J. Pharm. Pharmacol. 1999, 51: 1065–1074
Received September 8, 1998
Accepted April 1, 1999

The Effect of Oren-gedoku-to on Experimental Colitis in Rats

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Abstract

In Japan and China, Oren-gedoku-to (a complex mixture of ingredients derived from plants) has been used as a herbal medicine in the treatment of inflammatory and ulcerative diseases. In other countries salicylazosulfapyridine has been used to treat inflammatory bowel disease. In this study, we have compared the effect of Oren-gedoku-to with salicylazosulfapyridine on trinitrobenzene–sulphonic acid-induced colonic damage in rats, a model representative of ulcerative colitis and Crohn's disease in man.

Oren-gedoku-to was administered orally for one or two weeks over a range of doses. Tissue damage scores, body weight, spleen weight, colon wet weight and colon wall thickness were measured, and colonic tissue levels of interleukin-8 (IL-8), leukotriene B_4 (LTB_4), prostaglandin E_2 (PGE_2), and myeloperoxidase activity were examined.

The results indicated that Oren-gedoku-to was effective in the treatment of inflammatory bowel disease in the rat model. Histological observation showed a quicker healing process of the lesions, and a reduction of inflammatory cell infiltration following administration of Oren-gedoku-to.

The precise mechanism of action for Oren-gedoku-to is still unclear; however, the reduction of IL-8, LTB_4, and PGE_2 observed suggests that the mechanism may be different from salicylazosulfapyridine (which has no effect on IL-8). There may be a potential benefit in offering combination therapy for the treatment of inflammatory bowel disease.

Oren-gedoku-to is a traditional Chinese herbal medicine consisting of a mixture of *Coptidis rhizoma*, *Scutellariae radix*, *Phellodendri cortex* and *Gardeniae fructus*. It is used to treat chronic inflammatory and ulcerative diseases such as Behcet's disease, rheumatic arthritis and peptic ulcer. We have studied the anti-inflammatory and anti-ulcerative effects of Oren-gedoku-to in a rat model of chronic colitis.

The pathological features of inflammatory bowel disease, such as Crohn's disease and ulcerative colitis, are marked by the presence of mucosal ulceration, and the infiltration of neutrophils and lymphocytes in mucous membranes. Although immunologic mechanisms have been postulated as playing an important role in the pathogenesis of these diseases, the etiology remains obscure. Salicylazosulfapyridine has been used frequently for the treatment of these diseases (Sutherland et al 1993), however its side effects, which are often severe, represent a major clinical problem. Consequently, traditional Chinese herbal medicines have recently generated increasing interest for the treatment of these diseases. We have reported (Zhou et al 1995) the effect of traditional Chinese herbal medicines on ulcerative colitis.

Several experimental models of colitis induced in various animal species have been reported (Ohkusa 1985; Sharon & Stenson 1985; Morris et al 1989). These models are useful in investigating the effects of drugs on damaged tissue. Hoshino et al (1994) studied the effect of a traditional Chinese herbal medicine using a model of colitis induced by dextran sulphate sodium in hamsters. In this study, we have examined the effect of Oren-gedoku-to on colonic damage using a rat model (Morris et al 1989) for chronic colon inflammation that possesses some of the histopathologic features of inflammatory bowel disease. Colonic inflammation was induced in rats by intrarectal administration of

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trinitrobenzene–sulphonic acid (TNB) in ethanol. TNB disrupts the epithelial barrier and induces mucosal injury.

A test for evaluating inflammation in the intestine has been established based on the determination of myeloperoxidase activity, a marker of tissue neutrophil infiltration (Krawisz et al 1984). We have measured myeloperoxidase activity three days, one week, and two weeks after TNB administration. Infiltration by neutrophils is a striking histological feature during active inflammatory bowel disease. The migration of circulating neutrophils into an inflamed site involves the response to several chemotactic factors, such as leukotriene B_4 (LTB_4) (Ford-Hutchinson et al 1980), platelet-activating factor (PAF) (Lee & Snyder 1985), and interleukin-8 (IL-8) (Baggiolini et al 1989; Leonard & Yoshimura 1990). Therefore, to investigate the effect of Oren-gedoku-to on these mediators, we measured the levels of IL-8, LTB_4, and PGE_2 in colonic tissues one week after TNB administration.

Also, we have addressed the effect of induced colitis on splenatrophy and have measured the wet weight of spleen. A relationship between splenatrophy and colitis in both man and rat has been suggested by several investigators (Pereira et al 1987; Tubaro et al 1993), however, definitive association has yet to be confirmed. The mechanism by which splenatrophy occurs in severe colitis remains unknown also.

Materials and Methods

Animals

One hundred and sixty eight Sprague–Dawley albino male rats (180–220 g), obtained from Saitama Breeding Laboratories (Saitama Prefecture, Japan) were used. They were kept in a restricted access room with a controlled temperature (22°C ± 2°C) and a 12-h light–dark cycle. Three animals were housed in each cage, and were fed standard pellets with tap water freely available. One hundred and forty four rats were divided into groups of 18 and colitis was induced by administration of 40 mg TNB. Twenty-four rats were divided into groups of six and colitis was induced by administration of 30 mg TNB.

All of the animal studies adhered to the guidelines established by the Tokyo Medical and Dental University for the care and use of laboratory animals.

Drugs

Oren-gedoku-to (Lot No. 15), a gift from Tsumura (Tokyo), was suspended in distilled water and shaken for 30 min. The aqueous Oren-gedoku-to was adjusted to the following dosages: 0·5, 1·0, and 2 g kg^{-1}.

