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Preventive Effects of Unsei-in and Oren-gedoku-to, Chinese Traditional Medicines, Against Rat Paw Oedema and Abdominal Constriction in Mice

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Abstract

Oren-gedoku-to and Unsei-in are complex mixtures of ingredients derived from plants. These two drugs are clinically most frequently used in the treatment of Behçet's disease, we have investigated the anti-inflammatory and analgesic actions of Oren-gedoku-to and Unsei-in using a battery of tests; rat paw oedema induced by five different agents; abdominal constriction; mouse ear swelling; and dye leakage tests, designed to clarify the therapeutic potential of these medicines.

The findings in this study that these medicines are able to inhibit the rat paw oedema, suppress the abdominal constriction and inhibit the increased dye permeability confirm the analgesic and anti-inflammatory effects of these compounds.

From these results there seems a clear rationale for exploring the effectiveness of these Chinese medicines in the treatment of chronic and acute inflammatory diseases.

Oren-gedoku-to, which is a mixture of *Coptis root* (Franch), *Skutellaria* root (Georgi), *Phellodendron* bark (Rupr) and *Capejasmine* fruit (Ellis), is a traditional Chinese medicine and is used to treat hypertension, melena, apoplexia, nasal bleeding and palpitation. In addition to these cardiovascular diseases, it has been reported that Oren-gedoku-to can improve chronic inflammatory diseases, such as Behçet's disease and rheumatic arthritis (Shimizu 1975).

On the other hand, Unsei-in, which consists of *Coptis* root (Franch), *Skutellaria* root (Georgi), *Phellodendron* bark (Rupr), *Capejasmine* fruit (Ellis), *Angelica* root (Diels), White peony root (Pall), *Chuanxiong* rhizome (Franch) and prepared rhizome of Rehmannia (Libosch), is used to treat eczema, oral aphtha, pityriasis rosea Gibert and skin eruptions such as urticaria (Kaneko 1986).

These findings suggest that these drugs may have some anti-inflammatory effects. These Chinese medicines are clinically widely used but their properties and mechanism of action are still unclear. This study was carried out to investigate the anti-inflammatory effects of these medicines.

Materials and Methods

Male ddy mice (20–25 g) and Wistar rats (150–200 g) were obtained from Kanamaru Animal Laboratories (Tokyo). They were housed in temperature-controlled (22–24°C) cages with 12-h light-dark cycle, and were allowed free access to food and water. The experiments were carried out at a room temperature of 22–24°C, and humidity of 55–60%.

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Drugs

Unsei-in (Lot No.57, Tsumura, Tokyo), Oren-gedoku-to (Lot No.15, Tsumura, Tokyo) and ketoprofen (Capisten, Kissei, Tokyo) were gifts from the respective companies. Carrageenin, bradykinin, prostaglandin E_2, acetic acid and xylene were purchased from Wako Pure Chemical Industries Incorporated, Tokyo.

Adjuvant-induced rat paw oedema test

Carrageenin was prepared as a 1% suspension in saline. A volume of 0·1 mL was injected through a 26-gauge needle into the plantar tissue of the right hind paw. Immediately thereafter, the volume of the injected foot was measured. Measurement of foot volume was made using a plethysmometer (Unicon, Tokyo) as follows. The paw of the unanaesthetized rat was immersed in water exactly to an ink mark on the skin at the level of the lateral malleolus and water displacement recorded in mL on a linear scale. Thirty-two rats were randomly allocated to 4 equal groups. One group served as control, and received vehicle alone at the same time as the other groups were given the test solution. Unsei-in (0·25 or 0·5 g kg^{-1}) and Oren-gedoku-to (0·25 or 0·5 g kg^{-1}) was given orally via a stomach tube once a day for 5 days. Ketoprofen (10 mg kg^{-1}) was used as a positive control. Rat paw oedema was measured at 30 min, and then every hour until 6 h after the injection of each inciter.

Other phlogistic compounds used in this study to induce rat paw oedema were egg albumin (10% solution in saline, administered in 0·1 mL), bradykinin (10 μg in 0·1 mL saline, and 10 μg in carrageenin suspended solution), and prostaglandin E_2 (1 μg in carrageenin suspended solution). The methods described by Matsuda & Tanihara (1992)

were used. Unsei-in (0·5 g kg^{-1}) and Oren-gedoku-to (0·5 g kg^{-1}) was given orally via a stomach tube once a day for 5 days.

