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Protective Effect of DA-9601, an Extract of *Artemisiae Herba*, against Naproxen-induced Gastric Damage in Arthritic Rats

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Gastrointestinal irritation is the most frequent adverse effect in patients chronically taking nonsteroidal antiinflammatory drugs (NSAIDs) for the treatment of arthritic conditions. Gastroprotective effect of DA-9601, a new antiulcer agent from *Artemisiae Herba* extract, against NSAID was evaluated in a rat model of arthritis that is similar in many aspects to human rheumatoid arthritis. Daily oral dosing of naproxen (30 mg/kg), one of the most commonly used NSAID, induced apparent gastric lesions as well as a significant decrease in mucosal prostaglandin E_2 (PGE_2) and prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$) levels. Coadministration of DA-9601 prevents naproxen-induced mucosal injury and depletion of prostaglandins, in a dose-related manner. DA-9601 did not alter the antiinflammatory or analgesic effect of naproxen. The present results suggest that DA-9601 may be useful as a mucoprotectant against NSAIDs in clinical practice.

Key words : DA-9601, *Artemisiae Herba,* Nonsteroidal antiinflammatory drug, Prostaglandin, Arthritis, Naproxen

INTRODUCTION

Nonsteroidal antiinflammatory drugs (NSAIDs) are widely used in clinical medicine for the treatment of many degenerative and inflammatory disorders, and are the mainstay of therapy for the pain and inflammation associated with diseases such as osteoarthritis (OA) and rheumatoid arthritis (RA) (Simon and Mills, 1980; Kastrup, 1983). The most common adverse effect in arthritis patients with prolonged use of NSAIDs is gastrointestinal (GI) symptom, such as nausea, vomiting, dyspepsia, ulceration and bleeding (Caruso and Porro, 1980; Roth and Bennett, 1987). NSAIDs-associated gastroenteropathy is most common among patients who use these drugs for the treatment of arthritis. Particularly, RA patients have been reported to be more susceptible to NSAIDs-induced ulcer than other NSAIDs users (Fries *et al.*, 1989). And it was also been proven that arthritic rats are more vulnerable to NSAIDs-induced gastric damage than healthy rats (McCafferty *et al.*, 1995). It is clear that suppression of prostaglandin synthesis contributes both to antiinflammatory and toxic effects in the upper GI tract (Scheon and Vender, 1989; Rainsford, 1993), but the exact pathogenesis of this disorder is not fully understood.

The authors recently reported DA-9601, a quality-controlled extract of dried aerial parts of *Artemisiae Herba* (Aeyop), possesses cytoprotective action against various noxious agents (Oh *et al.*, 1996) through enhancement of prostaglandins and mucus secretion from gastric mucosa (Oh *et al.*, 1997) with low toxicity (Kim *et al.*, 1996). The method of extraction and formulation of DA-9601 was described previously in detail (Yang, 1995). And DA-9601 is now under clinical trial for the treatment of acute and chronic gastritis in Korea.

This study was undertaken to examine gastroprotective effect of DA-9601 against naproxen, one of the commonly used NSAIDs, and its effect on mucosal eicosanoids levels in adjuvant-induced arthritic rats.

MATERIALS AND METHODS

Animals

Male Sprague-Dawley rats (250~300 g), aged 7 weeks of age were purchased from Charles River Japan (Kanagawa, Japan). Before an experiment, the rats were

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deprived of rodent chow (Cheil, Korea) except drinking water for about 20 hours. During the experiments, rats were kept under standard laboratory conditions in Specific Pathogen Free (SPF) barrier facility.

