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Effects of Oren-gedoku-to and Unsei-in, Chinese Traditional Medicines, on Interleukin-8 and Superoxide Dismutase in Rats

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Abstract

This study was conducted to elucidate the mechanisms of action of two Chinese traditional drugs, Oren-gedoku-to and Unsei-in, which have been used for many years in the treatment of inflammatory disorders.

In rats with acetic acid-induced inflammation, both drugs reduced interleukin-8 concentrations in the serum. Neither drug significantly affected superoxide dismutase activity in the serum, although Unsei-in increased superoxide dismutase activity in liver after 1 month of administration. Oren-gedoku-to showed no significant effect on liver superoxide dismutase activity.

It was considered that these medicines exert their anti-inflammatory effects mainly on the early stages of inflammation, wherein increased capillary permeability and migration of leucocytes occur.

The Chinese traditional blended medicine Oren-gedoku-to contains four components: *Coptis* rhizome, *Scutellaria* root, *Phellodendron* bark, and *Gardenia* fruit. Another Chinese traditional blended medicine, Unsei-in, contains *Rehamannia* root, Japanese angelica root, *Cnidium* rhizome,and peony root, as well as each component of Oren-gedoku-to. These traditional medicines have been clinically used for many years in the treatment of various diseases, such as Behçet's disease and rheumatoid arthritis, but they are mainly supported by clinical experience in China and Japan. There are already numerous clinical reports on Oren-gedoku-to and Unsei-in (Shimizu 1975; Kaneko 1986; Hashimoto 1986).

Recently, pharmacological studies of Oren-gedoku-to have been carried out to elucidate its anti-inflammatory action, haemostatic effect, anti-hypertensive action, and inhibitory effect on gastric mucosal lesions (Arakawa et al 1981; Adachihala et al 1983). However, sufficient experimental studies on Unsei-in have not yet been done.

The present study was undertaken to clarify the anti-inflammatory effects of Oren-gedoku-to and Unsei-in, using acetic acid-induced inflammation in the rat, with respect to interleukin-8 (IL-8) production and superoxide dismutase (SOD).

Materials and Methods

Animals

Male 5-week-old Wistar rats (120–150 g) were obtained from Kanamaru Animal Labo (Tokyo). They were housed in temperature-controlled (22–24°C) cages, with a 12-h light-dark cycle, and 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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Materials

Oren-gedoku-to (Lot no. 15, Tsumura, Tokyo), Unsei-in (Lot no. 57, Tsumura, Tokyo) and ketoprofen (Capisten, Kissei, Matumoto) were donated by the respective companies. Acetic acid and the Rat IL-8 kit (IBL Co., Lat. Gunma) and the SOD Wako test (Wako Pure Chemical Industries Inc., Tokyo) were obtained commercially.

In all experiments, Oren-gedoku-to and Unsei-in were respectively dissolved in water and administered orally to animals ($0{\cdot}5\,g\,kg^{-1}$) for 5 days or 1 month. Ketoprofen ($10\,mg\,kg^{-1}$, i.m.) was also given to a respective group as a positive control.

IL-8 production in the rat

Each of Oren-gedoku-to or Unsei-in were administered, $0{\cdot}5\,g\,kg^{-1}$ for 5 days to a respective group of 8 rats. Acetic acid (0·7% v/v in saline, $10\,mL\,kg^{-1}$) was injected intraperitoneally into the rats. After 24 h, blood was collected from the rat heart; after storage at 4°C overnight, the serum was separated and centrifuged at 1800 *g* for 10 min. The IL-8 production in serum was determined using a Rat IL-8 kit with sandwich enzyme-linked immunosorbent assay (Ishikawa,1993).

