SHORT COMMUNICATION

Effect of the Antiulcer Polysaccharide Fraction from Bupleurum falcatum L. on the Healing of Gastric Ulcer Induced by Acetic Acid in Rats

T. Matsumoto,1 X. B. Sun,1,2 T. Hanawa,1 H. Kodaira,3 K. Ishii3 and H. Yamada1*

1Oriental Medicine Research Centre, The Kitasato Institute, Tokyo, Japan
2Academy of Traditional Chinese Medicine and Materia Medica of Jilin Province, Jilin Province, China
3Department of Molecular Pharmacology, School of Pharmaceutical Sciences, Kitasato University, Tokyo, Japan

An antiulcer polysaccharide fraction (BR-2) from Bupleurum falcatum L. was examined for its effect on the healing of chronic ulcers induced by acetic acid in rats. When BR-2 was administered orally to the rats, it was shown to be effective in the healing of acetic acid-induced chronic ulcer. This result suggests that the use of herbal prescriptions containing B. falcatum L. may prove useful for the treatment of peptic ulcers. Copyright © 2002 John Wiley & Sons, Ltd.

Keywords: acetic acid; chronic ulcer; antiulcer; polysaccharide; Bupleurum falcatum L.

INTRODUCTION

Kampo medicines (Chinese and Japanese herbal medicines) containing the roots of Bupleurum falcatum L. (Umbelliferae) have been used widely for the treatment of gastroenteric diseases. In our search for pharmacologically active compounds from crude drugs of plant origin, we found that the polysaccharide fraction (BR-2) of B. falcatum L. showed a potent inhibitory activity against HCl/ethanol-induced gastric lesions in mice, and the active polysaccharides, bupleurans 2IIb and 2IIc, were isolated and characterized as pectic polysaccharides (Yamada et al., 1989; Yamada et al., 1991a, 1991b; Hirano et al., 1994). BR-2 significantly protected against a wide variety of experimental acute gastric mucosal lesions including 99.5% ethanol-induced, water immersion and restraint stress-induced lesion, and pylorus-ligated ulcer in mice or rats (Sun et al., 1991). However, it is not known whether BR-2 is effective in the healing of chronic ulcer.

Human peptic ulcers are characterized by repeated recurrence or relapse. An experimental gastric ulcer induced by acetic acid is the only model for recurrence or relapse after the healing of the ulcer has been demonstrated (Ito et al., 1984). It has been reported that the experimental ulcer induced by acetic acid in the rat is a useful animal model for evaluating the healing effects of antiulcer agents because the repair process closely resembles that of the human peptic ulcer from macroscopic and histological observations (Takagi et al., 1969; Okabe and Pfeiffer, 1972). In the present study, the effect of BR-2 on this ulcer model was examined to determine whether BR-2 is effective in the healing of chronic ulcer.

* Correspondence to: H. Yamada, Kitasato Institute for Life Science, Kitasato University, 5-9-1 Shirokane, Minato-Ku, Tokyo 108-8641, Japan (present address).
Email: yamada-h@kitasato.or.jp

MATERIALS AND METHODS

Materials. The roots of B. falcatum L., complying with the standards of the Japanese Pharmacopoeia (13th edition) were purchased from Uchida Wakanyaku Co. Ltd (Tokyo, Japan). A voucher specimen was deposited at the herbarium of the Oriental Medicine Research Centre of the Kitasato Institute. An antiulcer polysaccharide fraction (BR-2) was prepared from a hot water extract of B. falcatum L. as described previously (Yamada et al., 1989; Sun et al., 1991). BR-2 contains the antiulcer polysaccharides, bupleurans 2IIb and 2IIc, at a concentration of 33.1% and 24.5%, respectively (Yamada et al., 1991a).

Animals. Male Wistar strain SPF rats (200–220 g), obtained from SLC (Shizuoka, Japan), were housed at an ambient temperature of 22°±1°C with constant humidity (55%). They had free access to food (CE-2, CLEA Japan Inc., Tokyo, Japan) and water ad libitum.