Gastroprotective evaluation in arthritic rats

Gastroprotective effect of DA-9601 (lot. L-07) against naproxen (Sigma Chemical Co., St. Louis, USA) was evaluated in rats with 14 day established polyarthritis. Arthritis similar to that described by Katz *et al.* (1986) was induced in male rats by injection of 0.1 ml of *Mycobacterium butyricum* (DIFCO Laboratories, Detroit, MI) suspended in mineral oil (7.5 mg/ml) into the subplantar surface of the right hind paw. Development of edema in the controlateral paw indicated presence of polyarthritic condition. To examine the gastroprotective activity of DA-9601 against naproxen, 30 mg/kg of naproxen was administered orally to rats for five consecutive days from 14 days after induction of arthritis, and graded doses of DA-9601, 200 mg/kg of cetraxate (Jeil Pharmaceutical Co., Seoul, Korea) or 5 ml/kg of the vehicle (5% hydroxypropylmethylcellulose suspension) were orally administered 60 min after naproxen for the same period of 5 days. At 24 hrs after the last drug administration, rats were sacrificed by ether anesthesia. The stomachs were then removed, opened along the greater curvature, layed out flat, and the optical lesion area was counted to obtain lesion index (mm^2) by dissecting microscope and recorded by a veterinary pathologist unaware to the treatment. Then, lesion inhibition ratio of each condition was calculated as described below.

$$\text{Lesion inhibition (\%)} = \frac{\{\text{lesion area of control } (mm^2) - \text{lesion area of test group } (mm^2)\}}{\text{lesion area of control } (mm^2)} \times 100$$

Antiinflammatory and analgesic evaluation in arthritic rats

Tarsotibial thickness (uninjected left hind limb) was measured by digital caliper (Mitutoyo, Japan) immediately after arthritis induction and again 14 and 19 days later for an index of antiinflammatory effect of naproxen. And 19 days after induction of arthritis, rats were tested for their tendency to vocalize after flexion of the tarsotibial joint of the noninjected paw by an observer blinded to the treatment using the method described by Capetola *et al.* (1980). Briefly, 24 hours after the last drug administration, an unbiased observer flexed the noninjected paw of each rat for 5 times and recorded the number of vocalization. A reduction in the mean number of vocalizations compared to controls indicated analgesic activity.

Histologic observation

Samples (1 cm × 1 cm) of grossly normal and grossly inflamed gastric tissue were fixed in 10% neutral buffered formalin and processed by routine techniques prior to embedding in paraffin. Thick sections (4 μm) were mounted on glass slides and stained with hematoxylin and eosin. The sections were examined under a light microscope (BH-2, Olympus) by a veterinary pathologist unaware of the treatment.

Mucosal eicosanoids assay

Mucosal eicosanoids levels were measured using a commercially available EIA kits (Amersham, UK). Immediately after biopsy and gross observation, mucosal specimens were frozen in liquid nitrogen and stored at -70°C until measurement of prostaglandin E_2 (PGE_2), 6-keto prostaglandin $F_{1\alpha}$ ($PGF_{1\alpha}$) and leukotriene B_4 (LTB_4). Tissue specimens were processed for the assay using the method described previously by Lipscomb and Rees (1996). The final eicosanoid concentration is expressed in pg/mg of wet biopsy weight.

Statistical analyses

The numeric data including lesion index, paw thickness and eicosanoid concentration are expressed as mean ± standard error. Comparison between groups were made using one-way analysis of variance followed by the Bonferroni test. A p value of $\leq 5\%$ was considered significant. For evaluation of analgesic activity, the relative potency between groups was compared using the Mann-Whitney test.

RESULTS

Gastroprotective evaluation in arthritic rats

Repeated oral administration of naproxen induced multifocal and linear mucosal erosion/ulcers in arthritic rats, while arthritis alone induced minimal or no gastric lesion. The lesion index was 43.3±4.5 mm^2 in naproxen group. Co-administration with DA-9601 inhibited the naproxen-induced damage in a dose-related manner (Table I). Even at a low dose of 50 mg/kg, DA-9601 significantly reduced the lesion index ($p < 0.05$). The prevention of naproxen-induced damage reached the maximum at 100 mg/kg, the inhibition being 73.2%. At 200 mg/kg of DA-9601, however, there was no further protection against naproxen-induced the damage. Cetraxate (200 mg/kg) also significantly reduced the lesion index with an inhibition of 46.2% compared to the naproxen group ($p<0.05$). Histologic examination confirmed the result of macroscopic observation. Light microscopy showed focal but extensive exfoliation of the surface epithelial cells and submu-