Salicylazosulfapyridine (Sigma Chemical Company, St Louis, MO) was suspended in distilled water and shaken for 30 min. The aqueous salicylazosulfapyridine was adjusted to the following dosages: 0·1, 0·25 and 0·5 g kg^{-1}.

TNB was also obtained commercially from Sigma Chemical Company (St Louis, MO). It was dissolved in 50% ethanol and shaken for 30 min before use.

Colitis induction

Inflammation of the colon was induced by the method described by Morris et al (1989). A single instillation of TNB in the colon causes ulceration, releases mediators of inflammation and induces inflammatory cell infiltration for five weeks. To produce inflammatory bowel disease in rats a dose of 5–30 mg TNB is required. It is reported that a dose of 40 mg TNB induces splenatrophy (Tubaro et al 1993). Therefore, we used doses of 30 and 40 mg to allow us to study the spleen atrophy response as well as the inflammatory responses seen at lower doses.

The rats were fasted for 14 h, but had free access to drinking water. They were lightly anaesthetized with pentobarbital sodium (20 mg kg^{-1}, i.p.), and then 0·25 mL 50% ethanol containing 30 or 40 mg TNB was infused intrarectally via a catheter. The tip of the catheter was inserted after removing distal stools so that it was set at 7 cm proximately to the anus. To clear the TNB/ethanol solution from the cannula 0·5 mL air was injected and then the anus was clipped. The rats were left for 5 min in a supine Trendelenburg's position. A control group of rats was subjected to the same surgical procedures but received saline instead of TNB.

Drug treatment

The rats treated with 40 mg TNB were randomly allocated into seven groups. Six groups were treated with Oren-gedoku-to (0·5, 1, or 2 g kg^{-1}) or salicylazosulfapyridine (0·1, 0·25, or 0·5 g kg^{-1}) orally via a stomach tube once a day for two weeks. One group was used as a control, and received an equal volume of vehicle. Observations were made on days 3, 7 and 14 after the intracolonic administration of TNB. The rats were killed by pentobarbital sodium injection (90 mg kg^{-1}, i.p.) and a 7-cm segment of the distal colon was removed for functional and morphological studies. The rats treated with 30 mg TNB were randomly allocated

into three groups. Two groups were administrated Oren-gedoku-to ($1\,g\,kg^{-1}$) or salicylazosulfapyridine ($0{\cdot}25\,g\,kg^{-1}$) orally via a stomach tube once a day for one week. The remaining group served as a control and were administered an equal volume of distilled water. The measurements of colonic levels of IL-8, LTB_4 and PGE_2, and plasma levels of IL-8 were made one week after TNB administration.

Body weight measurement

The rats were weighed on an electric balance every day before and after TNB treatment for one week between 0900 and 1000 h.

Spleen weight measurement

On days 3, 7, and 14 after administration of 40 mg TNB, six rats from each group were randomly selected and killed by pentobarbital sodium injection ($90\,mg\,kg^{-1}$, i.p.). Their spleens were carefully removed and weighed immediately.

Colonic inflammation and damage assessment

On days 3, 7, and 14 after administration of 40 mg TNB, six rats from each group were randomly selected and killed by pentobarbital sodium injection ($90\,mg\,kg^{-1}$, i.p.). Macroscopic damage and microscopic colonic lesions were assessed as follows.

Macroscopic damage assessment. The distal colons were removed, opened by longitudinal incisions, and assigned code numbers. Each colon was assessed by a semiquantitative scoring system, in a blind trial procedure, described previously (Vilaseca et al 1990). Colonic adhesions to surrounding tissues, strictures, mucosal ulcerations, and wall thickening were noted (Table 1).

Histological evaluation of colonic damage. After macroscopic scoring, the mass of colon (7 cm from the anus) was taken out and immediately weighed. Two tissue samples (2×10 mm) were excised from each colon. The tissue samples were fixed in 4% buffered formaldehyde, and prepared for routine paraffin embedding. Sections of tissue (5-μm thick) were cut with a microtome, mounted on slides, and then stained with haematoxylin–eosin. Histological assessment by light microscopy was performed using a blind method on coded slides using the criteria of Vilaseca et al (1990) as outlined in Table 1. As an index of tissue oedema, colonic wall thickness was used by measuring the distance from the serosal surface to the luminal surface of the mucosa. Ulcer diameter was also measured.

Table 1. Criteria for the assessment of colonic damage.

Macroscopic score		
Adhesions	None	0
	Minimal	1
	Involving several bowel loops	2
Strictures	None	0
	Mild	2
	Severe, proximal dilatation	3
Ulcers	None	0
	Linear ulceration <1 cm	1
	Two linear ulcers <1 cm	2
	More sites of ulceration or one large ulcer >1 cm	3
Wall thickness	Less than 1 mm	0
	1–3 mm	1
	More than 3 mm	2
	Maximum score	10
Microscopic score		
Ulceration	No ulcer, epithelization	0
	Small ulcers <3 mm	1
	Large ulcers >3 mm	2
Inflammation	None	0
	Mild	1
	Moderate	2
	Severe	3
Depth of the lesion	None	0
	Submucosa	1
	Muscularis propria	2
	Serosa	3
	Maximum score	8

The mucosa, submucosa, and muscularis propria were separately evaluated for infiltration of leucocytes, such as neutrophils, eosinophils, macrophages, and lymphocytes. A scale of 0 to 3 was used to semiquantify inflammatory cell infiltration: 0, 0–10 leucocytes per high-power field; 1, 11–25 leucocytes per high-power field; 2, 26–50 leucocytes per high-power field; 3, $\geq$51 leucocytes per high-power field. At least eight high-power fields from each section were examined (Zipser et al 1987).

