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SKI306X, an oriental herbal mixture, suppresses gastric leukotriene B_4 synthesis without causing mucosal injury and the diclofenac-induced gastric lesions

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Abstract

SKI306X compound is a herbal mixture. This plant was in oriental medicine and was clinically approved for the treatment of osteoarthritis (OA) in Korea. SKI306X was previously found to have anti-inflammatory, analgesic and cartilage protective effects in several experimental models. In this study, SKI306X was investigated for its gastro-sparing effects on the gastric mucosa comparing with those of diclofenac, a conventional NSAID, and celecoxib, a cyclooxygenase-2 (COX-2) specific inhibitor. To investigate acute gastric damaging properties of SKI306X, the stomach of the animals was histologically and immuno-histochemically examined after single or repeated administration, and SKI306X demonstrated excellent gastric tolerability. SKI306X did not cause significant gastric irritation, erosion, or ulceration up to the orally administered dose of 2 g/kg and the intraperitoneal (i.p.) dose of 125 mg/kg. In contrast, diclofenac caused mucosal erosion, ulceration and bleeding at clinically effective doses. To determine the mode of gastro-sparing action, eicosanoid synthesis was examined in gastric mucosa and blood. SKI306X significantly decreased gastric and blood leukotriene B_4 (LTB_4) production. However, SKI306X showed either no effect or a slight increase in levels of prostaglandin E_2 (PGE_2). In addition, gastro-protective effects of SKI306X were exhibited by suppressing diclofenac-induced erosion and ulceration of gastric mucosa in a rat model and the possible mechanism of these effects were investigated. These studies demonstrated that SKI306X did not produce any significant damage up to dose of 2 g/kg and was effective in significantly protecting the

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damage associated to diclofenac-induced gastric ulcerations. SKI306X could spare the gastric mucosa through significantly suppressing gastric leukotriene (LT) synthesis.
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Keywords: SKI 306X; Gastric mucosa; Prostaglandin; Leukotriene

Introduction

Non-steroidal anti-inflammatory drugs (NSAIDs), exerting their effects by blocking cyclooxygenases (COXs), are used widely in the treatment of inflammation, fever, pain and thrombosis and also represent the major symptomatic therapy in OA and rheumatoid arthritis (RA). Each year in the United States, about 60 million people including arthritis patients are prescribed NSAIDs (Miller and Prichard, 1990; Jobanputra and Nuki, 1994; Polison, 1996). However, this use is limited by their tendency to cause significant damage in the gastrointestinal (GI) tract. The ulcerogenic effects of NSAIDs appear to be attributable to their ability to inhibit gastric prostaglandin (PG) synthesis (Vane, 1971; Whittle, 1981) resulting in decreased gastric mucosal blood flow and, in turn, increased susceptibility to injury induced by topical irritants (Robert, 1979). Indeed, several NSAID-associated GI complications are the most commonly reported serious adverse drug in patients with RA. These compounds non-selectively inhibit the two isoforms of the COX (COX-1 and COX-2) and thus prevent the up-regulation of PG formation, which otherwise lead to an increase of vascular permeability, edema, hyperalgesia, pyrexia, and inflammation. The constitutive isoform COX-1 is responsible for the production of PGs involved in prostanoid-mediated physiological functions such as gastric cytoprotection, maintenance of renal homeostasis, and maintenance of normal platelet functions. The second isoform, COX-2, has been identified and has been demonstrated to be highly expressed in response to inflammatory or mitogenic stimuli (Mitchell et al., 1993; Wallace et al., 1994a,b). Thus, it is proposed that COX-2 is responsible for the production of PGs associated with inflammatory conditions. Developing NSAID which do not exhibit gastric toxicity is a considerable challenge, and several different strategies have been employed. The primary reason for the development of COX-2 specific inhibitors, or coxibs, was to decrease the risk of GI toxicity. In this study, we investigated diclofenac-caused gastrointestinal ulceration by inhibiting gastric mucosal biosynthesis of cytoprotective PG, but celecoxib, proved for the treatment of the signs and symptoms of OA and RA, does not cause severe ulceration on the upper GI mucosa and platelets (Bensen et al., 1999; Emery et al., 1999; Simon et al., 1998). SKI306X is clinically approved for the treatment of OA in Korea. Therefore, in this study, we investigate the affect of SKI306X to GI tract and compare to that of diclofenac or celecoxib. Also we demonstrate that SKI306X exerts gastro-protective effects.

SKI306X is a fractionated extract of three oriental herbal ingredients, *Clematis mandshurica*, *Trichosanthes kirilowii* and *Prunella vulgaris* which have been widely used for the treatment of inflammatory diseases such as lymphadenitis and arthritis in Far East Asia (Park et al., 1995; Kim et al., 1996). In previous studies, the anti-inflammatory and analgesic activities of this herbal extract were reported in various in vitro and in vivo experimental models. SKI306X has been reported to have protective effects in articular cartilage (Choi et al., 2002). Double blind, placebo-controlled clinical study confirmed the clinical efficacy and tolerability of SKI306X in patients with OA in the knee (Jung et al., 2001) which was comparable to those of diclofenac SR. Another clinical study is underway to determine the efficacy of SKI306X in RA patients. In the present study, SKI306X was found to have not only gastro-

sparing effects on healthy gastric mucosa but also healing effects on NSAID-induced ulcer in rat. Finally it was attempted to understand the mechanism underlying the gastric-sparing properties of this drug.

Materials and methods

Animals

Male, Sprague–Dawley rats (Charles River Breeding Farms, Montreal, Quebec, Canada) were used for the study. All animals had free access to tap water and pellet food (Agribrands, Purina, Korea). The animal experiments have been carried out according to the internationally accredited guidelines. On the day of dosing the rats were in the weight range 200–225 g and were housed 3 to a cage in suspended polypropylene cages with wire grid floors. The room temperature and relative humidity controls were set at 21 °C and 50%, respectively. Prior to experiments, the rats were deprived of food, but not water, for 18 h.