Bradykinin-induced capillary permeability test

Unsei-in (0·5 g kg^{-1}) or Oren-gedoku-to (0·5 g kg^{-1}) was given for 5 days to two groups of 8 rats, respectively. On day 5, following a final 30-min administration of the drug, brilliant blue 6B dye (1%, 30 mg kg^{-1}) was injected into the tail vein, immediately followed by bradykinin (10 μg in 0·1 mL) injected intradermally into the abdominal wall. After 30 min the animals were killed and their skin was removed. After removal of the subcutaneous tissue, the area of blue colouration was measured. A small spot (0·25 cm^2) was selected from a uniformly blue area and was removed with a hole punch. The portion of skin was immersed in 10 mL acetone solution (70% v/v in saline) overnight and the absorbance of the resulting solution was measured in a UV spectrophotometer at 590 nm.

Xylene-induced mouse ear oedema

Twenty mice were randomly divided into two equal groups. Unsei-in or Oren-gedoku-to was administered orally, via a stomach tube, once a day (0·25 or 0·5 g kg^{-1}) for 5 days to the two groups respectively. The method described by Brown (1964) was used. Briefly, xylene (0·03 mL) was applied topically to the right ear of each mouse, and 15 min later the mice were killed. Both ears of each mouse were removed and weighed. The left ear of each mouse served as the control.

Dye dilution

The method described by Whittle (1964) was used. Brilliant blue 6B (1%, 25 mg kg^{-1}) was administered to the mice (n = 10) through the tail vein and after 5 min acetic acid (0·7% v/v in saline, 0·1 mL per 10 g) was given intraperitoneally. The mice were killed 20 min later and the peritoneum was opened. The abdominal cavity was washed with distilled water and the fluid was collected and filtered with glass wool. The absorbance of the peritoneal lavage was measured on a UV spectrophotometer at 590 nm.

Mouse abdominal constriction test

This experiment was performed according to the method of Whittle (1964). Either Unsei-in or Oren-gedoku-to was administered for 5 days (0·25 or 0·5 g kg^{-1}) to two groups of 10 mice respectively. Acetic acid (0·7% v/v in saline, 0·1 mL per 10 g) was injected intraperitoneally to mice and abdominal constrictions monitored for 15 min.

Ketoprofen (10 mg kg^{-1}) was given intraperitoneally to a third group of mice and acted as a positive control. The abdominal constriction in mice treated with vehicle alone was used as the negative control.

Statistical analysis

All data are expressed as the mean ± s.d. The variances of the means were tested for homogeneity of distribution using the F-test. Where the variances were found to be normally distributed, the mean differences were compared using Student's t-test. $P < 0·05$ was taken as the level of significance.

Results

Adjuvant-induced rat paw oedema test

All three of the adjuvants (carrageenin, egg albumin and bradykinin) used alone resulted in a marked swelling of the injected paw over the time course of the experiments. The time scale of the oedematous reaction however varied in each case. Carrageenin caused a slow progressive swelling, which remained high for at least 4 h before beginning to decline at the end of the experiment (Fig. 1A).

Both bradykinin and egg albumin in contrast, produced an extremely rapid swelling within the first 30 min post injection, thereafter declining almost linearly to virtually control levels (Fig. 1B, C) throughout the remainder of the experiment. The combination of carrageenin and bradykinin (Fig. 2A) produced a swelling profile with an initial rapid swelling followed by a more delayed and prolonged increase to about 80% of the original paw volume.