Myeloperoxidase activity measurement

Myeloperoxidase activity in the colon was measured by the method of Krawisz et al (1984). Subsequent to weighing on an analytical balance, the distal segment of the colon (200–400 mg) was suspended in 1·0 mL ice-cold 0·5% hexadecyltrimethylammonium bromide (Wako Pure Chemical Industries, Ltd, Osaka, Japan) in 50 mM phosphate buffer (pH 6·0), and then homogenized three times for 30 s using a tissue homogenizer (OMNI GLH-2017, Yamato Scientific Co., Ltd, Tokyo, Japan). The probe was rinsed twice with 1·0 mL buffer. After the homogenate had been thawed and refrozen three times, it was centrifuged

for 15 min at 4000 *g*. To measure myeloperoxidase activity spectrophotometrically, 0·1 mL supernatant was combined with 2·4 mL 60 mM phosphate buffer (pH 6·0), containing 0·202 mg mL^{-1} *O*-dianisidine hydrochloride (Sigma Chemical Co., St Louis, MO). After pre-incubation for 10 min at 25°C, 0·5 mL 0·0029% hydrogen peroxide (Wako Pure Chemical Industries, Ltd, Osaka, Japan) was added and incubated for 10 min at 25°C. To stop the reaction 0·5 mL 0·1% sodium azide was added. The absorbance at 460 nm was measured on a spectrophotometer (DU-640, Beckman Instruments, Inc., Fullerton, CA). A unit of myeloperoxidase activity was defined as that converting 1 μmol hydrogen peroxide to water in 1 min at 25°C, and was divided by colonic wet weight. The myeloperoxidase assay was performed blind using coded tubes.

IL-8 measurement

Samples of colon (100–150 mg) obtained at 7 days were immediately washed in cold phosphate-buffered saline (PBS; pH 7·4), blotted, weighed, and homogenized for 1 min in 0·01 M PBS, on ice. The homogenate was then centrifuged at 9000 *g* at 4°C for 30 min, and the supernatant was stored frozen at −79°C. Plasma was prepared from cardiac blood using EDTA as an anticoagulant and stored frozen at −79°C until analysis. Supernatant was diluted 1 : 10 to 1 : 50 in assay buffer, IL-8 levels were quantified with a rat IL-8 ELISA system (Amersham Pharmacia Biotech UK Limited, Amersham, UK) according to the manufacturer's instructions. Plasma diluted 1 : 4 in assay buffer was also measured by ELISA system. The detection limit of this assay was 4·7 pg mL^{-1}. The absorbance at 450 nm was measured on a 96-well plate reader. The levels of IL-8 in colonic tissue were expressed as ng immunoreactive IL-8 (g wet tissue)$^{-1}$.

LTB_4 and PGE_2 measurement

Samples (100–150 mg) for LTB_4 and PGE_2 assay were obtained as described above. LTB_4 and PGE_2 extraction were performed according to Wallace et al (1989). Briefly, samples were suspended in 1 mL 20 mM Tris buffer (pH 7·4), homogenized for 1 min and incubated in a water bath (37°C) for 20 min. The samples were then centrifuged at 9000 *g* for 1 min and the supernatant was frozen at −79°C. Supernatant was diluted 1 : 2 to 1 : 25 in assay buffer, LTB_4 and PGE_2 were measured with an enzyme immunoassay (EIA) system (Amersham Pharmacia Biotech UK Limited, Amersham, UK). The detection limit was 0·31 and 1 pg mL^{-1}, respectively. The LTB_4 and PGE_2 were expressed as ng (g wet tissue)$^{-1}$.

Statistical analysis

Unless otherwise stated, data are expressed as a mean ± s.d. Parametric data were analysed using Student's *t*-test. Nonparametric data were analysed with the Mann–Whitney U-test. With all statistical analysis, $P < 0.05$ was considered significant.

Results

Effects of Oren-gedoku-to and salicylazosulfapyridine on rat body weight

The effects of Oren-gedoku-to and salicylazosulfapyridine on rat body weight are shown in Figure 1. Body weight is expressed as the percent of weight on the day before treatment. The body weight of rats treated with 40 mg TNB decreased throughout the 5-day post-treatment period, but increased slightly on the 7th day. The groups administrated Oren-gedoku-to (2 g kg^{-1}) or salicylazosulfapyridine (0·5 g kg^{-1}) showed quick recovery of their body weight.

Effects of Oren-gedoku-to and salicylazosulfapyridine on spleen weight

As shown in Table 2, spleen weight was reduced on day 14 after 40-mg TNB administration. The