Preparation and composition of SKI306X

SKI306X was prepared by extracting a mixture of three medicinal herbs (dried root of *C. mandshurica*, dried root of *T. kirilowii* and dried flower and stem of *P. vulgaris* at 1:2:1 (w/w), respectively) with 30% (v/v) ethanol solution. After the extracted solution was filtered and evaporated in vacuo, the residue was partitioned between *n*-butanol and water. The *n*-butanol layer was evaporated in vacuo and lyophilized for a complete removal of the residual solvent to yield dark-brown powder. SKI306X was standardized for quality control, according to previous report. SKI306X was analyzed to find standard materials by HPLC. We identified oleanolic acid and rutin in SKI306X as standard materials (Park et al., 1995). *C. mandshurica, T. kirilowii*, and *P. vulgaris* were also prepared as above method.

Preparation of test drugs

All drugs were prepared freshly on each day of dosing. SKI306X was suspended in 0.5% carboxymethylcellulose at the highest concentration: lower concentrations were prepared by serial dilution. Diclofenac and celecoxib were also suspended in 0.5% carboxymethylcellulose at the concentration required.

Materials

EIA kits for PGE_2 and LTB_4 were provided from Cayman (Ann Arber, MI, USA). Diclofenac, carboxymethylcellulose, A23187 and other reagents were obtained from Sigma Chemical Co. (St. Louis, MO, USA). ELISA kit for myeloperoxidase (MPO) was provided from Immundiagnostik (Bensheim, Germany).

Experimental procedure

Adverse effect on gastric mucosa

Rats were divided into 2 groups, single administration and 5 time administration, of 6–10 rats each. Each group of 6–10 rats was given SKI306X (125, 500, 2000 mg/kg), diclofenac (2.5, 10, 40 mg/kg),

celecoxib (50, 200, 1000 mg/kg) or vehicle orally. In case of single administration, 8 h later after administration, the rats were sacrificed and the stomach was removed. In case of repeated administration, test materials were orally administered daily. The rats were sacrificed after the 8 h fifth administration. Any macroscopically visible lesions were measured to calculate a gastric damage score. This assessment of injury was performed by an individual unaware of the treatments. The stomach was then fixed in neutral buffered formalin and sections of the tissue were processed by routine techniques for subsequent histological evaluation. H and E staining was done and Cyclooxygenase-2 (COX-2), 5-lipoxygenase (5-LO) antibodies were used for immuno-staining of the tissues. MPO activity was determined by the method described by Bradley et al. (1982) with modifications. Samples of 100–150 mg were homogenized in 0.5% hexadecyltrimethylammonium bromide in 50 mM potassium phosphate buffer, pH 6.0. Suspensions were centrifuged at 9000×*g* for 30 min and the resulting supernatant was assayed using MPO ELISA kit. The final values were expressed as mU per mg protein.

Measurement of gastric eicosanoid production

In the same animals as above, samples of corpus region of the stomach were excised (prior to the stomach being fixed in formalin), finely minced with scissors, then suspended in 1 ml of 10 mM sodium phosphate buffer (pH 7.4). The samples were incubated at 37 °C for 20 min and then centrifuged at 9000×*g*. The supernatant was frozen for subsequent measurement of PGE_2 and LTB_4 concentration by ELISA.

Measurement of eicosanoid production by blood

Prior to killing the rats used in the studies described above, 1 ml of blood sample was taken by cardiac puncture. The blood sample was stimulated with A23187 (10 μM) and allowed to incubate at 37 °C for 30 min. After centrifugation at 9000×*g*, the levels of PGE_2 and LTB_4 in the supernatant were measured, as above.

Gastro-protective activity

Two experimental methods were performed to evaluate the possible protective or therapeutic effect of SKI306X in rat model of diclofenac-induced gastric injury with single or 5 time repeated administration. Diclofenac (40 mg/kg) was administered once to groups of rats to induce gastric mucosal lesion. Control group received vehicle and test groups received SKI306X for specified days.

In method I, the protective or therapeutic effect of single administration of SKI306X at the doses of 62.5 and 125 mg/kg i.p. on the gastric injury orally induced by diclofenac simultaneously was investigated. Diclofenac and SKI306X were i.p. administered and orally together. 8 hr after administration, the rats were sacrificed and the stomach was removed. In method II, the test material was administered orally eight hours after diclofenac was orally administered and then test material was administered orally for 4 days. Twenty-four hours after the fourth administration, the rats were sacrificed and the stomach was removed. In the same animals, samples of corpus region of the stomach were excised (prior to the stomach being fixed in formalin) and frozen for subsequent measurement of PGE_2 and LTB_4 concentrations by ELISA as above. Prior to killing the rats used in the above studies, 1 ml of blood was taken by cardiac puncture. The blood sample was stimulated with A23187 (10 μM) and allowed to incubate at 37 °C for 30 min. After centrifugation at 9000×*g*, the levels of PGE_2 and LTB_4 in the supernatant were measured, as above.

Statistics

All data were expressed as mean ± S.E.M. Statistical significances among groups were tested by one-way analysis of variance (ANOVA) and Dunnett's test using Sigma Stat (Jandel Co., San Rafael, CA, USA). Differences were considered significant when p was less than 0.05. All experiments were done duplicated.