Acetic acid induced gastric ulcerogenesis. Rats were fasted for 20 h, but were provided with water ad libitum. This ulcer model was produced according to the modified method of Takagi et al. (1969). Rats under diethyl ether anaesthesia were subjected to coeliotomy to expose the stomach. 100 μL of glacial acetic acid was applied to the surface of the serosa at the junction of the fundus and antrum on the abdominal side in the glandular stomach through a plastic tube (6 mm ID) for 1 min, and then immediately the serosa of the stomach was washed with 0.9% NaCl. Before oceliorrhaphy, in order to prevent bacterial infection, the surgical wound area was treated with 3 drops of penicillin G solution (1 × 10^5 U/mL). After the operation, the rats were allowed free access to water and food. To assess the effect of BR-2 on the healing of ulcers, rats with ulcers were given BR-2 or atropine sulphate, as a positive control, intragastrically.
twice a day (9:00–10:00 a.m. and 5:00–6:00 p.m.) in a volume of 1.0 mL/100 g of body weight for 14 consecutive days starting on the day (day 1) after the acetic acid treatment. The control group was received water alone as a vehicle (1.0 mL/100 g of body weight). The rats were killed by an overdose of diethyl ether on day 15. The stomach was removed and incised along the greater curvature, followed by rinsing gently with 0.9% NaCl. The longitudinal and abscissal length of each ulcer was measured quickly under a stereoscopic microscope, and the multiplied product was used as the ulcer index. The healing ratio was calculated as follows:

\[
\text{Healing ratio (\%)} = \frac{\text{Ulcer index of control} - \text{Ulcer index of tested}}{\text{Ulcer index of control}} \times 100
\]

**Preparation of histochemical specimens.** After the ulcer size was measured as mentioned above, the stomach tissue was immersed in 5% formaldehyde–PBS solution for 1 week. The formalin-fixed tissue was then cut vertically against the serosa along the long diameter, and then embedded in paraffin. The sections were transferred onto a slide glass using the water flotation method, and stained with haematoxylin and eosin (HE) for ordinary light microscopic study.

**Statistical analyses of data.** Data are expressed as the mean ± SE. The statistical significance of the differences of the means was performed by one-way analysis of variance (ANOVA) followed by the Bonferroni/Dunn post hoc test.

**RESULTS AND DISCUSSION**

When rats were administered BR-2, it was shown to be effective in the healing of acetic acid-induced ulcer. The consecutive oral administration of BR-2 at doses of 50, 100 and 200 mg/kg twice a day for 14 days, produced a significant acceleration of the ulcer healing by 43.1%, 41.2% and 51.9%, respectively (Table 1). Oral administration of atropine sulphate at 10 mg/kg also significantly promoted the healing of the acetic acid induced ulcer (Table 1). Microscopic study also showed a significant healing effect by administration of BR-2 (Fig. 1). During

**Table 1. Effect of BR-2 on acetic acid-induced ulcers in rats**

<table>
<thead>
<tr>
<th>Treatment (p.o.)</th>
<th>Dose (mg/kg)</th>
<th>n</th>
<th>Ulcer index (mm²)</th>
<th>Healing ratio (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control (vehicle)</td>
<td>—</td>
<td>10</td>
<td>16.0 ± 2.1</td>
<td>—</td>
</tr>
<tr>
<td>BR-2</td>
<td>50</td>
<td>8</td>
<td>9.1 ± 1.6</td>
<td>43.1</td>
</tr>
<tr>
<td>BR-2</td>
<td>100</td>
<td>8</td>
<td>9.4 ± 2.2</td>
<td>41.2</td>
</tr>
<tr>
<td>BR-2</td>
<td>200</td>
<td>8</td>
<td>7.7 ± 2.4</td>
<td>51.9</td>
</tr>
<tr>
<td>Atropine sulphate</td>
<td>10</td>
<td>9</td>
<td>2.7 ± 1.1</td>
<td>83.1</td>
</tr>
</tbody>
</table>

BR-2 or atropine sulphate was administered orally to the rats twice a day (9:00–10:00 a.m. and 5:00–6:00 p.m.) in a volume of 1.0 mL/100 g of body weight for 14 consecutive days from the day after the formation of acetic acid ulcer. The rats were killed on day 15 after the acetic acid treatment. The data represent the mean ± SE. Significant differences from control, *p < 0.05, †p < 0.01, ‡p < 0.001.

this experiment, no bacterial infection by surgical operation was observed.