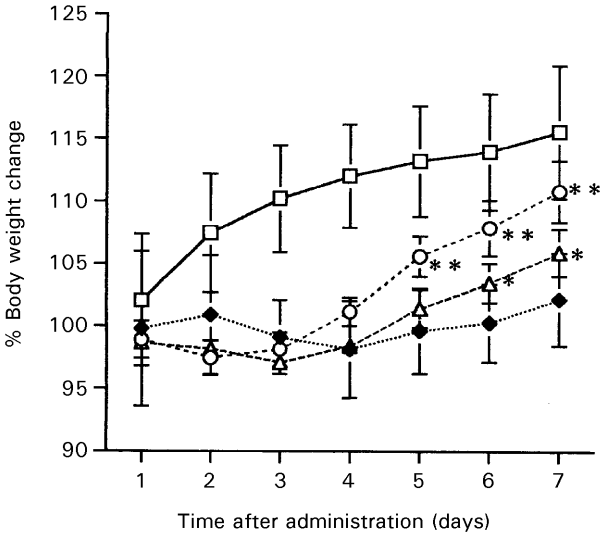

Figure 1. Effects of Oren-gedoku-to and salicylazosulfapyridine on rat body weight (blank, □; Oren-gedoku-to, △; salicylazosulfapyridine, ○; TNB, ◆) on day 1 to day 7 after 40 mg TNB administration. Salicylazosulfapyridine (0·5 g kg^{-1}) increased body weight on day 5 to day 7. Oren-gedoku-to (2 g kg^{-1}) increased body weight on day 6 and day 7. Results are mean ± s.d. (n = 6). $*P < 0.05$, $**P < 0.001$, compared with TNB group.

Table 2. Effects of Oren-gedoku-to and salicylazosulfapyridine on wet weight of spleen two weeks after treatment.

Treatment	Wet weight of spleen (mg)
Normal	1025·5 ± 118·2
TNB only	613·50 ± 69·62#
TNB + Oren-gedoku-to (0·5 g kg^{-1})	623·17 ± 59·33#
TNB + Oren-gedoku-to (1 g kg^{-1})	638·67 ± 60·51#
TNB + Oren-gedoku-to (2 g kg^{-1})	780·5 ± 46·58*
TNB + salicylazosulfapyridine (0·1 g kg^{-1})	626·5 ± 57·21#
TNB + salicylazosulfapyridine (0·25 g kg^{-1})	636·17 ± 44·31#
TNB + salicylazosulfapyridine (0·5 g kg^{-1})	630·50 ± 51·25#

Spleen atrophy occurred two weeks after 40 mg TNB administration and was improved by Oren-gedoku-to (2 g kg^{-1}). Salicylazosulfapyridine had no effect. Results are expressed as mean ± s.d., n = 6. #$P < 0·001$ compared with normal rats. *$P < 0·02$ compared with TNB control.

weight loss was recovered by using Oren-gedoku-to at a dose of 2 g kg^{-1}. Salicylazosulfapyridine was not effective in preventing splenatrophy at any dose.

Effects of Oren-gedoku-to and salicylazosulfapyridine on macroscopic damage

Figure 2A shows the mean macroscopic scores obtained from rats killed 3, 7 and 14 days after 40-mg TNB administration. On day 7 and 14, macroscopic damage scores were lower in rats treated with Oren-gedoku-to (2 g kg^{-1}). On days 3 7 and 14, macroscopic damage scores were lower in rats treated with salicylazosulfapyridine (0·5 g kg^{-1}) than in the control group, but there were no significant differences in the other groups.

Figure 3 shows photographs of macroscopic views of the colons of control, Oren-gedoku-to- and salicylazosulfapyridine-treated rats killed at various times after dosing. The development of chronic inflammatory lesions in the colons was mitigated by Oren-gedoku-to and salicylazosulfapyridine at large doses.

Effects of Oren-gedoku-to and salicylazosulfapyridine on the colon weight

Table 3 shows the mean wet weight of distal colons (7 cm) on day 3, 7 and 14 after TNB administration. Wet weight was maximal on day 3. Wet weight of the colon was lower on days 7 and 14 in the groups treated with Oren-gedoku-to (2 g kg^{-1}) or salicylazosulfapyridine (0·5 g kg^{-1}).

Effects of Oren-gedoku-to and salicylazosulfapyridine on colonic wall thickness

As outlined in Table 3 the mean thickness of the intestinal wall at the site of grossly visible ulceration and inflammation was increased on day 3, 7 and 14 after TNB administration; the thickness of the intestinal wall showed a peak on day 3. The wall thickness of the colon decreased on days 7 and 14 in the groups administered Oren-gedoku-to (2 g kg^{-1}) or salicylazosulfapyridine (0·5 g kg^{-1}).