Results

Gastric adverse event of SKI306X on gastric mucosa

The damages on gastric mucosa caused by the oral administration of SKI306X, the clinical approved herbal extract for OA, were evaluated with those of diclofenac and celecoxib. Figs. 1 and 2 clearly demonstrate that single and repeated administrations of SKI306X at all doses caused little damage on gastric mucosa, which is comparable to those of celecoxib, but, as expected, the

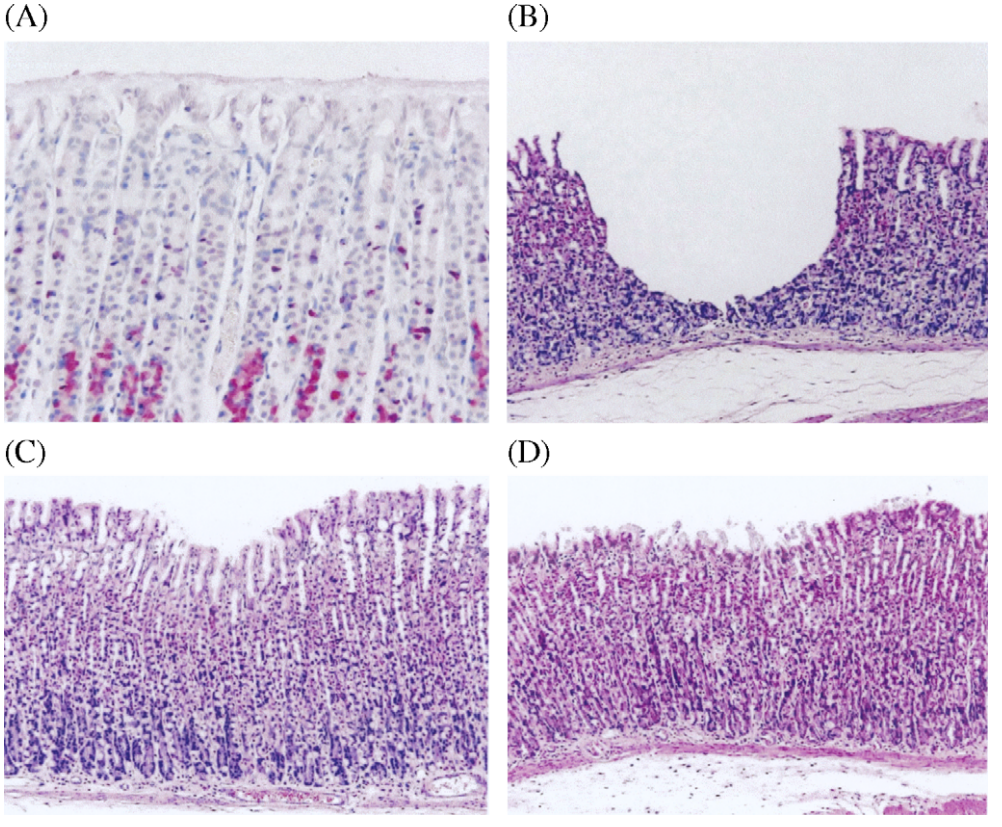

Fig. 1. Microscopic appearance of gastric mucosa after repeated treatment of SKI306X, diclofenac, celecoxib or the vehicle in rats. All drugs were administered orally to rats one time per day for 5 days. Stomachs were removed 8 h after the last 5th dose, H and E staining, magn., 150 × (n=6); (A) vehicle-treated, (B) diclofenac (40 mg/kg)-treated, (C) celecoxib (1000 mg/kg)-treated and (D) SKI306X (2000 mg/kg)-treated animals.

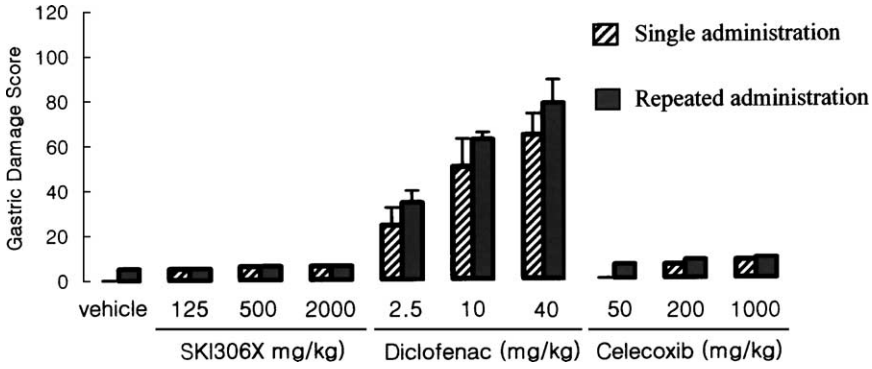

Fig. 2. Score of gastric damage caused by the oral administration of SKI306X, diclofenac, celecoxib or the vehicle. Damage was scored by an observer unaware of the treatment. Each column represents the mean ± S.E.M. of 6 rats per group. Only diclofenac caused damage that was significantly greater than that in the vehicle-treated group ($p < 0.001$).

administration of diclofenac caused gastric damages directly proportional to the doses. H and E staining shows mucosal damage caused by diclofenac characterized by necrosis ranging the full thickness of the mucosa up to the muscularis mucosae. On the other hand, a few regions of

