Studies of Aloe. V. 1) Mechanism of Cathartic Effect. (4)

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Aloe-emodin-9-anthrone (AE-anthrone), produced from barbaloin in the rat large intestine, caused not only an increase in the intestinal water content but also stimulated mucus secretion. This might play an important role in the occurrence of diarrhea. It was demonstrated that the amount of AE-anthrone produced in the rat large intestine (maximal amount: 568 μg/rat at 4 h after injection) was enough to cause both of these effects, which were observed following intracecal administration of barbaloin (31.1 mg/kg). These results together with our previous data, which showed a relationship between increase in the intestinal water content and the stimulation of peristalsis, confirm that AE-anthrone is the principal agent responsible for the cathartic effect of barbaloin. We also propose that the increase in water content is a more important factor than stimulation of peristalsis in the induction of diarrhea by barbaloin.

Keywords barbaloin; aloe-emodin-9-anthrone; water content; mucus secretion; fecal excretion; cathartic effect mechanism

In general, diarrhea is induced by an increase in water content and/or stimulation of peristalsis in the large intestine. The stimulation of peristalsis and consequent diarrhea induced by barbaloin, the main laxative component of aloe, was previously suggested to result from an increase in the water content of the large intestine in the rat, 1) because this increase preceded the acceleration of charcoal transport observed following intracecal administration of barbaloin. Moreover, aloe-emodin-9-anthrone (AE-anthrone), a decomposition product of barbaloin produced in the large intestine, has been found to cause an increase in the water content of the rat large intestine by several mechanisms, including inhibition of rat colonic Na+K+-adenosine triphosphatase, and increase of paracellular permeability across the colonic mucosa. 2)

To clarify whether AE-anthrone plays a principal role in the cathartic action of barbaloin, further investigation of the relationship between the amount of AE-anthrone produced from barbaloin and the increment in water content of the large intestine was deemed necessary. The effect of AE-anthrone on mucus secretion was also studied, because an increase was found in mucus-like substances after injection of AE-anthrone into the rat colon, and it seemed that mucus might act as a lubricant on diarrhea. The quantity and consistency of the feces excreted after intracecal administration of barbaloin to rats was also investigated to elucidate the characteristics of the cathartic action.

MATERIALS AND METHODS

Chemicals The barbaloin and AE-anthrone used in this study were obtained as described previously. 2) Sep-Pak® C18 cartridges (Sep-Pak C18) were purchased from Japan Waters Co., Ltd. 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 cathartic response to barbaloin (31.1 mg/10 ml/kg) was examined in these rats by a previously described method. 3) Those with definite diarrhea were selected and used for the determination of AE-anthrone in the large intestine and the observation of fecal excretion.

Thin Layer Chromatogram-Densitometry Thin layer chromatography (TLC) was performed on Silica gel 60 silanised pre-coated plates using a mixture of 70% MeOH—NH4OH (100:0.5) as the developing solvent. Immediately after air-drying, the TLC plate was covered with a clean glass plate to prevent discoloration. TLC densitograms were obtained using a Shimadzu LS-910 chromatogram scanner with a sample wavelength of 590 nm and a reference wavelength of 780 nm.

Time Course of Fecal Excretion Cecal intubation was carried out as reported, 1) and the rats were used in the experiment from the third day after intubation. During the experiment the rats were kept in individual cages with a wire-mesh floor through which the feces fell onto blotting paper. After administration of barbaloin (31.1 mg/5 ml/kg, in 5% gum arabic solution) via the cecal tube, the number of normal (hard) and soft or fluid (soft) fecal pellets were counted at hourly intervals for 9 h and then over a 15 h period.

Quantification of AE-Anthrone Produced in the Rat Large Intestine after Intracecal Administration of Barbaloin At 1, 2, 3, 4 and 5 h after the administration of barbaloin the rats were killed by exposure to ether, and the ceum and colon were excised after ligating both ends. Five milliliters of 0.1% p-nitrosodimethylaniline (p-NDM) pyridine solution was injected into each segment and allowed to stand for several minutes. The segments were then dissected, their contents were centrifuged, and the residue was extracted with 2 ml pyridine. The combined supernatants were extracted twice with 20 and 5 ml chloroform. The combined chloroform layers were evaporated to dryness under reduced pressure. The residue was redissolved in 5 ml of mixture A [50% MeOH—NH4OH (100:0.5)], and applied to a Sep-Pak C18 cartridge which had been previously washed with 50% MeOH. The Sep-Pak C18 cartridge was washed with 5 ml of mixture A, and eluted with 25 ml of mixture B [50% MeOH—NH4OH (100:5)]. The eluate was evaporated to dryness under reduced pressure, and the residue
redissoled in a known volume of AcOEt. The amount of AE-anthrone in the AcOEt solution was determined by TLC-densitometry.