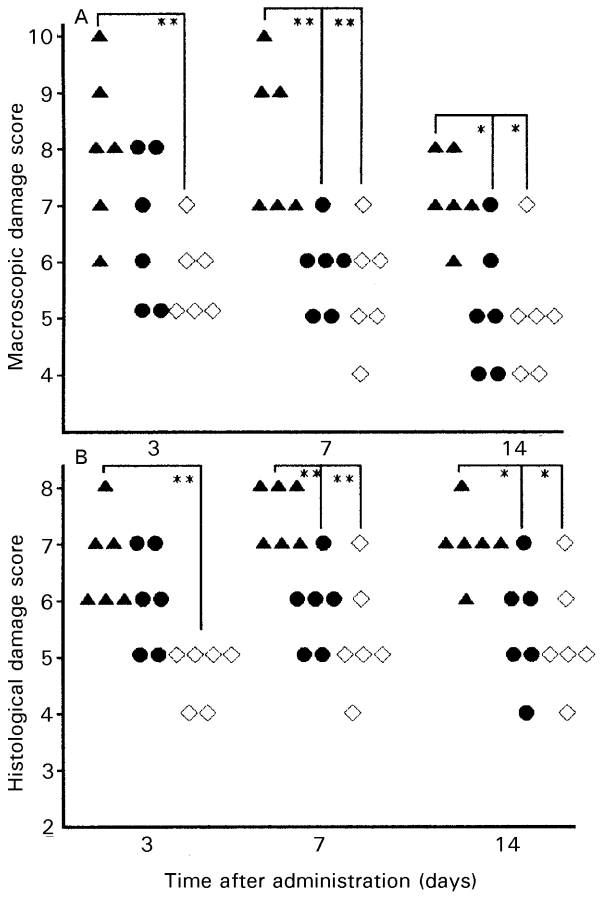

Figure 2. Effects of Oren-gedoku-to and salicylazosulfapyridine on macroscopic (A) and microscopic (B) damage score (TNB, ▲; Oren-gedoku-to, ●; salicylazosulfapyridine, ◇). Salicylazosulfapyridine (0·5 g kg^{-1}) reduced the colonic damage score (macroscopic or microscopic) on days 3, 7 and 14 significantly. Oren-gedoku-to (2 g kg^{-1}) decreased the colonic damage score (both macroscopic and microscopic) on days 7 and 14. Results are mean ± s.d. (n = 6). *$P < 0·02$, **$P < 0·01$, compared with TNB (40 mg) group.

Effects of Oren-gedoku-to and salicylazosulfapyridine on ulceration of the colon

The ulceration of the colon was examined on days 3, 7 and 14 after the induction of severe colitis by 40 mg TNB. Peak ulceration was on day 7. The mean diameter of the colonic ulcers was smaller on days 7 and 14 in the groups administered Oren-gedoku-to (2 g kg^{-1}) or salicylazosulfapyridine (0·5 g kg^{-1}) (Table 4). There were no significant differences in the other Oren-gedoku-to or salicylazosulfapyridine groups.

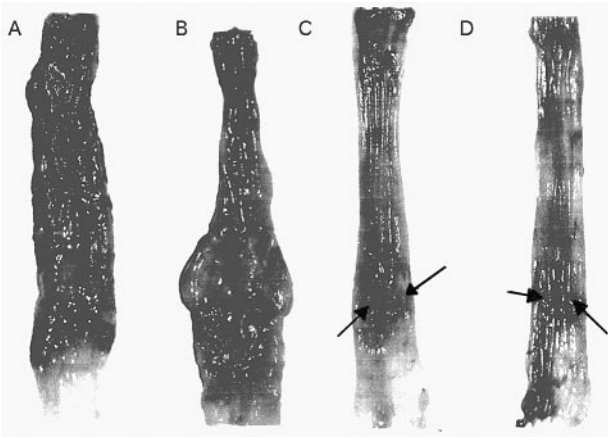

Figure 3. A. Colon of rat given 40 mg TNB on day 3 after administration. Note the extensive hyperaemia, oedema, and ulceration of the colon. This colon was given a damage score of 10. B. Colon of rat given TNB alone on day 7 after administration. Note the extensive large ulceration of the colon and the grossly visible enlargement of the colon. This colon was given a damage score of 10. C. Colon of rat on day 7 after treatment with Oren-gedoku-to ($2\,g\,kg^{-1}\,day^{-1}$). Arrow indicates an ulcer surrounded by grossly normal tissue. This colon was given a damage score of 6. D. Colon of rat on day 7 after treatment with salicylazosulfapyridine ($0{\cdot}5\,g\,kg^{-1}\,day^{-1}$). Arrow shows a discrete, transverse ulcer surrounded by grossly normal tissue. This colon was given a damage score of 4.

Effects of Oren-gedoku-to and salicylazosulfapyridine on histological damage score

The histological appearance of tissues examined on days 3, 7, and 14 after TNB administration showed tissue damage characterized by oedema, haemorrhage, epithelial exfoliation, and infiltration of polymorphonuclear leucocytes, macrophages, eosinophils, and lymphocytes. The peak of inflammatory cell infiltration was on day 7. As shown in Figure 2B, the histological damage score of the distal colon was lower on days 7 and 14 in the group administered Oren-gedoku-to ($2\,g\,kg^{-1}\,day^{-1}$), and on days 3, 7 and 14 in the group administered salicylazosulfapyridine ($0{\cdot}5\,g\,kg^{-1}\,day^{-1}$). Figure 4 shows the histological sections of the intestines of the TNB control rats at various stages. Eosinophilic cell infiltration in glandular mucosa was noted in a section obtained from a rat killed on day 3 (Figure 4A). In a section obtained from a rat killed on day 7 extensive ulceration and inflammation were noted (Figure 4B); areas of ulceration are separated by intact glandular mucosa. Extensive mucosal thickening and infiltration of polymorphonuclear leucocytes, macrophages, eosinophils and lymphocytes through the mucosa and submucosa were observed in Figure 4C, which was a section obtained from a rat on day 14, the preservation of goblet cells and crypt distortion were also noted.

Table 4. Effects of Oren-gedoku-to and salicylazosulfapyridine on intestinal ulceration.

Time after treatment	Diameter of ulcer (mm)		
	TNB only	TNB + Oren-gedoku-to ($2\,g\,kg^{-1}$)	TNB + salicylazosulfapyridine ($0{\cdot}5\,g\,kg^{-1}$)
7 days	$3{\cdot}77 \pm 0{\cdot}25$	$3{\cdot}01 \pm 0{\cdot}39$*	$2{\cdot}99 \pm 0{\cdot}37$*
14 days	$3{\cdot}15 \pm 0{\cdot}24$	$2{\cdot}6 \pm 0{\cdot}15$*	$2{\cdot}62 \pm 0{\cdot}15$*

Salicylazosulfapyridine ($0{\cdot}5\,g\,kg^{-1}$) and Oren-gedoku-to ($2\,g\,kg^{-1}$) reduced intestinal ulceration on day 7 and 14 compared with TNB 40-mg group. Results are mean ± s.d., n = 6. *$P < 0{\cdot}005$ compared with TNB control group.