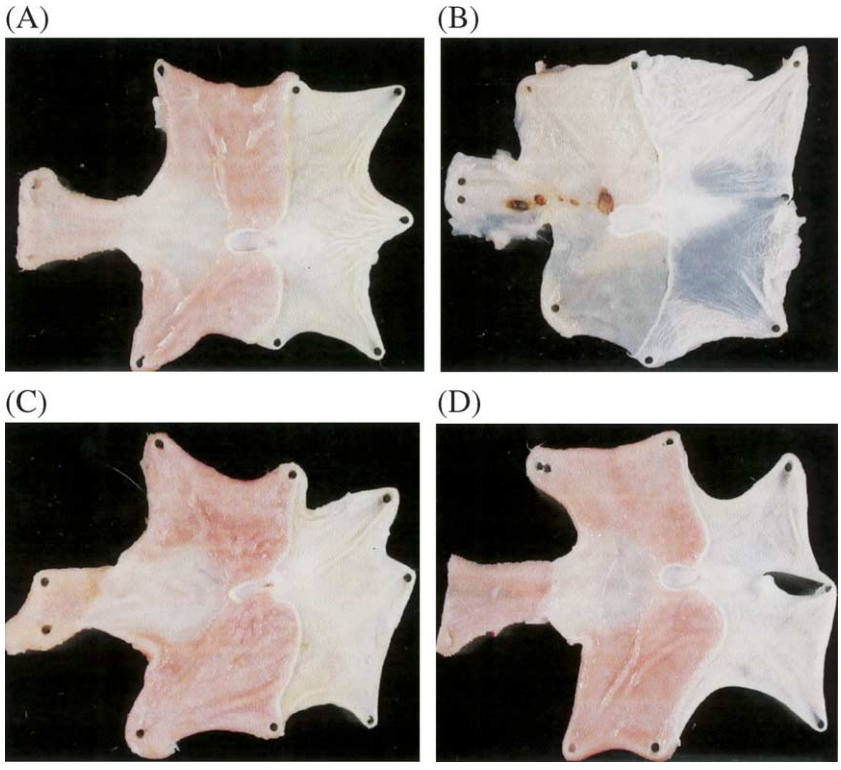

Fig. 3. Macroscopic appearance of the stomach after repeated treatment of SKI306X, diclofenac, celecoxib or the vehicle (0.5% carboxymethylcellulose) in rats. All drugs were orally administered to rats for 5 days. Stomachs were removed 8 h after the 5th administration; (A) vehicle-treated, (B) diclofenac (40 mg/kg)-treated, (C) celecoxib (1000 mg/kg)-treated and (D) SKI306X (2000 mg/kg)-treated animals.

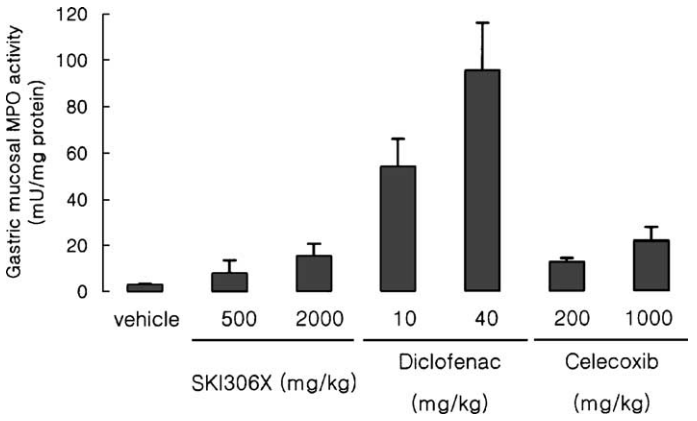

Fig. 4. Effects of the repeated administration of SKI306X, diclofenac, celecoxib or the vehicle on the potentiation of gastric myeloperoxidase activity. Each column is the mean ± S.E.M. of 6 rats per group. Only diclofenac showed MPO activity that was significantly greater than that in the vehicle-treated group ($p < 0.05$).

superficial epithelial sloughing were noted in the gastric mucosa of the SKI306X- and celecoxib-treated rats, which were also seen in the rats treated with the vehicle (Figs. 1 and 3). Also, the treatment of diclofenac was found to increase gastric mucosal MPO activity in a dose-dependant manner but the treatment of SKI306X or celecoxib did not increase the mucosal activities (Fig. 4, Table 1).

Effects on gastric eicosanoid synthesis

The administration of celecoxib, not to mention diclofenac, had strong inhibitory effects on the PGE_2 synthesis in both of stomach and blood. However, the single or repeated administrations of

Table 1
Effects of SKI306X, diclofenac and celecoxib on gastric and blood PGE_2 synthesis

Treatment (mg/kg)	Gastric PGE_2 synthesis (pg/mg)		Blood PGE_2 synthesis (pg/ml)	
	Single dose	Repeated dose	Single dose	Repeated dose
Vehicle	687.4 ± 21.8	975.2 ± 11.8	1135 ± 12.3	1410.2 ± 19
SKI306X 125	628.2 ± 11.3	912.1 ± 28.9	1278 ± 3.8	1358.2 ± 21.6
SKI306X 500	315.8 ± 14.2*	1289.4 ± 10.2	1487 ± 21.5	1489.2 ± 15.2
SKI306X 2000	19.2 ± 5.6**	1658.5 ± 9.6	1589 ± 3.8	1497.8 ± 25.1
Diclonefac 2.5	695.1 ± 15.2	684.5 ± 18.2-18.2*	1243 ± 25.6	1352.4 ± 23.9
Diclonefac 10	21.9 ± 2.2**	2.5 ± 0.1**	59.58 ± 3.6**	658.4 ± 38.5
Diclonefac 40	12.4 ± 5.8**	2.1 ± 0.5**	12.1 ± 6.3**	12.5 ± 18.9**
Celecoxib 50	128.4 ± 12.6*	847.5 ± 12.6	825.4 ± 8.9	1286.4 ± 12.8
Celecoxib 200	117.2 ± 6.3*	638.2 ± 15.2	598.5 ± 13.6	485.7 ± 8.5*
Celecoxib 1000	8.9 ± 1.2**	12.5 ± 2.1*-2.1**	138.5 ± 2.6**	29.8 ± 3.6**

Each data represents the mean ± S.D. Each group consisted of 6 rats. **$p < 0.05$, *$p < 0.01$ compared to vehicle-treated group. In single dose, all drugs were administrated orally 8 h prior to remove stomachs. In repeated dose, all drugs were orally administered for 5 days. Stomachs from rats were removed 8 h after the 5th administration.

