Anti-inflammatory Effects of a Combined Herbal Preparation (RAH13) of Phellodendron amurense and Coptis chinensis in Animal Models of Inflammation

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In an attempt to develop an antiinflammatory herbal remedy that is as potent as current synthetic medicines, the cortex of Phellodendron amurense Rupr (Rutaceae) and the rhizomes of Coptis chinensis Franch (Ranunculaceae) were combined in a 2:1 ratio. This ratio was chosen based on in vitro experiments and traditional Asian medicine prescriptions. The combined ethanol extract, named RAH13, was evaluated for antiinflammatory properties using animal models of acute inflammation such as the croton oil-induced ear edema test and an acetic acid-induced capillary permeability test. Models of chronic inflammation were also tested using the cotton pellet test and a delayed-type hypersensitivity (DTH) test. Oral administration of RAH13 at a dose of 200 mg/kg showed in vivo antiinflammatory activity as potent as the effects associated with 100 mg/mL of celecoxib or 1 mg/kg of dexamethasone. These effects were seen in both acute and chronic inflammation models, suggesting that RAH13 may be effective in controlling some inflammation-related diseases. Copyright © 2007 John Wiley & Sons, Ltd.

Keywords: Phellodendron amurense Rupr. (Rutaceae); Coptis chinensis Franch (Ranunculaceae); delayed-type hypersensitivity (DTH); croton oil-induced ear edema; acetic acid-induced capillary permeability; cotton pellet-induced granuloma.

INTRODUCTION

Aspirin is presently one of the most widely used antiinflammatory drugs worldwide. However, a major factor limiting its use is the potential for gastrointestinal toxicity. A new generation of non-steroidal antiinflammatory drugs (NSAIDs) called cyclooxygenase (COX)-2 selective inhibitors (e.g. celecoxib, rofecoxib and valdecoxib), have been developed in an attempt to improve gastrointestinal tolerance (Zhang et al., 2006). The cardiovascular safety of these drugs was questioned several years ago; however, they appear to be well tolerated (Mukherjee et al., 2001). One of the major problems encountered in developing antiinflammatory medicines to date has been the occurrence of side effects. Therefore, antiinflammatory drugs with less severe side effects must be developed. There is an interest in natural products, given that they have recently become attractive as a source material for the development of new medicines. In addition, they are increasingly being used to treat various clinical diseases such as rheumatoid arthritis (Lipsky and Tao, 1997) and lupus erythematosus (Qin et al., 1981).

In Asian medicine, the cortex of Phellodendron amurense Rupr has been used to treat patients who suffer from gastroenteritis, abdominal pain and diarrhea (Uchiyama et al., 1989). This substance has long been used as a traditional herb for the treatment of cancer in Asian medicine as it is believed to have antiinflammatory, immunostimulatory and antitumor activities (Park et al., 1999). On the other hand, the rhizomes of Coptis chinensis Franch are a widely used treatment for fever, cough and angina (Jiangsu New Medical College, 1986). Coptis chinensis Franch has also been prescribed alone or in combination with other traditional herbs for the treatment of diabetes (Jia et al., 2003).

Berberine is the major active component in both plants and possesses strong antiinflammatory (Akhter et al., 1977) and antimicrobial (Amin et al., 1969) properties. Furthermore, it also has antihypertensive (Bova...
et al., 1992), anticholinergic (Tsai and Ochillo, 1991), antiarrhythmic (Wang and Zheng, 1997) and anticancer (Anis et al., 1999; Lin et al., 2004) effects. More recently, the cholesterol-lowering and anti-diabetic effects of berberine have been studied (Leng et al., 2004; Kong et al., 2004). The combination of these two medicinal materials (Phellodendron amurense Rupr and Coptis chinensis Franch) may have several beneficial effects, including fewer and less severe side effects than a single pure drug and increased efficacy through synergistic interactions (Lee, 2000). To address these questions, the cortex of Phellodendron amurense Rupr with the rhizomes of Coptis chinensis Franch were combined in a 2:1 ratio. This is the same ratio that is commonly used in herbal medicinal prescriptions in Korea.

This study was performed in several experimental animal models of inflammation to investigate the anti-inflammatory effects of the herbal combination of Phellodendron amurense Rupr cortex and Coptis chinensis Franch rhizomes (RAH13). This was done in order to develop an anti-inflammatory herbal medicine that demonstrates effects as potent as those associated with dexamethasone or celecoxib.