Determination of superoxide dismutase (SOD) in the rat

Both medicines were administered $0{\cdot}5\,g\,kg^{-1}$ for 5 days and for 1 month to respective groups of rats (four groups in total). In two groups, for each medicine, on day 5, 30 min after the administration of the medicines, acetic acid (0·7% v/v in saline, $10\,mL\,kg^{-1}$) was injected intraperitoneally into rats. After 24 h, blood was collected from the rat heart and centrifuged at 1800 *g* for 10 min. In the other groups, each medicine was administered for 1 month, serum was separated from blood taken from the heart, and SOD was determined using the SOD Wako test. $O_2^{\cdot-}$ is formed from xanthine by the action of xanthine oxidase (XOD) contained in the enzyme solution. The $O_2^{\cdot-}$ thus produced reduced nitroblue tetrazolium (NBT) and forms diformazan. When SOD is contained in a sample, partial $O_2^{\cdot-}$ is dismutated into H_2O_2 and O_2 and the production of

Table 1. Superoxide dismutase test procedure.

	Test assay		Blank assay	
	sample (mL)	Blank (mL)	sample (mL)	Blank (mL)
Sample	0·1	0·1 (water)	0·1	0·1 (water)
Colour reagent	1·0	1·0	1·0	1·0
Enzyme solution	1·0	1·0	–	–
Blank solution	–	–	1·0	1·0
	Mix, incubate accurately for 20 min at 37°C			
	2·0	2·0	2·0	2·0
	Mix well and measure absorbance at 560 nm			

SOD activity (ratio % of inhibition) = [1 − (A − C)/(B − D)] × 100
where A is absorbance of test assay sample, B is absorbance of test assay blank, C is absorbance of blank assay sample, and D is absorbance of blank assay blank.

Table 2. Effects of Unsei-in and Oren-gedoku-to on serum interleukin-8 production and superoxide dismutase in serum and liver homogenate in rats with acetic acid-induced inflammation.

	Serum IL-8 (pg mL^{-1})	Serum SOD (% inhibition)	Liver SOD (% inhibition)
Control	53·8	16·4 ± 3·2†	32·5 ± 4·2
Oren-gedoku-to	32·4 ± 0·4*	20·5 ± 4·1	40·1 ± 5·1
Unsei-in	36·8 ± 0·6*	20·7 ± 3·8	43·3 ± 4·7*
Ketoprofen	22·2 ± 0·3**	23·2 ± 3·1*	–

Values are means ± s.e.m. (n = 10). *Significantly different to controls $P < 0.05$, **$P < 0.01$.
†Serum SOD control in normal rats (in non-inflammatory state) = 20·71% inhibition (significantly different from control in inflamed rats, $P < 0.05$).

diformazan is markedly inhibited by competing for the superoxide radicals. The SOD activity of the sample is determined by measuring the inhibition rate of diformazan production. The method is shown in Table 1.

Statistical analysis

All data are expressed as the mean ± s.e.m. The variances of the means were tested for homogeneity of distribution using the *F*-test. When 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

Effects of Oren-gedoku-to and Unsei-in on IL-8 production in rats with acetic acid-induced inflammation are shown in Table 2. These medicines reduced IL-8 production: the amount produced by the Oren-gedoku-to group was 32·4 pg mL^{-1}, and that by the Unsei-in group was 36·9 pg mL^{-1}; these values were significantly ($P < 0.05$) different from that of the control group (53·8 pg mL^{-1}).

Table 2 also shows the effects of Oren-gedoku-to and Unsei-in on rat serum SOD in the acetic acid-induced group and the normal group. SOD activity was shown as an inhibition rate (%). Serum SOD was more increased in the acetic acid-induced group than in the normal group ($P < 0.05$), but the drugs did not show any effect on the serum SOD. However, SOD was increased in the liver homogenate of the rats treated with Unsei-in for 1 month. Oren-gedoku-to showed no significant effects either on SOD of rat serum or liver homogenate.

Discussion

In a previous study (Wang & Mineshita 1996), we showed that Oren-gedoku-to and Unsei-in have inhibitory effects on rat paw oedema caused by carrageenin alone or plus bradykinin or prostaglandin E_2 (PGE_2), and egg white, and they suppress the abdominal constriction response.