SKI306X had little effect on the blood level of PGE_2. Similarly, repeated administration of SKI306X did not show an inhibitory effect on gastric PGE_2 synthesis. But single administration of SKI306X was found to decrease the level of PGE_2 in stomach in a dose-dependent manner. Unlike the case of PGE_2 synthesis, SKI306X and the control drugs exerted quite the reverse effects on LTB_4 synthesis. While the administration of diclofenac or celecoxib, regardless of single or repeated administrations, has shown little effect on the levels of LTB_4 in stomach and blood, the administration of SKI306X significantly reduced the levels of LTB_4 in stomach and blood in a dose-dependent manner, except that the blood level of LTB_4 was not changed by the single administration of the herbal formula up to the doses of 2000 mg/kg (Table 2). These findings are strongly supported by the immuno-histological studies (Fig. 5). COX-2 and 5-LO immuno-staining of the tissues showed the similar results with PGE_2 and LTB_4 synthesis by the stomach. SKI306X caused a significant suppression of 5-LO synthesis and increased slightly COX-2 synthesis in a superficial area of gastric mucosa.

Gastroprotection

According to these mechanism studies, the possibility for SKI306X to have additional activity of gastro-protection was evaluated by using the rat model of diclofenac-induced ulcer. Oral administration of diclofenac (40 mg/kg) resulted in extensive erosion, hemorrhagic and ulcerogenic damage to the rat stomach. When diclofenac (oral administration, 40 mg/kg) and SKI306X (i.p. treatment, 62.5, 125 mg/kg) were treated to rats together, the extent of diclofenac-induced damage was reduced. SKI306X can be suggested that it has effect to suppress the procedure of gastric damaging by diclofenac. In addition, it was clearly demonstrated that repeated oral administration of SKI306X at the doses of 500 and 2000 mg/kg reduced erosion and hemorrhagic damages in the rat stomach induced by diclofenac (Fig. 6). In these experiments, diclofenac (40 mg/kg) significantly increased the level of LTB_4 synthesis in rat stomachs and SKI306X (i.p. treatment, the doses of

Table 2
Effects of SKI306X, diclofeneac and celecoxib on gastric and blood LTB_4 synthesis

Treatment (mg/kg)	Gastric LTB_4 synthesis (pg/mg)		Blood LTB_4 synthesis (pg/ml)	
	Single dose	Repeated dose	Single dose	Repeated dose
Vehicle	35.9 ± 11.2	45.5 ± 8.5	4035 ± 125	4110 ± 191
SKI306X 125	18.4 ± 6.3*	27.9 ± 7.9*	4271 ± 148	3829 ± 217
SKI306X 500	12.6 ± 4.2**	19.4 ± 2.2**	3192 ± 201	2125 ± 152*
SKI306X 2000	9.2 ± 3.6**	8.5 ± 3.6**	3143 ± 89	147 ± 25**
Diclofenac 2.5	45.8 ± 15.2	42.5 ± 6.2	4124 ± 256	4218 ± 239
Diclofenac 10	31.9 ± 10.2	49.5 ± 4.1	4287 ± 165	4165 ± 285
Diclofenac 40	42.4 ± 12.8	52.1 ± 11.5	4044 ± 519	4125 ± 189
Celecoxib 50	38.9 ± 20.6	47.5 ± 10.6	4825 ± 104	4286 ± 128
Celecoxib 200	41.2 ± 6.3	38.2 ± 5.8	4059 ± 136	4015 ± 85
Celecoxib 1000	11.5 ± 2.3*	31.7 ± 3.6	4117 ± 210	3985 ± 136

Each data represents the mean ± S.D. Each group consisted of 6 rats. **$p < 0.05$, *$p < 0.01$ compared to vehicle-treated group. In single dose, all drugs were administrated orally 8 h prior to remove stomachs. In repeated dose, all drugs were orally administered for 5 days. Stomachs from rats were removed 8 h after the 5th administration.