MATERIALS AND METHODS

Preparation of RAH13 extract. The cortex of Phellodendron amurense Rupr (200 g) and rhizomes of Coptis chinensis Franch (100 g) were dried in the shade and powdered. The powder was then extracted with 50% ethanol. The first extraction consisted of submerging the powder in 50% ethanol at 80 °C for 4 h. The ethanol extract was then drained off and this process was repeated two more times. The combined ethanol extract (RAH13) was filtered, freeze-dried (ca. 27 g) and kept at −20 °C. The RAH13 (200 mg/kg) was suspended in 0.5% (w/v) aqueous carboxymethyl cellulose (CMC) to prepare it for animal administration.

Animals. Female Balb/c and male ICR mice (4–6 weeks old) were obtained from Charles River Laboratories Inc. (Japan). Animals were maintained under standard environmental conditions in plastic cages at 21–24 °C on a 12 h light:dark cycle, with free access to pellet food and water. All experiments were conducted according to the ethical guidelines for the investigation of experimental pain in conscious animals.

Acute toxicity. ICR mice were divided into two groups of six mice each. Following an overnight fast, the experimental group was given a 5 g/kg oral dose of RAH13, while the control group received only the vehicle (0.5% CMC). Animals were observed during a 24 h period following treatment and mortality was recorded for each group at the end of the observation period.

Croton oil-induced ear edema. For the croton oil-induced ear edema test, ICR mice were fasted for 4 h but allowed free access to tap water throughout the study. The mice were divided into three groups: RAH13 (200 mg/kg), celecoxib (100 mg/kg; positive control) or vehicle (negative control). Each group consisted of six mice, and all drugs were orally administered. After 1 h, ear edema was induced by the topical application of croton oil dissolved in acetone (total volume of 15 µL) to the inner and outer surfaces of the right ear. The left ear received only acetone as a control. The edema rate was calculated 4 h after topical application with croton oil as follows: edema rate = (right ear thickness – left ear thickness)/left ear thickness × 100.

Acetic acid-induced vascular permeability test. Vascular permeability induced by acetic acid in mice was determined according to the Whittle method with some modifications (Yesilada et al., 1988). After ICR mice (n = 6 per group) were fasted for 4 h, RAH13, celecoxib or 0.5% CMC was orally administered to the respective group. After 1 h, each mouse was injected intravenously through the tail vein with brilliant blue (Sigma, St Louis, Missouri, USA) in a saline solution. Acetic acid was then injected intraperitoneally. After 20 min, the mice were killed and the viscera were exposed and irrigated with distilled water into a 50 mL tube. The dye exudates collected from the abdominal cavity were washed with distilled water. This washed solution was centrifuged and then a NaOH solution was added to the supernatant. The optical density (OD) of the supernatant was measured at a wavelength of 590 nm using a spectrophotometer (Emax).

Cotton pellet-induced granuloma. For the cotton-pellet induced granuloma test, ICR mice (n = 6 per group) were shaved and then anesthetized. Sterile pre-weighted cotton pellets were implanted in the axilla region of each mouse through a single needle incision. RAH13, celecoxib, or 0.5% CMC were administered once daily for 7 days. On day 8, the mice were anesthetized and the cotton pellets were surgically removed and excised from extraneous tissues. The pellets were incubated at 37 °C for 24 h and then dried at 60 °C to a constant weight. The incremental changes in the dry weight of the pellets were regarded as a measure of granuloma formation.

Delayed type hypersensitivity (DTH). DTH was induced by a modification of a technique that has been previously described (Blaylock et al., 1988). Balb/c mice were first sensitized on the skin of the shaved abdomen by epicutaneous application of a 25 µL mixture of acetone and olive oil (4:1) containing 2% 4-ethoxymethylene-2-phenyloxazolone. The mice were then divided into three groups of six mice each: RAH13, dexamethasone (positive control), or vehicle (negative control). On days 1, 3 and 5 after sensitization, RAH13 (200 mg/kg), dexamethasone (1 mg/kg) or a vehicle control was orally administered to the respective groups. On day 6, all mice were challenged on both sides of the right ear with 10 µL of 0.5% oxazolone dissolved in the acetone and olive oil mixture. The inhibitory effects of RAH13 and dexamethasone on the DTH reaction were analysed by comparing the amount of swelling with that of the control 0.5% CMC-fed mice. The intensity of the DTH reaction was expressed as follows: (right ear thickness – left ear thickness)/right ear thickness × 100 (measured 24 h after challenge with 0.5% oxazolone).