Water Flux and Mucus Secretion after Injection of AE-Anthrone into Rat Colon Segments Surgery was carried out as described previously. The rats, which had received only water for 18 h prior to the experiment, were anesthetized with diethylether. The entire colon was rinsed with approximately 100 ml saline at 37 °C, and any solution remaining in the colon was expelled as thoroughly as possible by passing a stream of air through it. Both ends of the colon segment were then ligated, and 2 ml of 5% gum arabic-saline solution, either alone or containing AE-anthrone at a concentration of $10^{-3}$ or $10^{-4}$ M (i.e., 0.51 mg or 0.051 mg/rat), was injected. After 1 or 2 h, the residual fluid was collected from the segment, the volume was measured, and the fluid was centrifuged at 16000 x g for 20 min at 4°C. The supernatant and mucus-like fractions separated from each sample of colonic fluid were measured.

The amount of mucus in the mucus-like fractions was estimated by assaying the total protein-bound hexose (TPBH) content. The mucus-like fraction was dispersed by ultrasonication, and precipitated twice using 10% trichloroacetic acid and 95% ethanol. After redissolving the precipitate in 1 ml 1 M NaOH, orcinol reagent (8.5 ml) was added and the TPBH content was determined spectrophotometrically, using galactose as a standard, at 505 nm.

Statistical Evaluation The results except those in Fig. 1 were expressed as mean ± S.D. Statistical significance was assessed using Student’s t test.

RESULTS

Time Course of Fecal Excretion After the intracecal administration of barbaloyn, the first diarrhea was observed at 4 h, and all of the rats exhibited diarrhea by 9 h. In a few animals, however, normal feces was still being excreted at 8 h, and the time of maximal release of soft feces could not be confirmed, as shown in Fig. 1.

Quantification of AE-Anthrone Produced in the Rat Large Intestine after Intracecal Administration of Barbaloyn The amount of AE-anthrone produced in the rat large intestine was determined by 7 h after intracecal administration of barbaloyn, and the results are shown in Fig. 2.

AE-anthrone was detectable in both the cecum and colon at 1 h after administration. The amount in both these areas then increased abruptly, and peaked 4 h later (cecum: 508 µg/rat, colon: 83 µg/rat). Thereafter, the amount of AE-anthrone decreased markedly, to about 100 µg/rat in the cecum (p < 0.01) and about 50 µg/rat in the colon, but was still detectable 7 h after administration. The amount of AE-anthrone in the colon was thought to be similar to that in the cecum, but was found to be less than 90 µg/rat. This suggests that AE-anthrone produced in the cecum might be changed to another compound which does not react with p-NDMA before or after movement into the colon, or that only a small amount of AE-anthrone is produced in the colon.

Effect of AE-Anthrone on Water Flux and Mucus Secretion in the Rat Colon The results of the measurement of residual fluid volume (supernatant fraction volume plus mucus-like fraction volume) and the determination of TPBH in the mucus-like fraction are shown in Table I. Over the course of the observation period, both the residual fluid volume and the supernatant fraction volume decreased in the control group due to absorption of water. However, in the AE-anthrone treatment group, the volume of residual fluid increased.
TABLE I. Effect of AE-Anthrone on Volume of Residual Fluid (Mucus-like Fr. and Supernatant Fr. and Supernatant Fr.) and TPBH in Mucus-like Fr.