Table I. Protective effect of DA-9601 on naproxen-induced mucosal lesion in the arthritic rat

Naproxen (mg/kg)	Treatment[a]	Dose (mg/kg)	Number of rats	Gastric lesion (mean±S.E., mm^2)	Inhibition[b] (%)
-	Vehicle[c]	-	19	0.2±0.1*	-
30	Vehicle[c]	-	18	43.3±4.5	-
		50	18	22.6±1.9*	47.8
30	DA-9601	100	18	11.6±1.0*	73.2
		200	18	13.9±2.5*	67.9
30	Cetraxate	200	11	23.3±3.9*	46.2

[a]DA-9601 or cetraxate was gavaged one hour after naproxen
[b]Lesion inhibition *vs.* naproxen vehicle, see *Materials and Methods*
[c]5% hydroxypropylmethylcellulose
*p<0.05 (Bonferroni test) compared to naproxen vehicle

Fig. 1. Naproxen-induced mucosal injury. A photomicrograph (Hematoxylin-Eosin stain, ×100) shows extensive mucosal erosion and submucosal edematous change by 30 mg/kg of naproxen for 5 consecutive days. There was no evidence of intramucosal bleeding. Note mild infiltration of inflammatory cells in the submucosal space.

cosal edema in which inflammatory cells were infiltrated (Fig. 1). Coadministration of DA-9601 (100 mg/kg) dramatically inhibited naproxen-induced erosions and edematous change of gastric wall, as depicted in Fig. 2. The epithelial cell damage was also inhibited with a dose of 50 mg/kg or 200 mg/kg of DA-9601. There were apical erosions in some of the mucosal surface at these dose levels, but there was no evidence of deep (>2 mm) injury. In cetraxate-treated rats, deep erosions were often observed focally, but no ulceration was found.

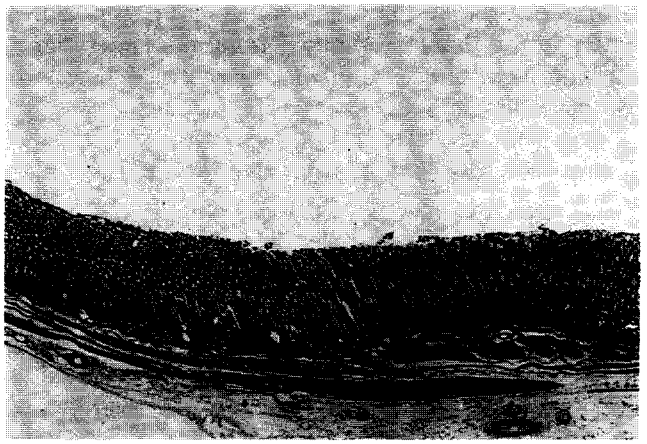

Fig. 2. A photomicrograph of gastric wall from a rat receiving 100 mg/kg of DA-9601 and 30 mg/kg of naproxen concomitantly for 5 days. No abnormalities were observed (Hematoxylin- Eosin stain, ×40).

Antiinflammatory and analgesic evaluation in arthritic rats

2 weeks after intraplantar inoculation of *Mycobacterium butyricum*, polyarthritis was induced. Naproxen treatment for 5 days significantly reduced paw edema when compared to control rats (14.8±0.4 *vs.* 10.8±0.3). DA-9601 did not affect the antiinflammatory activity or potency of naproxen (Table II), nor did cetraxate alter the activity of naproxen. When tested for analgesic activity, naproxen caused a significant decrease in the mean number of vocalizations in both vehicle and DA-9601 treated rats (Table III). None of the naproxen-treated groups exhibited any statistically significant analgesic activity after coadministration with DA-9601 or cetraxate.