Statistical analysis. Results were expressed as the mean ± SEM. Statistical significance was determined using a Student’s t-test. Values of p < 0.05 were considered significant.
### RESULTS

#### Effect of RAH13 on acute toxicity

To test the acute toxicity of RAH13, mice were given a 5 g/kg oral dose of RAH13. None of the mice receiving this dose of RAH13 died during the 24 h period following ingestion of the compound (data not shown).

#### Effects of RAH13 in animal models of acute inflammation

RAH13 was evaluated for antiinflammatory activity in animal models of acute inflammation. First, in the croton oil-induced mouse edema test (Table 1), oral administration of RAH13 (200 mg/kg/10 mL of 0.5% CMC) significantly suppressed the croton oil-induced ear swelling response in mice. The level of edema inhibition was 26% at a RAH13 dose of 200 mg/kg, while celecoxib (100 mg/kg/10 mL of 0.5% CMC) inhibited edema by 24% compared with the control. This result suggests that the antiinflammatory effects of RAH13 are similar to that of celecoxib. Next, RAH13 was further tested for antiinflammatory effects in another model of acute inflammation, i.e. the vascular permeability test. As shown in Table 2, the dye leakage induced by acetic acid was significantly inhibited by 26% and 40% in response to 200 mg/kg of RAH13 and 100 mg/kg of celecoxib, respectively (compared with the control group). The antiinflammatory activity of RAH13 was less effective than that of 100 mg/kg celecoxib in this model. However, the antiinflammatory effects of RAH13 were statistically significant compared with the control group.

#### Effects of RAH13 in animal models of chronic inflammation

RAH13 was further evaluated for antiinflammatory activity in animal models of chronic inflammation. In the cotton pellet granuloma model, inflammation and granuloma developed during a period of several days. The inflammation response involves infiltration of macrophages, neutrophils and the proliferation of fibroblasts, which are the basic sources for granuloma formation (Warren, 1972). As shown in Table 3, when RAH13 and celecoxib were tested for antiinflammatory activity, both RAH13 and celecoxib markedly inhibited granuloma formation surrounding the pellets compared with the vehicle control group. RAH13 administration resulted in a 17% inhibition in granuloma weight of the cotton pellet, while the celecoxib inhibition was 15.4%. Another model of chronic inflammation, DTH, which measured the cell-mediated immune response, was also used for the evaluation of antiinflammatory effects of RAH13. As shown in Table 4, in the control group, the ear thickness of the immunized right ear was increased by 80% compared with that of the non-immunized left ear. However, the difference in ear thickness was lower in both the RAH13 group (48%) and the dexamethasone group (53%). RAH13 inhibited the cell-mediated inflammatory response by 40%, while dexamethasone inhibited it by 33%. These results show that RAH13 is able to induce antiinflammatory effects as potent as dexamethasone, although RAH13 was used at a 200-fold higher concentration than dexamethasone.

### DISCUSSION

Many antiinflammatory drugs currently in use have been developed to control inflammatory disorders involving localized increases in the number of leukocytes and various other complex mediator molecules (Mantri and Witiak, 1994). However, most have side effects. We have been interested in using natural products for the development of new drugs in an attempt to treat a variety of inflammatory disease without side effects. The *in vitro* antiinflammatory effects of many natural products from Asian herbal medicinal prescriptions used...
for inflammatory disease were tested. The cortex of Phellodendron amurense Rupr and the rhizomes of Coptis chinensis Franch were selected on the basis of antiinflammatory effects and traditional medicinal prescriptions. Phellodendron amurense Rupr and Coptis chinensis Franch were then combined in a 2:1 ratio and named RAH13.

The preliminary study demonstrated that RAH13 reduces the production of macrophage-mediated inflammatory modulators such as nitric oxide (NO) and prostaglandin E2 (PGE2) in LPS/IFN-γ-activated mouse peritoneal macrophages (unpublished data). These results suggest that RAH13 may have potent in vivo antiinflammatory effects by interfering, at least in part, with NO and PGE2 biosynthesis pathways. RAH13 also inhibited the proliferation of concanavalin A-stimulated splenocytes (unpublished data). Therefore, the present study was performed to further demonstrate the in vivo antiinflammatory actions of RAH13 in a number of experimental mouse models that represent different phases of inflammation.