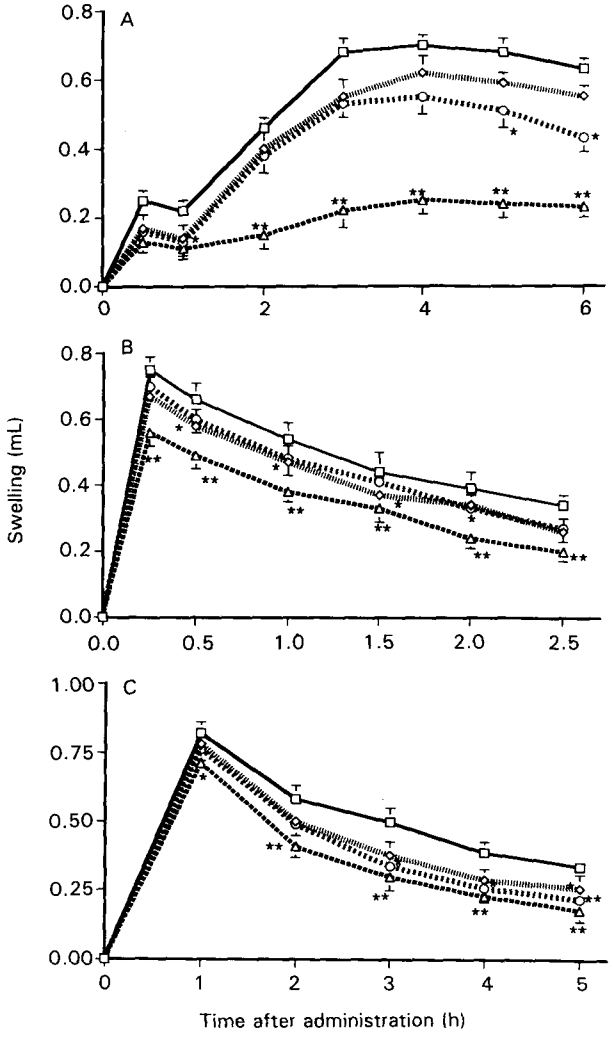

FIG. 1. Effects of Oren-gedoku-to (0·5 g kg^{-1}) and Unsei-in (0·5 g kg^{-1}) on rat hind paw oedema induced by (A) carrageenin (1 mg), (b) bradykinin (10 μg) and (C) egg white (10 mg). $^{**}P < 0·01$, $^{*}P < 0·05$, significantly different from the control. Error bars are means ± s.d. (n = 8). □ Control; ◇ Oren-gedoku-to; ○ Unsei-in; △ ketoprofen.

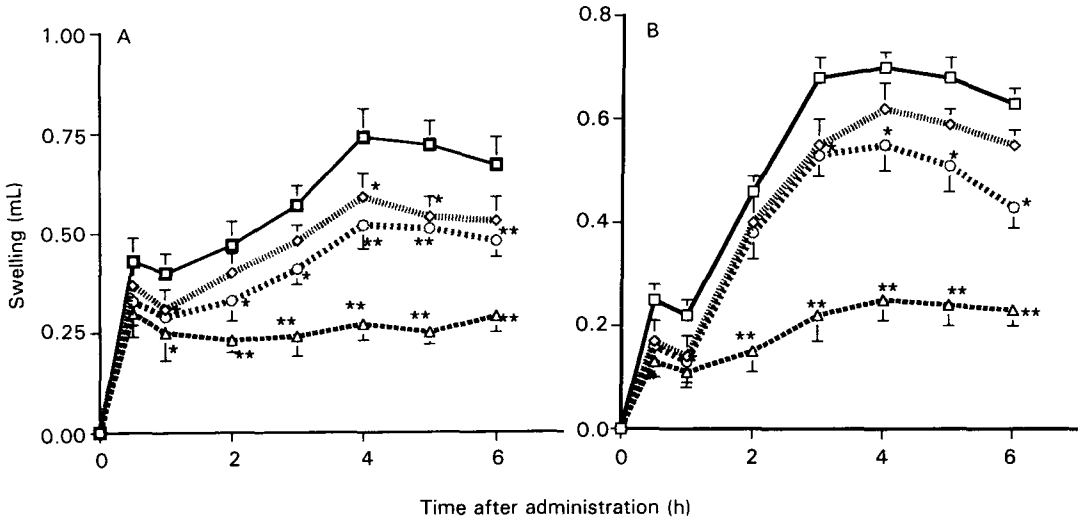

FIG. 2. Effects of Oren-gedoku-to (0·5 g kg^{-1}) and Unsei-in (0·5 g kg^{-1}) on rat hind paw oedema induced by (A) carrageenin (1 mg) plus bradykinin (10 μg) and (B) carrageenin (1 mg) plus prostaglandin (1 μg). $^{**}P < 0{\cdot}01$, $^{*}P < 0{\cdot}05$, significantly different from the control. Error bars are means ± s.d. (n = 8). □ Control; ◇ Oren-gedoku-to; ○ Unsei-in; △ ketoprofen.

Carrageenin together with PGE_2 gave a pattern of swelling that was not dissimilar to that of carrageenin alone in control animals (Fig. 2B).

Bradykinin-induced capillary permeability

The region of capillary permeability induced by bradykinin and the inhibition of the rate of dye leakage was suppressed by both Unsei-in and Oren-gedoku-to as shown in Table 1. In addition, the actual amount of leaked dye was smaller in the Oren-gedoku-to- and Unsei-in-treated groups. Table 2 shows that the rate of leakage caused by acetic acid was inhibited by these drugs.

Xylene-induced ear oedema

Xylene produced a rapid and extensive swelling of the right (treated) ear in all control animals. Both Unsei-in and Oren-gedoku-to inhibited this swelling as shown by a 28·3% (Unsei-in) and 26·3% (Oren-gedoku-to) reduction in right ear weight gain compared with the vehicle-treated control group (Table 3).

