeal administration will have to be done.

The increase in water content observed just before the appearance of diarrhea was marked in comparison with the initial increase in water content. Therefore, we speculate that other factors are partly responsible for the diarrhea induced by barbaloin. In fact, mucoid diarrhea was sometimes observed after the administration of barbaloin, and we also found an increase in the mucus-like substance after the injection of AE-anthron into the rat colon segment. We think that this mucus may act as a barrier blocking the absorption of water, and as a lubricant during diarrhea. Therefore, we will investigate the effect of AE-anthron on mucus secretion and the relationship between the amount of AE-anthron and this phenomenon, to further elucidate the cathartic mechanism of barbaloin.

REFERENCES