Effects of Oren-gedoku-to and salicylazosulfapyridine on myeloperoxidase activity in inflamed colonic tissues

The mean myeloperoxidase activities in inflamed colonic tissues were increased on days 3, 7 and 14, with the maximum on day 3 after TNB administration (Table 5). In the group that had been treated with Oren-gedoku-to ($2\,g\,kg^{-1}$), the myeloperoxidase activity of colonic tissues was lower on days 7 and 14. In the group that had been treated with

Table 3. Effects of Oren-gedoku-to and salicylazosulfapyridine on wet weight of colon and intestinal wall thickness.

Time	Wet weight of colon (mg/7 cm)				Intestinal wall thickness (mm)			
	Normal	TNB only	Oren-gedoku-to ($2\,g\,kg^{-1}$)	Salicylazosulfapyridine ($0{\cdot}5\,g\,kg^{-1}$)	Normal	TNB only	Oren-gedoku-to ($2\,g\,kg^{-1}$)	Salicylazosulfapyridine ($0{\cdot}5\,g\,kg^{-1}$)
Day 3	$675{\cdot}83 \pm 22{\cdot}36$	$2341{\cdot}3 \pm 182{\cdot}8$†	$2238{\cdot}5 \pm 87{\cdot}3$†	$2205{\cdot}8 \pm 166{\cdot}9$†	$0{\cdot}665 \pm 0{\cdot}06$	$3{\cdot}53 \pm 0{\cdot}12$†	$3{\cdot}33 \pm 0{\cdot}28$†	$3{\cdot}28 \pm 0{\cdot}31$†
Day 7	$680{\cdot}5 \pm 15{\cdot}06$	$2105{\cdot}5 \pm 101{\cdot}9$†	$1790{\cdot}5 \pm 69{\cdot}01$*	$1787{\cdot}7 \pm 123{\cdot}5$*	$0{\cdot}73 \pm 0{\cdot}11$	$3{\cdot}37 \pm 0{\cdot}28$†	$2{\cdot}86 \pm 0{\cdot}16$*	$2{\cdot}72 \pm 0{\cdot}26$*
Day 14	$670{\cdot}67 \pm 23{\cdot}36$	$1740{\cdot}7 \pm 84{\cdot}08$†	$1523{\cdot}5 \pm 66{\cdot}77$*	$1561{\cdot}5 \pm 92{\cdot}5$*	$0{\cdot}67 \pm 0{\cdot}06$	$3{\cdot}1 \pm 0{\cdot}18$†	$2{\cdot}68 \pm 0{\cdot}17$*	$2{\cdot}66 \pm 0{\cdot}19$*

Salicylazosulfapyridine ($0{\cdot}5\,g\,kg^{-1}$) and Oren-gedoku-to ($2\,g\,kg^{-1}$) decreased the wet weight of the colon and intestinal wall thickening on days 7 and 14 compared with the group administered 40 mg TNB. Results are mean ± s.d. n = 6. *$P < 0{\cdot}05$ compared with TNB control group. †$P < 0{\cdot}001$ compared with normal rats.

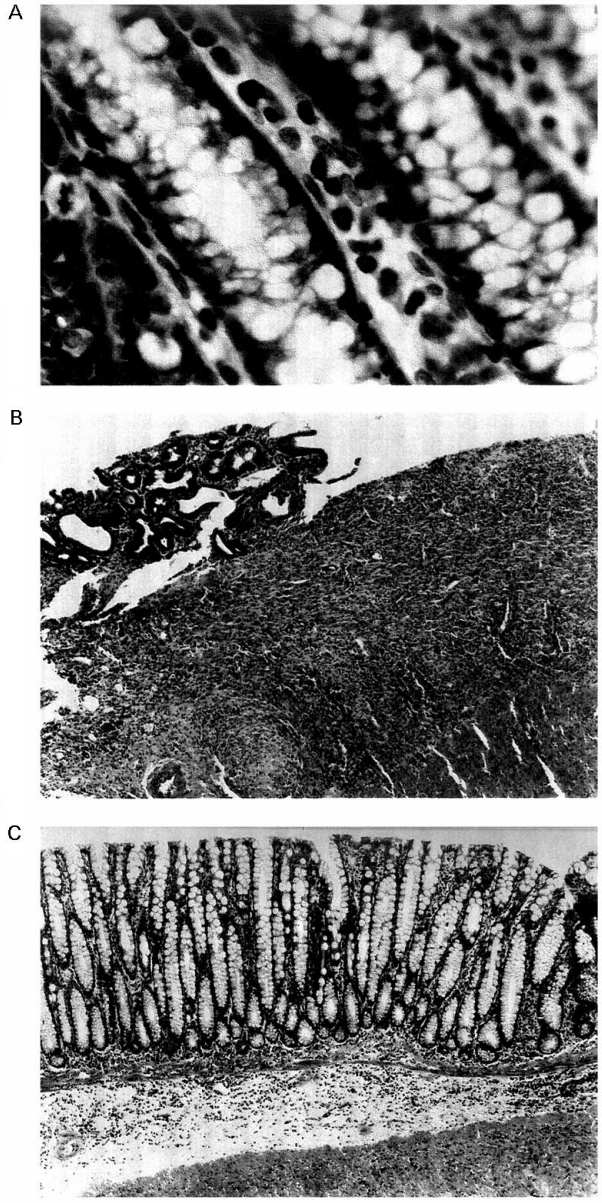

Figure 4. Histological sections of rat intestine. A. Eosinophilic cell infiltration in glandular mucosa in a sample from a rat on day 3 after 40 mg TNB administration. Magnification, ×100 (haematoxylin & eosin). B. Ulceration and inflammation in a sample from a rat on day 7 after 40 mg TNB administration. Areas of ulceration are separated by intact glandular mucosa. Magnification, ×40 (haematoxylin & eosin). C. Light micrograph of a sample from a rat on day 14 after 40 mg TNB administration. Note the mucosal thickening and preservation of goblet cells. Magnification, ×40 (haematoxylin & eosin).

salicylazosulfapyridine ($0{\cdot}5\,g\,kg^{-1}$), myeloperoxidase activity was lower on days 3, 7, and 14.