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>Compound</th>
<th>Residual fluid (ml)</th>
<th>Mucus-like fr. (ml)</th>
<th>Supernatant fr. (ml)</th>
<th>TPBH weight (µg)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>Control</td>
<td>1.576 ± 0.153</td>
<td>0.277 ± 0.147</td>
<td>1.259 ± 0.194</td>
<td>83.3 ± 24.5</td>
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<td></td>
<td>AE-anthrone 10^{-4}M</td>
<td>1.940 ± 0.255&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.362 ± 0.146</td>
<td>1.608 ± 0.322&lt;sup&gt;a&lt;/sup&gt;</td>
<td>110.2 ± 47.8</td>
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<tr>
<td></td>
<td>10^{-3}M</td>
<td>2.086 ± 0.179&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.429 ± 0.148&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.647 ± 0.164&lt;sup&gt;a&lt;/sup&gt;</td>
<td>160.1 ± 56.4&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>2</td>
<td>Control</td>
<td>1.095 ± 0.177</td>
<td>0.356 ± 0.148</td>
<td>0.752 ± 0.166</td>
<td>122.3 ± 52.2</td>
</tr>
<tr>
<td></td>
<td>AE-anthrone 10^{-4}M</td>
<td>1.401 ± 0.233&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.423 ± 0.152</td>
<td>0.972 ± 0.199&lt;sup&gt;a&lt;/sup&gt;</td>
<td>145.7 ± 78.0</td>
</tr>
<tr>
<td></td>
<td>10^{-3}M</td>
<td>2.158 ± 0.226&lt;sup&gt;a&lt;/sup&gt;</td>
<td>0.696 ± 0.208&lt;sup&gt;a&lt;/sup&gt;</td>
<td>1.461 ± 0.257&lt;sup&gt;a&lt;/sup&gt;</td>
<td>265.1 ± 58.1&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
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Each value represents the mean ± S.D. of 18–37 experiments. <sup>a</sup> p<0.01 as compared with corresponding control.

significantly compared with that in the control group, although at the 10^{-4}M (51 µg/rat) dose the effect did not continue. This effect was mainly responsible for the increase in supernatant fraction volume. A significant increase in the volume of both the supernatant and mucus-like fractions was caused at 10^{-3}M (510 µg/rat) of AE-anthrone. At 1 h after the injection of AE-anthrone (10^{-3}M), a significant increase in the amount of TPBH, which represents the amount of mucus in the mucus-like fraction, was already apparent. By 2 h, a marked increase in the amount of TPBH was observed, and was accompanied by a significant increase in the mucus-like fraction volume. At this point we observed an increase in the viscosity of the mucus, and a jelly-like mass was easily isolated from the supernatant fraction.

DISCUSSION

Our results show that AE-anthrone not only causes an increase in the water content of the rat large intestine but also stimulates mucus secretion, and that it is produced from barbaloin in situ in sufficient amounts to stimulate mucus secretion. When the amount of AE-anthrone produced from barbaloin was less than 50 µg/rat, it appeared to affect only the water flux, but when produced in large amounts, it affected both mucus secretion and water flux. Moreover, it seemed that the time required to stimulate mucus secretion markedly in rats after intracelal administration of barbaloin was over 5 h, i.e., 4 h was needed for the decomposition of barbaloin to AE-anthrone and at least one hour more was necessary for the onset of effect of AE-anthrone.

Diarrhea was still not observed 2 h after the intracelal administration of AE-anthrone to rats (data not shown), although the substance caused a marked increase in the amount of mucus from 2 h following its injection into the colon segment. Therefore, it seems that part of the AE-anthrone administered intracelally reacts with contents of the cecum, and loses its stimulating activity for secretion of water content and mucus.

During the observation of fecal excretion, diarrhea usually occurred from 6 h after the intracelal administration of barbaloin. Barbaloin requires several hours for its activation even after intracelal administration, as activation depends on the activity of intestinal bacteria. 5

We previously reported<sup>1</sup> the relationship between the water content of the large intestine and large intestinal charcoal transport as a measure of the degree of stimulation of peristalsis after intracelal administration of barbaloin to rats. A marked increase in the water content of the large intestine was induced 1 h before acceleration of charcoal transport, which was observed at 3.5 and 6.5 h after administration. The increase in water content observed at 5.5 h after administration was more sudden and marked than that at 3.5 h, and 1 h later the stimulation of peristalsis was accompanied by diarrhea. These results suggested that at 3.5 h mucus secretion had not yet been stimulated, whereas at 6.5 h it had been and this induced diarrhea. Mucus contains a high proportion of water, and the stimulation of its secretion contributes to the increase in the water content of the large intestine. The mucus may also facilitate the movement of intestinal contents by acting as a lubricant. Therefore, it appears that an increase in the intestinal water content, which includes an increase in mucus, is an important factor in the production of diarrhea by barbaloin, and that stimulation of mucus secretion plays a particularly important role.

We have demonstrated here that AE-anthrone is produced in sufficient quantities in the large intestine to cause an increase in both water content and mucus. Both these increases might be more important than stimulation of peristalsis in the induction of diarrhea by barbaloin. Therefore, AE-anthrone is suggested to be the principal agent responsible for the cathartic effects of barbaloin.

REFERENCES