Previously, we reported that BR-2 has a potent protective activity against a wide variety of experimental gastric mucosal lesions, and this may be due to a reinforcement of resistance of the mucosal barrier by a protective coating of polysaccharide, an antisecreatory activity on acid and pepsinogen, and an oxygen radical scavenging activity (Sun et al., 1991; Matsumoto et al., 1993). Therefore, the combination effect of both the reinforcement of resistance of the mucosal barrier and the reduction of aggressive factors might be involved in the accelerated healing effect of BR-2 on acetic acid-induced gastric ulcer. However, a dose-related effect on ulcer healing was not observed with BR-2 administration. The reason is unknown at present, however, one possibility may be that the ulcer healing effect reached a plateau at a dose of over 50 mg/kg in this model.

Recently, it was reported that a sulphated mucopolysaccharide of animal origin, heparin, accelerated the healing of acetic acid-induced gastric ulcers, and this action was related to its effects in increasing the levels of gastric mucosal prostaglandin E2 (PGE2), the gastric mucosal blood flow, basic fibroblast growth factor (bFGF), epidermal growth factor (EGF) and constitutive nitric oxide synthase (cNOS) activity in the gastric mucosa (Li et al., 1998, 1999). It has been reported that the protective effect of BR-2 on experimental gastric lesions is not due to the action of endogenous PGs (Sun et al., 1991), therefore it must be assumed that the mode of action of heparin and BR-2 were different.

It has been assumed widely that polysaccharides are not absorbed from the digestive tract because of their high molecular weight. Recently, however, we have demonstrated that the antiulcer polysaccharide, bupleuran 2IIc, can be detected in the liver by using a specific antibody after oral administration of BR-2 to mice (Sakurai et al., 1996), and these results suggested that at least part of the orally administered bupleuran 2IIc was incorporated into the circulation. Previously, we reported that the protective effect of BR-2 against HCl/ethanol induced gastric lesions was observed by not only the oral route but also by intraperitoneal and subcutaneous administrations (Sun et al., 1991). Therefore it is presumed that BR-2 may exert its antiulcer activity not only from the luminal side of the stomach, but also from the subluminal side via the circulation.

In our preliminary experiments atropine sulphate accelerated ulcer healing of acetic acid induced ulcer, so we used it as a positive control. In this study, atropine sulphate showed a significant healing effect (Table 1), and this result accorded with that of Bacchi et al. (1994), who reported that atropine sulphate (5.3 mg/kg, p.o., daily) showed a significant healing effect on day 10th after the induction of ulcer by acetic acid. In contrast to our results Ito et al. (1994) reported that atropine sulphate was ineffective in healing acetic acid-induced gastric ulcers. The reason for these differences is not known at present. However, it may attributable to differences in experimental conditions. Ito et al. (1994) used SD strain rats as the experimental animal; in contrast, both we and Bacchi et al. used Wistar strain rats. The sensitivity to atropine and/or functions of cholinergic neurons of the stomach in Wistar and SD strain rats may have some differences, but in order to resolve the question, further study is required.

In conclusion, our results suggest that the polysaccharide from B. falcatum L. may be responsible for antiulcer activity, and supports the use of herbal prescriptions containing B. falcatum for the treatment of peptic ulcers.

Acknowledgements

The authors would like to thank Ms M. Murakami for her technical assistance. A part of this work was supported by Tsumura & Co., Tokyo.

REFERENCES