In this study, we found that IL-8 production was reduced in rats that were treated with Oren-gedoku-to and Unsei-in. IL-8 is a member of a recently described family of low-molecular-weight cytokines. This neutrophil chemoattractant/activator is produced by a wide variety of cell types, including monocytes, endothelial cells, and fibroblasts. Its production is elicited by inflammatory stimuli, such as lipopolysaccharide and phytohemagglutinin, as well as by pro-inflammatory cytokines, specifically tumour necrosis factor (TNF) and interleukin-1 (IL-1). Many studies have reported that IL-8 produced locally in inflammatory lesions plays an important role in the inflammatory process, and that IL-8 levels in various body fluids may help to illuminate the pathogenesis of inflammatory disease accompanied by leucocytosis, leucocyte infiltration, or both (Yue et al 1992). Since the identification of IL-8 in psoriatic scales, and in the synovial fluid of rheumatoid arthritis, and in other non-rheumatoid joint disease, many reports have been published on human IL-8 (Schroeder et al 1989; Brennan et al 1990). Yue et al (1994) have reported that IL-8 stimulates smooth muscle cells to produce PGE_2, which can inhibit IL-8-induced smooth muscle cell proliferation. It has also been reported that IL-8 plays a causative role in acute inflammation by recruiting and activating neutrophils (Harada et al 1994).

We reported that Oren-gedoku-to and Unsei-in inhibited the rat paw oedema induced by carageenan plus PGE_2, and that they also had an analgesic effect, and the present study showed that these medicines reduced IL-8 production. Together, these results suggest that both Oren-gedoku-to and Unsei-in tended to inhibit the inflammatory stage, that increased capillary permeability and migration of leucocytes.

On the other hand, SOD has been used systemically for the treatment of inflammatory, degenerative, and ischaemic disease, and it has been applied locally onto the skin and mucosal lesions in progressive systemic sclerosis, systemic lupus erythematosus, Behçet's disease, herpes simplex, and burns (Mizushima et al 1991). The anti-inflammatory activity of SOD has been seen in several animal models of induced inflammation, as well as in clinical trials with humans. Some

studies have reported that the participation of superoxide in the inflammatory response has been inferred from the anti-inflammatory effect of parenterally administered SOD, and that SOD inhibits the migration of neutrophils to sites of inflammatory challenge (Petrone et al 1980). The free radical or the oxidative species derived from it are toxic to cells and tissues, and this has been assumed to account for the anti-inflammatory activity of SOD. Some Chinese medicines can eliminate or inhibit the production of oxygen radicals; for example, Oren-gedoku-to has SOD's effect of eliminating oxygen radicals, the property brought about by the actions of *Sculellaria* root and *Phellodendron* bark (Takase et al 1991; Mizukawa et al 1993; Kobayashi et al 1994). The present study showed that Oren-gedoku-to and Unsei-in had a tendency to increase serum SOD in acetic acid-induced inflammation, but it was not quite significant, and in the rat liver Unsei-in increased SOD significantly after one month's treatment.

The process of inflammation can be broken down into three phases: the first phase features increased capillary permeability; the second phase is represented by the migration of leucocytes; and the proliferation of connective tissue is the hallmark of the third phase. Our results suggested that Oren-gedoki-to and Unsei-in can be considered to exert their anti-inflammatory effects mainly on the first and second phases, because they inhibit capillary permeability, and because they reduce IL-8 production (thus inhibiting migration of leucocytes) and increase SOD (a functional oxygen radical scavenger). Our laboratory has just begun studying IL-8. The present study utilized CINC (cytokine-induced neutrophil chemoattractant), which is the rat counterpart of human growth-regulated gene product (GRO). Further studies will be required on other types of inflammation, such as adjuvant arthritis and air-pouch granuloma, to look at the chief responses brought about by these medicines.

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