Table II. DA-9601 does not alter the antiinflammatory activity of naproxen in the arthritic rat

Naproxen (mg/kg)	Treatment[a]	Dose (mg/kg)	Number of rats	Thickness of uninjected tarsotibial joint (mm)[b]		
				Day 0	Day 14	Day 19
-	Vehicle[c]	-	19	5.45±0.05	12.84±0.26	14.83±0.36*
30	Vehicle	-	18	5.50±0.07	12.81±0.35	10.80±0.28
		50	18	5.44±0.04	13.47±0.27	10.97±0.22
30	DA-9601	100	18	5.46±0.05	13.40±0.36	11.44±0.21
		200	18	5.54±0.05	13.29±0.29	11.03±0.29
30	Cetraxate	200	11	5.44±0.33	12.93±0.33	10.80±0.20

[a]DA-9601 or cetraxate was gavaged one hour after naproxen
[b]Mean±S.E.
[c]5% hydroxypropylmethylcellulose
*p<0.05 (Bonferroni test) compared to naproxen vehicle

Table III. DA-9601 does not alter the analgesic activity of naproxen in the arthritic rat

Naproxen (mg/kg)	Treatment[a]	Dose (mg/kg)	Number of rats	Number of vocalizations[b] (Mean±S.E.)
-	Vehicle[c]	-	19	3.16±0.76*
30	Vehicle[c]	-	18	1.17±0.62
		50	18	1.11±0.76
30	DA-9601	100	18	1.17±0.51
		200	18	1.11±0.58
30	Cetraxate	200	11	1.18±0.60

[a]DA-9601 or cetraxate was gavaged one hour after naproxen
[b]Noninjected paw flexed at the tarsotibial joint for 5 times
[c]5% hydroxypropylmethylcellulose

Mucosal eicosanoids assay

Repeated administration of naproxen resulted in significant decrease in mucosal PGE_2 and $PGF_1\alpha$ levels and also protected against gastric lesions (Table IV). The mean values of PGE_2 and $PGF_1\alpha$ in control gastric mucosa were 274.0±23.3 and 31.5±9.5 pg/mg of mucosa, respectively. Naproxen lowered the PGE_2 level (163.2±31.3) and PGF_1 (10.3±7.7) level ($p<0.05$ *vs.* control). However, treatment with DA-9601 or cetraxate completely protected naproxen-induced PG suppression, or even elevated the mucosal levels of these two eicosanoids above the control values. Nei- ther naproxen nor DA-9601 altered the mucosal LTB_4 level.

DISCUSSION

Despite the frequent side effects associated with use of NSAIDs, more than 30 billion dollars are spent each year for NSAID therapy in the United States alone. About 1.2% of the whole population take NSAIDs regularly, and many more people take them intermittently (Graham, 1989). As many as 60% of all patients who take NSAIDs have silent (asymptomatic) gastrointestinal (GI) damage resulting from erosions or intramucosal hemorrhages (Soll *et al.*, 1991). Upper GI bleeding is the most frequent serious complication of long-term NSAIDs therapy, especially in elderly patients (Gabriel *et al.*, 1991). The dual-injury theory contends that after initial direct NSAIDs-induced mucosal damage, the following cascade is set in motion: permeability of the gastric mucosal barrier is increased, luminal hydrogen ions and pepsin diffuse into the mucosal cells, and the damage is, in turn, potentiated by the inhibitory effects on prostaglandins (Schoen and Vender, 1989). Prostaglandins have a mucoprotective role and have been shown to stimulate secretion of mucus, increase the content of lipids and glycoproteins in the membranes, stabilize lysosomes and inhibit secretion of acid (Haw- key and Rampton, 1985). At this time, misoprostol, a synthetic prostaglandin E_1 derivative, is the only effective protectant against NSAID-induced mucosal damage, which is proven by many investigators (Fenn and Robinson, 1991; Graham *et al.*, 1993; Ballinger, 1994), though it shows a rather severe adverse reaction of diarrhea in many patients (Graham *et al.*, 1988a). The most frequent adverse effects of misoprostol are diarrhea, abdominal cramps and dyspepsia, which can be seen in 20 to 30% of patients (Agrawal *et al.*, 1991; Isdale and Wright, 1995).