Effects of Oren-gedoku-to and salicylazosulfapyridine on IL-8 in inflamed colonic tissues and plasma

The increase in IL-8 concentration in inflamed colonic tissues of TNB-administrated rats was striking (Table 6). It was decreased by the administration of Oren-gedoku-to ($1\,g\,kg^{-1}$) for one week; salicylazosulfapyridine ($0{\cdot}25\,g\,kg^{-1}$) had no influence on IL-8. In contrast, with respect to the plasma IL-8 concentration there were no significant differences among the colitis and control groups.

Effects of Oren-gedoku-to and salicylazosulfapyridine on LTB_4 and PGE_2 in inflamed colonic tissues

The administration of TNB resulted in a striking increase in the concentration of LTB_4 and PGE_2 in colonic tissues (Table 6). The levels were decreased by the administration of Oren-gedoku-to ($1\,g\,kg^{-1}$) or salicylazosulfapyridine ($0{\cdot}25\,g\,kg^{-1}$) for one week.

Discussion

Reports on the relationship between spleen atrophy or hyposplenism and colitis in man (Ryan et al 1978; Pereira et al 1987) has led us to use spleen weight as a parameter. In this study, splenatrophy was observed two weeks after 40-mg TNB administration, and was inhibited by Oren-gedoku-to ($2\,g\,kg^{-1}\,day^{-1}$). Although the mechanism of hyposplenism still remains uncertain, the involvement of a severe lymphoreticular dysfunction (McCarthy et al 1966) and the impairment of the splenic microcirculation (Ryan et al 1981) are reported to be involved. *Phellodendric cortex*, a component of Oren-gedoku-to, acts as an immune regulator (Mori et al 1994), and it is thought that this may explain how Oren-gedoku-to inhibits splenatrophy.

The mode of action of salicylazosulfapyridine has been a matter of dispute. Several studies have shown that salicylazosulfapyridine or one of its metabolites, 5-aminosalicylic acid, acts as an inhibitor of inflammatory mediators such as leukotrienes (Nielsen et al 1987), PAF (Eliakim et al 1988), IL-1 generation (Mahida et al 1991), and PGE_2 (Sharon et al 1978) released from intestinal cells. Its side effects such as anti-fertility (Wu et al 1989), fulminant hepatic failure, necrotizing pancreatitis (Rubin 1994) and folate deficiency (Jensen et al 1996) are also widely recognized. Our study shows that salicylazosulfapyridine was effective in attenuating the lesions in TNB-induced colitis dose-dependently, but was of no value in preventing splenatrophy.

Recently, Oren-gedoku-to has been used with positive effect for treatment of some chronic and intractable diseases, such as hypertension, cerebro-

Table 5. Effects of Oren-gedoku-to and salicylazosulfapyridine on myeloperoxidase activity of intestinal wet tissue.

Time after treatment	Myeloperoxidase activity (units (g wet tissue)$^{-1}$)			
	Normal	TNB only	TNB + Oren-gedoku-to (2 g kg^{-1})	TNB + salicyl-azosulfa-pyridine (0·5 g kg^{-1})
3 days	0·051 ± 0·047	1·282 ± 0·057	1·239 ± 0·053	1·174 ± 0·019*
7 days	0·057 ± 0·055	1·11 ± 0·018	0·977 ± 0·069*	0·979 ± 0·018*
14 days	0·071 ± 0·06	1·096 ± 0·036	0·981 ± 0·047*	0·945 ± 0·035*

Oren-gedoku-to (2 g kg^{-1}) and salicylazosulfapyridine (0·5 g kg^{-1}) decreased myeloperoxidase activity compared with TNB 40-mg group. Results are mean ± s.d., n = 6. *$P < 0·05$ compared with TNB control group.

Table 6. Effects of Oren-gedoku-to and salicylazosulfapyridine on IL-8, LTB_4, and PGE_2 levels in inflamed colon and IL-8 levels in plasma.

	Levels of tissue IL-8, LTB_4, and PGE_2 (ng (g wet tissue)$^{-1}$) and circulating IL-8 (ng mL^{-1})			
	Normal (n = 6)	TNB only (n = 5)	TNB + Oren-gedoku-to (n = 6)	TNB + salicylazosulfa-pyridine (n = 6)
IL-8 (colon)	1·85 ± 0·20***	13·70 ± 2·40	9·83 ± 1·63**	12·35 ± 1·54
IL-8 (plasma)	5·36 ± 1·87	6·59 ± 1·33	5·72 ± 0·81	6·38 ± 1·10
LTB_4 (colon)	4·35 ± 1·10**	26·86 ± 14·60	9·23 ± 4·28*	7·59 ± 2·07*
PGE_2 (colon)	401·10 ± 79·20**	882·50 ± 187·10	652·60 ± 69·90*	631·90 ± 83·90*