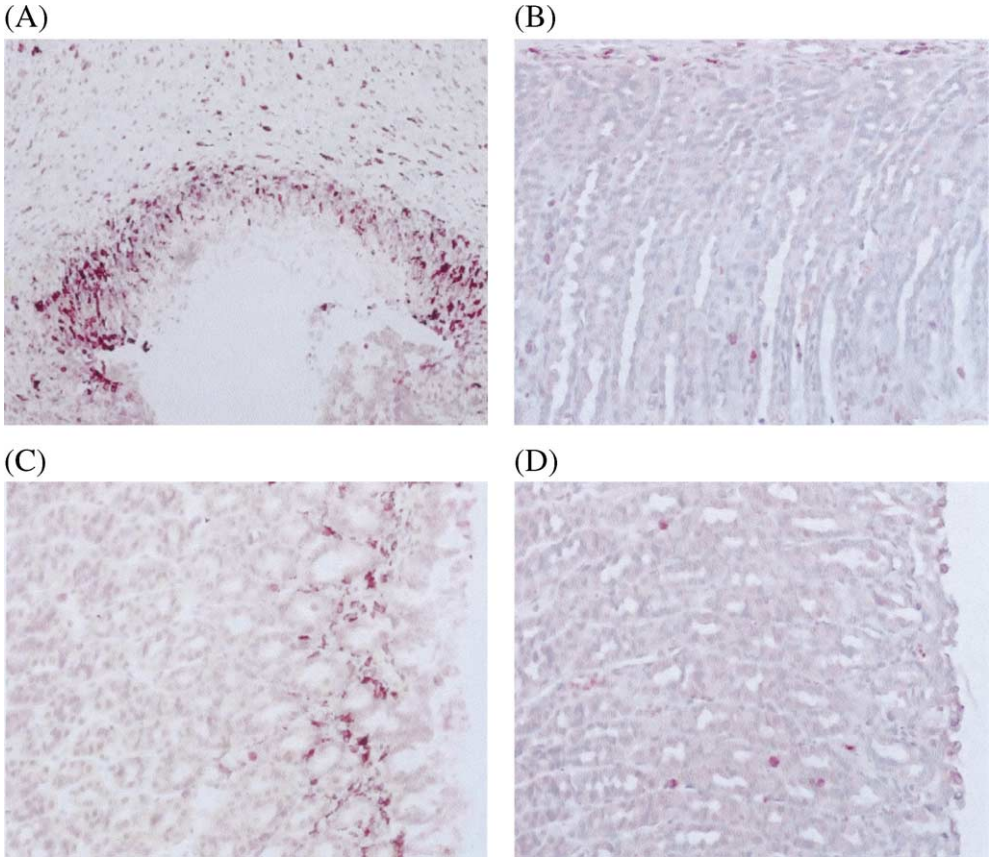

Fig. 5. Immunohistological appearance of gastric mucosal COX-2 and 5-LO after repeated treatment of SKI306X, diclofenac, celecoxib or the vehicle in rats, magn., 260 ×; (A) COX-2, diclofenac (40 mg/kg)-treated, (B) 5-LO, diclofenac (40 mg/kg)-treated, (C) COX-2, SKI306X (2000 mg/kg)-treated and (D) 5-LO, SKI306X (2000 mg/kg)-treated animals.

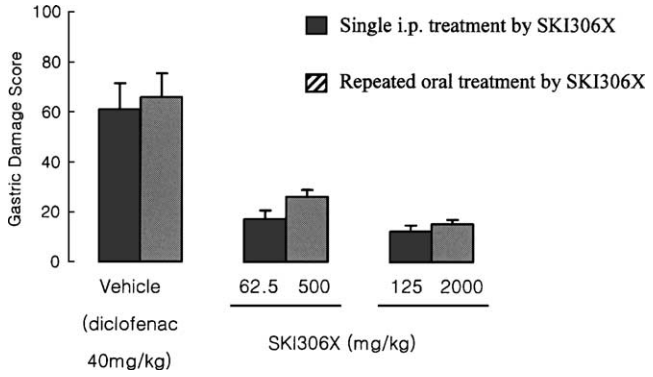

Fig. 6. Gastroprotective effects of SKI306X or the vehicle on diclofenac-induced gastric ulcer. Each column is the mean ± S.E.M. of 6 rats per group. SKI306X showed significant gastro-protective effect comparing to that of vehicle-treated group ($p < 0.05$).

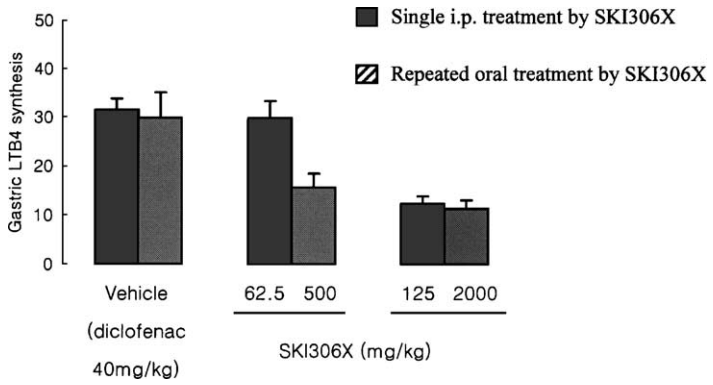

Fig. 7. Effects of SKI306X on diclofenac-induced gastric LTB_4 synthesis. Each column is the mean ± S.E.M. of 6 rats per group. SKI306X showed significant gastro-protective effect comparing to that of the vehicle-treated group ($p < 0.05$).

62.5 and 125 mg/kg and oral administration, the doses of 500 and 2000 mg/kg) also significantly reduced gastric LTB_4 synthesis (Fig. 7).

Discussion

In the present study, SKI306X at clinically effective doses which have been shown to exert anti-inflammatory and analgesic effects is capable of markedly suppressing gastric mucosal LT synthesis in single and repeated administration. However, SKI306X does not suppress gastric mucosal PG synthesis without causing significant gastric injury. Moreover, when was administered repeatedly to rats, SKI-306X produced virtually no gastric damage like celecoxib, while diclofenac produced penetrating antral ulcers. In this model, celecoxib at doses of 50–1000 mg/kg and SKI306X at dose of 125–2000 mg/kg have been shown to produce no gastric ulceration similar to vehicle control. SKI306X decreased mildly PGE_2 synthesis in single administration and increased slightly or did not change PGE_2 synthesis in the repeated administration by the mucosa. In addition, SKI306X increased slightly or did not change PGE_2 synthesis in single and repeated dose in blood. The gastric mucosa withstands a variety of endogeneous and exogeneous noxious factors due to the function of mucosal defense mechanisms. Gastric mucosal integrity requires continuous generation of PGE_2 and PGI_2. Majority of mucosal defense mechanisms are stimulated or facilitated by endogenous or exogenous PG. Suppression of PG synthesis by NSAID results in decreased susceptibility of mucosal to injury (Allen et al., 1993; Szabo, 1989; Tarnawski and Hollander, 1987; Wallace and Granger, 1996). SKI306X did not affect mucosal PGE_2 synthesis significantly in this model. Therefore, SKI306X can be suggested that this drug did not suppress mucosal susceptibility and has the mucosal sparing effect to injury. Moreover, SKI306X has the strong inhibitory effect for LTB_4 synthesis by the stomach. The role of LTs in gastric mucosa has been studies extensively in the recent years (Smuelsson, 1983; Lewis et al., 1990; Drazen et al., 1999). LTs have been found to stimulate pepsin secretion, to reduce mucosal blood flow and to interfere with gastric emptying. Lipoxygenase (LO) products impair gastric mucosal integrity and exacerbate the damaging effects of noxious agents. LTB_4 is the major chemotactic factor for leukocytes, whereas LTC_4 is one of the most potent vasoconstrictors. These findings suggest a