The in vivo antiinflammatory effects of RAH13 were first evaluated in animal models of acute inflammation, i.e. croton oil-induced ear edema and acetic acid-induced vascular permeability. Croton oil-induced ear edema has frequently been used to assess the antiinflammatory effects of natural products. Olajide et al. investigated using this model to explain the antiinflammatory activity of the aqueous extract of Bridelia ferruginea stem bark (Olajide et al., 2000). The mechanism of croton oil-induced inflammation involves an increase in phospholipase A2 activity (Kondoh et al., 1985; McColl et al., 1986). RAH13 at a dose of 200 mg/kg inhibited croton oil-induced inflammation at the same level as 100 mg/kg celecoxib. Acetic acid-induced vascular permeability in a mouse model is a commonly used vascular permeability assay (Winter et al., 1962). The inflammatory response is a physiological characteristic of vascularized tissues (Rang et al., 1999). RAH13 reduced the intensity of the peritoneal inflammation produced by acetic acid, indicating that it has the ability to inhibit the permeability of small blood vessels in the process of acute inflammation.

The in vivo antiinflammatory effects of RAH13 were also tested in animal models of chronic inflammation, i.e. cotton pellet-induced granuloma formation and DTH. These models are considered a valuable way to assess the action of antiinflammatory drugs on the proliferation phase of inflammation (Selye, 1953). RAH13 exhibited significant antiinflammatory activity in the cotton pellet-induced granuloma test. This result reflects the ability of RAH13 to inhibit the proliferative phase of the inflammation process. In addition, the inhibitory effects of RAH13 on cell proliferation were further confirmed in the DTH model. In the DTH model, ear swelling is primarily the result of in vivo functions of antigen-specific CD4+ T cells activated through multiple intracellular signaling pathways (Grabbe and Schwarz, 1998; Kobayashi et al., 2001; Pimentel-Muinos et al., 1994). In this study, RAH13 was as potent as dexamethasone in inhibiting the swelling response, although RAH13 was used at a much higher concentration. These data indicate that RAH13 may down-regulate the cell-mediated inflammatory response by directly inhibiting T-cell activation.

The results indicate that RAH13 possesses antiinflammatory properties. The work also provides scientific evidence in favor of the development of RAH13 as an herbal medicine to control several inflammatory diseases. However, the molecular mechanisms by which RAH13 inhibits inflammation in animal models must be clarified by in vitro studies. In addition, disease-specific animal models (e.g. collagen-induced arthritis) should be used to elucidate the clinical value of RAH13.

In summary, RAH13, which is a combined ethanol extract of the cortex of Phellodendron amurense Rupr and the rhizomes of Coptis chinensis Franch, was formulated to develop an antiinflammatory herbal medicine. This extract was tested on the basis of an in vitro experiment and traditional medicinal prescriptions. Thus, RAH13 was evaluated for in vivo antiinflammatory activity in a variety of antiinflammatory animal models. RAH13 at a dose of 200 mg/kg produced antiinflammatory effects as potent as celecoxib or dexamethasone, although RAH13 was used at a much higher concentration. Detailed studies are needed to clarify the molecular mechanisms mediating the antiinflammatory effects of RAH13 in order to develop it for clinical use.

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Table 4. Effect of RAH13 on oxazolone-induced DTH in mice

<table>
<thead>
<tr>
<th>Drug</th>
<th>Dose (mg/kg)</th>
<th>Left ear thickness (x 10^-2 mm ± SEM)</th>
<th>Right ear thickness (x 10^-2 mm ± SEM)</th>
<th>Differences in swelling ear thickness (%)</th>
<th>Inhibition (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>–</td>
<td>15.9 ± 1.3</td>
<td>28.6 ± 2.0</td>
<td>12.7</td>
<td>79.96 ± 9.42</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>1</td>
<td>15.7 ± 1.3</td>
<td>24.1 ± 2.1*</td>
<td>8.4</td>
<td>53.58 ± 5.99</td>
</tr>
<tr>
<td>RAH13</td>
<td>200</td>
<td>16.0 ± 0.8</td>
<td>23.8 ± 1.6*</td>
<td>7.6</td>
<td>47.99 ± 6.06</td>
</tr>
</tbody>
</table>

* Statistical significance p < 0.05, Student’s t-test vs control.
REFERENCES