Table 1. Inhibitory effect of Oren-gedoku-to (0·5 g kg^{-1}) and Unsei-in (0·5 g kg^{-1}) on bradykinin induced brilliant blue extravasation in the rat.

Treatment	Area (cm^2)	Inhibition (%)	Exudated dye (λ 10 mL^{-1})	Inhibition (%)
Control	2·30 ± 0·3		24·5 ± 1·7	
Oren-gedoku-to	1·56 ± 0·2	23·2*	18·9 ± 2·2	22·9*
Unsei-in	1·63 ± 0·2	20·7*	19·8 ± 2·9	19·8*
Ketoprofen	1·08 ± 0·2	46·8**	12·4 ± 1·9	49·3**

Values are means ± s.d., n = 10. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, significantly different from control.

Table 2. Inhibitory effect of Oren-gedoku-to (0·5 g kg^{-1}) and Unsei-in (0·5 g kg^{-1}) on the capillary permeability by the dye dilution method in mice.

Treatment	Leakage of dye (λ 10 mL^{-1})	Inhibition (%)
Control	69·4 ± 2·6	
Oren-gedoku-to	46·0 ± 0·7	28·1**
Unsei-in	49·7 ± 1·3	23·1*
Ketoprofen	45·1 ± 3·3	30·2***

Values are means ± s.d., n = 10. $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$, $^{***}P < 0{\cdot}001$, significantly different from control.

Table 3. Effects of Oren-gedoku-to and Unsei-in (0·5 g kg^{-1}) on mouse ear oedema induced by xylene (0·03 mL in ear). Inhibition of the rate of swelling was 28·3% for Unsei-in and 26·3% for Oren-gedoku-to.

	Ear swelling (mg)	Inhibition (%)
Control	45 ± 5	
Oren-gedoku-to	30 ± 2·5*	26.3
Unsei-in	29 ± 2·5*	28.3
Ketoprofen	17·5 ± 2·0**	60.0

Means ± s.d. (n = 10). $^{*}P < 0{\cdot}05$, $^{**}P < 0{\cdot}01$ significantly different from the control.

Table 4. Effects of Oren-gedoku-to and Unsei-in administered for 5 days (0·25 or 0·5 g kg^{-1}) on mouse abdominal constriction by acetic acid (0·7% v/v, 0·1 mL/10 g).

		Number of constrictions
Control		38 ± 4·2
Ketoprofen	10 mg kg^{-1}	7·5 ± 1·1**
Oren-gedoku-to	0·25 g kg^{-1}	17·0 ± 3·8*
	0·5 g kg^{-1}	14·0 ± 2·4*
Unsei-in	0·25 g kg^{-1}	22·0 ± 2·5*
	0·5 g kg^{-1}	15·0 ± 1·8*

Means ± s.d. (n = 10). *$P < 0·05$, **$P < 0·01$ significantly different from the control.

Mouse abdominal constriction test

In mice administered vehicle alone the number of constrictions during the 15 min test period was 38 ± 4·2. The reaction was significantly reduced to 22 ± 2·5 and 17 ± 3·8 by Unsei-in and Oren-gedoku-to respectively (Table 4).

Discussion

The principal aspect of this study was performed using the adjuvant-induced rat paw oedema test. Oh-ishi & Sakuma (1970) have made an analysis of the effects of antagonists of inflammation using a mathematical model for rat paw oedema. They used this to classify the various agents modifying the rat paw oedema. They described a model which provided a reasonably good description of the data in some selected cases of experimental oedema in rats. The model is constructed on the assumption that the swelling volume is a reflection of the dynamic balance between the swelling force, evoked by the inflammatory stimulus, and the antiswelling force, intrinsic to the animal. The model describes the swelling volume (Y) as a function of time (t) following administration of the inciter, such that:

$$Y = C/(A - B) \times (e^{-B_t} - e^{-A_t}) \quad (1)$$

The three parameters, A, B and C, are concerned with the decay constant of swelling force, the degree of the antiswelling force and initial velocity of swelling force, respectively. In the broad sense, the decay constant could be correlated with the antiswelling force of the animal. A matter of importance is to determine which of the model parameters is most likely to be influenced by a given class of anti-inflammatory agent.

General depression of the magnitude of swelling is brought about either by an increase in A or B, or through a decrease in C. The modification in the swelling curve due to pretreatment with an agent is, however, more likely to be the result of concomitant change in these parameters as has already been reported. The authors concluded that, based on their results, the very early phase of the swelling process is the most important.