place for LT inhibitors in the treatment of gastric ulcers. LTB_4 and the cysteinyl-leukotrienes also have powerful pro-inflammatory properties and the inhibition of this metabolic pathway led to the development of new therapeutic treatments for pathologies such as asthma, allergies and other inflammatory disorders. LTs are pro-inflammatory mediators that play an important role in disease states and have also been implicated as having a protective role in host defense against microbial infection (Guslandi, 1987).

The active ingredients of SKI306X such as oleanolic acid, rosmarinic acid and rutin are known to have multifunction including anti-inflammation. Among these compounds, rosmarinic acid is reported to have excellent activities such as: (i) antioxidant activity and/or biosynthesis of prostacyclin generated in the metabolism of arachidonic acid, and by scavenging the active oxygen generated from polymorphonuclear leukocytes; (ii) anti-inflammatory activity such as the inhibition of inflammatory metabolites and immuno-regulation; (iii) enhancement of blood circulation (James and Smolen, 1980; Yoshiyuki and Hiromichi, 1987; Englberger et al., 1988; Huang and Zhang, 1992; Zou et al., 1993). It has also been reported that oleanolic acid has not only remarkable anti-inflammatory and analgesic effects but also an excellent effect for chronic rheumatoid arthritis induced by Mycobacterium butyricum (Keijiro et al., 1980; Dai et al., 1989; Balanehru and Nagarajan, 1991; Singh et al., 1991). These reports suggest that the effects of these ingredients may contribute to the multifunction of SKI306X on inflammation and pain.

In previous studies, we reported that SKI306X displayed inhibitory effects of COX-2 expression/5-LO activity with good selectivity without COX-1, 2 activity inhibition and especially showed the efficient effect of 5-LO activity inhibition in an in vitro model (in press). While novel NSAIDs produced gastric injury, ML-3000, a new well-balanced dual inhibitor of COX/5-LO produced significantly less gastric injury (Laufer et al., 1994a,b). This suggests that the gastric safe action of SKI306X exerted by suppressing gastric LT synthesis with minimal affecting gastric PG synthesis.

Oral administration of diclofenac resulted in extensive gastric mucosal damage to the stomach. When diclofenac treated through oral administration and SKI306X treated i.p. together, the extent of diclofenac-induced damage was reduced. As this result, when SKI306X is treated for diclofenac-treated patients with diclofenac simultaneously, SKI306X can have the potent gastro-protective effect of significantly reducing gastric lesions and ulcers by diclofenac. According to these results, we investigated the gastro-protective effect of SKI306X on diclofenac-induced gastric damage. Diclofenac (40 mg/kg) was injected to rats in a single administration. After stomach showed the gastric mucosal ulcers, SKI306X was administrated for 5 days. SKI306X showed the effect of decreasing diclofenac-induced gastric damage. These results strongly suggest that SKI306X has a potential safety to gastric stomach in single or repeated administrations and a potential effect to ameliorate the progress of drug-induced gastric damage by protecting the gastric mucosa. More studies are in progress to investigate the action mechanism of SKI306X for proving the gastro-protective effects.

References

Allen, A., Flemstrom, G., Garner, A., Kivilaasko, E., 1993. Gastroduodenal mucosal protection. Physiological Reviews 73, 823–857.

Balanehru, S., Nagarajan, B., 1991. Protective effect of oleanolic acid and ursolic acid against lipid peroxidation. Biochemistry International 24, 981–990.

Bensen, W.G., Fiechtner, J.J., McMillen, J.I., 1999. Treatment of osteoarthritis with celecoxib, a cyclooxygenase-2 inhibitor: a randomized controlled trial. Mayo Clinical Process 74, 1095–1105.

Bradley, P.P., Priebat, D.A., Christensen, R.D., Rothstein, G., 1982. Measurement of cutaneous inflammation: estimation of neutrophil content with an enzyme marker. Journal of Investigate Dermatology 78, 206–209.

Choi, JH., Kim, DY., Yoon, JH., Yoon, HY., Yi, JB., 2002. Effects of SKI-306X, a new herbal agent, on proteoglycan degradation in cartilage explant culture and collagenase-induced rabbit osteoarthritis model. Osteoarthritis and cartilage 10, 471–478.

Dai, Y., Hang, B.Q., Li, P.Z., Tan, L.W., 1989. Effects of oleanolic acid on immune system and type I allergic reaction. Acta Pharmacologica Sinica 10, 381–384.

Drazen, J., Israel, E., O'Bryne, P., 1999. Treatment of Asthma with drugs modifying the leukotriene pathway. New England Journal of Medicine 340, 197–206.

Emery, P., Zeidler, H., Kvien, T.K., 1999. Celecoxib versus diclofenac in long-term management of rheumatoid arthritis: Randomized double-blind comparison. Lancet 354, 2106–2111.

Englberger, W., Hadding, U., Etschenberg, E., Graf, E., Leyck, S., Winkelmann, J., Parnham, M.J., 1988. Rosmarinic acid: a new inhibitor of complement C3-convertase with anti-inflammatory. International Journal of Immunopharmacology 10, 729–737.

Guslandi, M., 1987. Gastric effects of leukotrienes. Prostaglandins Leukotrienes and Medicine 26, 203–208.