Segments (100–150 mg) were obtained from five to six rats killed one week after 30 mg TNB administration. They were homogenized and centrifuged. IL-8, LTB_4, and PGE_2 levels in the supernatant were measured using an ELISA kit. Oren-gedoku-to (1 g kg^{-1}) or salicylazosulfapyridine (0·25 g kg^{-1}) were administrated orally once a day for one week. Results are expressed as mean ± s.d. *$P < 0·05$, **$P < 0·01$, ***$P < 0·001$ compared with TNB control.

vascular accidents, cardiovascular diseases and Behcet's disease due to its vasodepressor activity, haemostatic action and augmentation of cerebral blood flow (Shimizu 1975). Takase et al (1987, 1991) reported that it protected the mucosal barrier in rats; it is possible that these effects of Oren-gedoku-to are implicated in the inhibition of colonic damage. Studies have shown that Oren-gedoku-to has several different effects. Sekiya & Okuda (1982) showed it to be an inhibitor of leukotrienes and prostaglandin biosynthesis (attributed to one of its components, *Scutellariae Radix*, which contains baicalin and baicalein). Fushitani et al (1995) showed it to act as a scavenger of free radicals whilst Mori et al (1991) reported it to be an inhibitor of platelet aggregation (due to *Coptidis Rhizoma* and *Phellodendri Cortex* which affect the cyclic nucleotide metabolic system mainly through the action of Berberine). Wang et al (1997) reported it to be an attenuator of IL-8.

Ecosanoids are mediators of inflammation that are derived from membrane phospholipase A_2 and cyclooxygenase or 5-lipoxygenase, which can affect vascular permeability and can also promote the activation of neutrophils to release free radicals and proteases that have the potential to cause tissue necrosis. Both cyclooxygenase products (PGE_2, thromboxane A_2) and lipoxygenase products (LTB_4, LTC_4) of arachidonic acid have been found in colonic mucosa from patients with active inflammatory bowel disease (Ligumsky et al 1981; Sharon & Stenson 1984). In addition, several drugs that have been shown to have beneficial results in inflammatory bowel disease-treatment, including corticosteroids, salicylazosulfapyridine, and 5-aminosalicylic acid, have been shown to reduce production of leukotrienes in the inflamed colon (Peskar et al 1986). Those authors suggested that LTB_4 probably played an important role in the recruitment of neutrophils into the inflamed tissue in inflammatory bowel disease. Animal models have provided the evidence that leukotrienes play a role in the pathogenesis of colitis (Zipser et al 1987) and leukotriene synthesis inhibitors accelerated the healing process in a rat model of colitis (Wallace & Keenan 1990). More recently, interest has increased in IL-8 as a powerful neutrophil chemoattractant and activator. A large number of

studies have shown increased levels of IL-8 in inflammatory bowel disease (Izutani et al 1995; Daig et al 1996; Ina et al 1997), which has been shown to correlate with the macroscopic grade of local inflammation and with colonic myeloperoxidase activity in patients with the disease. Myeloperoxidase is an enzyme found predominantly in the azurophilic granules of neutrophils and has been used as a quantitative index of inflammation in several tissues, including the intestine (Krawisz et al 1984). For this reason we used myeloperoxidase activity as an index of inflammation. In agreement with previous studies, our results showed that myeloperoxidase activity correlated well with the macroscopic and microscopic grade of inflammation. Recent in-vitro studies demonstrated that IL-8 could function as an inducer of LTB_4, which is regarded as the major chemo-attractant in inflammatory bowel disease (Thomsen et al 1991). Harada et al (1994) also reported a sequential relationship among IL-8, LTB_4 and the degree of neutrophil infiltration within the intestinal mucosa using a model of TNB-induced colitis. Those results suggested that LTB_4 production in inflammatory bowel disease may be a secondary event, possibly a consequence of neutrophil infiltration induced by IL-8. This is in agreement with the results of our study in which IL-8, LTB_4, and PGE_2 levels in the inflamed tissues increased strikingly at one week after administration of TNB. Oren-gedoku-to ($1\,g\,kg^{-1}\,day^{-1}$) decreased all three of the mediators, IL-8, LTB_4 and PGE_2, in colonic tissues. In contrast, although salicylazosulfapyridine ($0{\cdot}25\,g\,kg^{-1}\,day^{-1}$) decreased LTB_4 and PGE_2 levels, it had no effect on IL-8. It has also been reported that salicylazosulfapyridine had no major influence on IL-8 in-vitro (De-Gendt et al 1998). These results suggested that the mode of action of Oren-gedoku-to was different from that of salicylazosulfapyridine.

In conclusion, our results demonstrated the anti-inflammatory and anti-ulcerative effects of Oren-gedoku-to on TNB-induced colitis in rats. Oren-gedoku-to inhibited myeloperoxidase activity and significantly decreased IL-8, PGE_2, and LTB_4 levels. Oren-gedoku-to may be useful for the treatment of inflammatory bowel disease. It is possible that the dose of salicylazosulfapyridine needed for treatment of inflammatory bowel disease could be reduced if combined with Oren-gedoku-to. This therapeutic strategy could reduce the incidence of severe side effects currently seen with high doses of salicylazosulfapyridine.

Acknowledgements

We thank Dr Neil Kitteringham, from the Department of Pharmacology, University of Liverpool, UK, for his valuable advice and encouragement. We thank also Dr T. Kuroiwa and Ms T. Tajima, from the Department of Neuropathology, and Assistant Professor K. Shiba and Mrs K. Sugimoto, from the Department of Hygiene, Tokyo Medical and Dental University, for fruitful advice and expert technical assistance.

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