Because of the significant gastrointestinal complications derived from NSAIDs, recent efforts have been focused on the prevention of the mucosal damage. For patients who need a relief from fever and pain rather than inflammation, the use of NSAIDs may be reduced or replaced by a less gastrointestinal (GI) toxic agent, such as acetaminophen (Bradley *et al.*, 1991). However, a considerable number of patients, for example RA patients, have no alternative but to use NSAIDs for therapy of the intractable illness. Katz and his colleagues (1986) suggested the following requirements for a drug to prevent NSAIDs-induced gastric irritation, (1) it must reduce the GI bleeding caused by NSAIDs, (2) it should not contribute to or exacerbate an existing arthritic condition, (3) it must not interfere with the antiinflammatory or analgesic efficacy of the NSAIDs, and (4) it should be effective against the side effects of prolonged NSAIDs administration. In our study,

Table IV. Effect of DA-9601 and naproxen on gastric mucosal eicosanoids in the arthritic rat

Naproxen (mg/kg)	Treatment[a]	Dose (mg/kg)	Number of rats	Mucosal eicosanoids (Mean±S.E., pg/mg mucosa)		
				PGE_2	$PGF_1\alpha$	LTB_4
-	Vehicle[b]	-	19	274.0±23.3	31.5±9.5	35.1±7.1
30	Vehicle[b]	-	18	163.2±31.3*	10.3±7.7*	44.4±8.2
		50	18	228.3±30.5	42.0±13.2	40.3±5.1
30	DA-9601	100	18	234.4±41.2	41.4±11.1	43.8±9.1
		200	18	263.0±38.3	42.5±10.1	40.3±10.2
30	Cetraxate	200	11	267.0±40.1	39.9±10.5	37.1±6.1

[a]DA-9601 or cetraxate was gavaged one hour after naproxen
[b]5% hydroxypropylmethylcellulose
*$p<0.05$ (Bonferroni test) compared to vehicle control

DA-9601 satisfies all these criteria, though the duration of naproxen treatment was rather short and clinical usefulness of the drug remains to be elucidated. The analgesic and antiinflammatory responses observed appear to be the outcome of naproxen treatment exclusively, since DA-9601 lacks these effects (Lee *et al.*, 1996).

As mentioned above, upper GI bleeding is the most frequent adverse effect in long-term NSAIDs consumers. Mucosal bleeding, however, was not prominent in our experiment. Instead of hemorrhage, mucosal erosions were major features of the damage, grossly and microscopically. With respect to mucosal prostaglandins, DA-9601 was found to protect the naproxen-induced reduction of PGE_2 and $PGF_1\alpha$, major endogenous cytoprotectants in human and animals, implying that preservation of mucosal PG plays an important role in mucoprotective action of DA-9601. Interestingly, naproxen did not alter mucosal leukotriene B_4 (LTB_4) levels in our study. McCafferty *et al.* (1995) confirmed the previous results obtained by others that migration and adherence of leukocytes within gastric microcirculation play an important role in NSAID-induced mucosal damage (Wallace *et al.*, 1993; Kitahora and Guth, 1987), while inhibition of 5-lipoxygenase (5-LO), a key enzyme to produce leukotrienes, has been reported to be beneficial for protection of NSAIDs-induced mucosal damage (Cho and Ogle, 1987). Thus, it is still controversial whether leukotrienes are really critical for the mucosal damage induced by NSAIDs or not.

In summary, gastric irritation and bleeding associated with long-term administration of NSAIDs remains a significant problem in patients with arthritic conditions. Based on the findings from this work DA-9601 may be useful in clinical practice for the prevention of gastric complications in chronic NSAIDs consumers including arthritic patients.

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