Huang, Y.S., Zhang, J.T., 1992. Effects of three water-soluble components of Salviae Miltiorrhizae (DanShen) on anti-oxidation in vitro. Acta Pharmaceutica Sinica 27, 96–100.

James, E., Smolen, W.G., 1980. Effects of indomethacin 5, 8, 11, 14-eicosatetraynoic acid, and p-bromophenacyl bromide on lysomal enzyme release and superoxide anion generation by human polymorphonuclear leukocytes. Biochemical Pharmacology 29, 533–538.

Jobanputra, P., Nuki, G., 1994. Nonsteroidal anti-inflammatory drugs in the treatment of osetoarthritis. Current Opinion in Rheumatology 6, 433–439.

Jung, Y.B., Roh, K.J., Jung, J.A., 2001. Effect of SKI-306X, a new herbal anti-arthritic agent, in patients with osteoarthritis of the knee: a double-blind placebo controlled study. American Journal of Chinese Medicine 29 (3-4), 485–491.

Keijiro, T., Eun-Hee, P., Hitoshi, K., 1980. Anti-Inflammatory activities of hederagenin and crude saponin isolated from Sapindus mukorissi Gaertn. Chemical and Pharmaceutical Bulletin 28, 1183–1188.

Kim, H.T., Ahn, J.S., Jeong, I.H., 1996. Subacute toxicity of SKI-306X, anti inflammatory herbal extract, in rats. Journal of Applied Pharmacology 1, 19–31.

Laufer, S., Augustin, J., Dannhardt, G., Kiefer, W., 1994a. 6, 7-Diaryldihydropyrrolizine-5-yl-acetic acids, a novel class of potent dual inhibitors of both cyclo-oxygenase and 5-lipoxygenase. Journal of Medicinal Chemistry 37.

Laufer, S., Tries, S., Augusti, J., Dannhart, G., 1994b. Pharmalogical profile of new pyrrolizine-derivative that inhibits the enzyme cyclo-oxygenase and 5-lipoxygenase. Arzneimittel-Forschung-Drug Research 44, 629.

Lewis, R., Austen, K., Soberman, R., 1990. Leukotrienes and other products of the 5-lipoxygenase pathway: biochemistry and relation to pathobiology in human disease. New England Journal of Medicine 323, 645–655.

Miller, L.G., Prichard, J.G., 1990. Current issue in NSAID theraphy 17, 589–601.

Mitchell, J.A., Akarasereenont, P., Thiemermann, C., Flower, R.J., Vane, J.R., 1993. Selectivity of nonsteroidal antiinflammatory drugs as inhibitors of constitutive and inducible cyclooxygenase. Proceedings of the National Academy of Sciences of the United States of America 90, 116930–116942.

Park, KS., Kim, HS., Ahn, JS., 1995. Preparation of anti-inflammatory herbal drug, SKI-306X. Yakhak Hoeji 39, 385–394.

Polison, R., 1996. Nonsteroidal anti-inflammatory drugs: practical and theoretical consideration in their selection. American Journal of Medicine 100 (Suppl. 2A), 31S–36S.

Robert, C.A., 1979. Cytoprotection by prostagladins. Gastroenterology 77, 761.

Simon, L.S., Lanza, F.L., Lipsky, P.E., 1998. Preliminary study of the safety and efficacy of SC-58635, a novel cycloxygenase 2 inhibitor: efficacy and safety in two placebo-cotrolled trials and studies of gastrointestinal and platelet effects. Arthritis and Rheumatism 41, 1591–1602.

Singh, G.B., Singh, S., Banl, S., Banerjee, S.K., Oupta, D., Banerjee, SK., 1991. Anti-inflammatory activity of oleanolic acid in rats and mice. Journal of Pharmacy and Pharmacology 44, 456–458.

Smuelsson, B., 1983. Leukotrienes: mediators of immediate hypersensitivity reactions and inflammation. Science 220, 568–575.

Szabo, S., 1989. Mechanisms of mucosal protection. In: Hollander, D., Tarnawski, A. (Eds.), Gastric Cytoprotection: A Clinician's Guide, pp. 49–60.

Tarnawski, A., Hollander, D., 1987. Cytoprotection of gastric and duodenal mucosa. The Royal Society of Medicine. Current Medical Literature. Gastroenterology 6, 3–9.

Vane, J.R., 1971. Inhibition of prostagladin synthesis as a mechanism of action for aspirin-like drugs. Nature 231, 232.

Wallace, JL., Granger, DN., 1996. The cellular and molecular basis of gastric mucosal defence. FASEB Journal 10, 731–740.

Wallace, J., Reuter, B., Cicala, C., McKnight, W., Grisham, M.B., Cirino, G., 1994a. A diclofenac derivative without ulcerogenic properties. European Journal of Pharmacology 257, 249–258.

Wallace, J., Reuter, B., Cicala, C., McKnight, W., Grisham, M.B., Cirino, G., 1994b. Novel nonsteroidal anti-inflammatory drug derivatives with markedly reduced ulcerogenic properties in the rat. Gastroenterology 107, 179–187.

Whittle, B.J.R., 1981. Temporal relationship between cyclooxygenase inhibition, as measured by prostacyclin biosynthesis and the gastrointestinal damage induced by indomethacin in the rat. Gastroenterology 80, 94.

Yoshiyuki, K., Hiromichi, O., 1987. Studies on the activities of tannins and related compounds: X. Effects of caffeetannins and related compounds on arachidonate metabolism in human polymorphonuclear leukocytes. Journal of Natural Products 50, 392–399.

Zou, Z.W., Xu, L.N., Tian, J.Y., 1993. Antithrombic and antiplatelet effects rosmarinic acid, a water-soluble component isolated from radix salviae militiorrhiaze (Danshen). Acta Pharmaceutica Sinica 28, 241–245.