It is reported that the carrageenin oedema shows three distinct phases, namely an initial release of histamine and 5-hydroxytryptamine (5-HT), a second phase mediated by kinins and a third phase, the mediator of which is suspected to be prostaglandin (Di Rosa et al 1971). Swelling of the rat paw reached a peak in 1 h then retained about the same degree of swelling for 5 h. The inhibitory effects of the two drugs were noted at 3 h then gradually increased for 2 h, especially for the case of Unsei-in. Thus, Unsei-in and Oren-gedoku-to exhibit inhibitory effects in the second and third phases where kinins and prostaglandin are suggested to be involved.

In this respect we intend to make further study on relation between these substances and chemical mediators of inflammation. The effect of the two drugs studied here tended to be most pronounced at the later stage of the inflammatory response. This was particularly so with the very rapid reaction observed when bradykinin was used to promote the rat paw oedema response. Nevertheless, with the carrageenin-induced reaction a clear effect of Unsei-in and Oren-gedoku-to was observed at the first estimate 30 min post-dosing.

According to previous reports, some anti-inflammatory effects of these drugs were considered to be due to berberine, baicalein and paeoniflorin constituents. Among the chemical constituents of *Skutellaria* root which is one of the component drugs of Oren-gedoku-to, baicalin, baicalein, and wagonin are reported to have major pharmacological effects and to interfere with the activity of sialidase and inhibit the production of sialic acid (Nagai et al 1987). It has also been reported that *Coptis* root which is one of the crude components of Oren-gedoku-to, has the effect of activating immunity and that the immunological mechanisms are closely related to the occurrence of adjuvant arthritis in rat (Van Eden et al 1985). Many reports on anti-inflammatory effects of some crude components of Unsei-in, such as the aqueous extract of *Angelica* root administered orally to mice inhibited both the abdominal constriction and capillary permeability, suggesting an analgesic effect and anti-inflammatory effect respectively (Tanaka et al 1971). The anti-adjuvant arthritic effects of *Angelica* root and White peony root are also reported (Toita et al 1983). Furthermore, studies on White peony root revealed anti-convulsive, sedative, analgesic and anti-inflammatory effects (Sugishita et al 1984; Harada 1985).

In this study, two Chinese drugs clearly inhibited the swelling, but it is not yet clear whether or not this action is a specific anti-inflammatory effect. Bradykinin and prostaglandin E_2 are probably involved in the pathogenesis of the inflammatory response concerned but it is not yet elucidated whether these drugs have specific anti-bradykinin or anti-prostaglandin effects.

With respect to the development of selective pharmacological antagonists, the unexpected failure of tripelenamine in depressing histamine-induced oedema may deserve a special emphasis. It is also known that bradykinin induces the production of prostaglandin E. Juan (1977) showed that the PGE-release induced by bradykinin was only partly mediated by liberating cathecholamines and that bradykinin stimulates PGE release by selectively activating a phospholipase A_2 without affecting cyclo-oxygenase and subsequent enzymes.

Matsuda & Tanihara (1992) studied the effect of sialic acid on rat paw oedema and suggested that its anti-inflammatory effect was via its antagonism of PGE_2, because the third phase was suppressed by sialic acid. We are of the opinion that this conclusion still needs to be confirmed by other methods.

Although, in many instances, both in terms of the preparation as well as the agent, pharmacological agonist-antagonist relationships have been well established in the rat paw oedema test, other examples are found in bradykinin-induced oedema, which is depressed by tripelenamine, BOL-148, bromelain, pyridinolcarbamate and indomethacin (Oh-ishi & Sakuma 1970). Considering these facts, the key mechanism of these Chinese medicines seems extremely complicated and not so clear cut.

There have been many reports on Unsei-in and Oren-gedoku-to concerning their pharmacological efficacy with respect to blood coagulation, microcirculation, hypertension and other cardiovascular diseases (Fujiwara & Iwasaki 1993), as well as gastro-intestinal disorders (Takase et al 1991a, b; Fushitani et al 1994). Mori et al (1991) showed a significant decline in platelet aggregation rate in both healthy controls and patients in response to Oren-gedoku-to, and that the maximal platelet aggregation time shortened in healthy controls, and was prolonged in patients following cerebral infarction. Recently the immuno-suppressive effect of Unsei-in was reported and it was suggested to have value as a new type of immuno-suppressant drug. These findings encourage us to make further investigations of these drugs in connection with their anti-inflammatory actions.

The findings in this study that these medicines are able to suppress abdominal constriction and inhibit the increased dye permeability confirm the analgesic and anti-inflammatory effects of these compounds. From these results there seems a clear rationale for exploring the potential use of these Chinese medicines in the treatment of chronic and acute inflammatory diseases. However studies are necessary to determine in which of the ingredients of these heterogeneous mixtures the pharmacological properties reside.

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