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Short communication

Effects of SA6541, a leukotriene A_4 hydrolase inhibitor, and indomethacin on carrageenan-induced murine dermatitis

Fumio Tsuji *, Yurika Miyake, Hiroshi Enomoto, Masato Horiuchi, Shiro Mita

Discovery Research Division, Santen Pharmaceutical, 3-9-19 Shimoshinjo, Higashiyodogawa-ku, Osaka 533-8651, Japan

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Abstract

We investigated the effects of *S*-(4-dimethylaminobenzyl)-*N*-[(2*S*)-3-mercapto-2-methylpropionyl]-L-cysteine (SA6541), a potent leukotriene A_4 hydrolase inhibitor, on early phase of carrageenan-induced dermatitis model. Carrageenan injection induced edema and neutrophil influx in the mouse ear. SA6541 inhibited edema formation and neutrophil influx. SA6541 also inhibited leukotriene B_4 production but not prostaglandin E_2 production in the mouse ear. On the other hand, indomethacin inhibited edema formation but not neutrophil influx. Indomethacin also inhibited prostaglandin E_2 production but not leukotriene B_4 production. Combination therapy with SA6541 and indomethacin strongly inhibited edema formation in comparison with treatment with either agent alone. These results suggest that leukotriene B_4 may be important in the pathogenesis of dermatitis. © 1998 Elsevier Science B.V.

Keywords: SA6541; Leukotriene A_4 hydrolase inhibitor; Leukotriene B_4; Indomethacin; Dermatitis

1. Introduction

Leukotriene B_4, a product of the action of 5-lipoxygenase on arachidonic acid, induces chemokinetic, chemotactic and aggregation responses in polymorphonuclear leukocytes (Ford-Hutchinson et al., 1980), degranulation (Showell et al., 1982) and increased adhesion of these cells to endothelial cell monolayers (Gimbrone et al., 1984). Evidence supporting a role of leukotriene B_4 in in vivo inflammatory processes has come from preclinical studies in animal models. Exogenous leukotriene B_4 was shown to induce polymorphonuclear leukocyte infiltration in animals (Wedmore and Williams, 1981; Movat et al., 1984; Ruzicka and Burg, 1987). Leukotriene B_4 has been found in a number of pathological fluids associated with the development of inflammation (Simmons et al., 1983; Aked and Foster, 1987), and both blockers of synthesis (i.e., 5-LO inhibitors) and antagonists of leukotriene B_4 receptors have been reported to inhibit the development of inflammatory processes (Fretland et al., 1990). Furthermore, some types of inflammatory responses were shown to be markedly reduced in 5-lipoxygenase knockout mice (Chen et al., 1994). In addition, administration of exogenous leukotriene B_4 induced inflammatory responses in normal subjects (Soter et al., 1983; Martin et al., 1989), and biologically relevant levels of leukotriene B_4 and its metabolites have been reported in numerous human tissues and fluids in pathological states (Lewis et al., 1990). Thus, prevention of leukotriene B_4 synthesis via inhibition of either 5-lipoxygenase or leukotriene A_4 hydrolase which produces leukotriene B_4 from leukotriene A_4, or alternatively inhibiting its action by blocking leukotriene B_4 receptors have been considered viable modes of action for novel anti-inflammatory drugs. To examine the role of leukotriene B_4 in dermatitis, we used *S*-(4-dimethylaminobenzyl) -*N*- [(2*S*)-3-mercapto-2-methylpropionyl]-L-cysteine (SA6541), a newly synthesized potent leukotriene A_4 hydrolase inhibitor. We reported previously that SA6541 inhibited leukotriene B_4 production and cell infiltration in 5-hydroperoxyeicosatetraenoic acid- and arachidonic acid-induced murine dermatitis models (Tsuji et al., 1998). In the present study, we investigated the effects of SA6541 on early phase of carrageenan-induced murine dermatitis.

2. Materials and methods

2.1. Animals

Inbred male ICR mice, 6–7 weeks old, were purchased from Japan (Hamamatsu, Japan). They were housed under

* Corresponding author. Tel.: +81-6-321-7034; fax: +81-6-321-2955.

a 12-h light–dark cycle (lights on at 07:00), with room temperature maintained at 23 ± 1°C, and humidity at 55 ± 10%. Food and water were freely available. All experiments were conducted in accordance with the recommendations of the Declaration of Helsinki.

2.2. Reagents

SA6541, an inhibitor of leukotriene A_4 hydrolase, was synthesized by the Central Research Laboratories of Santen (Osaka, Japan). Indomethacin, a cyclooxygenase inhibitor, was purchased from Wako (Osaka, Japan). These agents were suspended in 1% methyl cellulose solution (vehicle) for oral administration. λ-Carrageenan (Sigma, St. Louis, MO, USA) was used to induce dermatitis. This agent was suspended in saline.

2.3. Carrageenan-induced mouse ear dermatitis model

Mice were challenged intradermally with 25 μl of 1% carrageenan in the external ear using a microsyringe with a 27-gauge needle. At various time points after the intradermal challenge, the animals were killed by anesthetic overdose and the injection sites were punched out with either an 8 mm gasket punch for weight measurement, or a 6 mm gasket punch then placed in tubes containing 0.75 ml of 0.5% hexadecyltrimethylammonium bromide/80 mM phosphate buffer (pH 5.4) for myeloperoxidase activity measurement.

2.4. Administration of SA6541 or indomethacin in the dermatitis models

Animals in the control group were given the vehicle only orally. Other groups were orally dosed with SA6541 (100 mg/kg) or indomethacin (5 mg/kg) in the vehicle. One hour after oral dosing, mice were challenged intradermally with 25 μl of 1% carrageenan using a microsyringe with a 27-gauge needle. The animals were killed by anesthetic overdose and the injection sites were punched out 4 or 8 h after the challenge to evaluate edema formation or neutrophil influx, respectively. For the measurement of leukotriene B_4 or prostaglandin E_2, the injection sites were punched out with a 6 mm gasket punch 30 min after the challenge then placed in tubes containing 1 ml of ethanol.

2.5. Evaluation of edema formation

Evaluation of edema formation was performed by weighing the punched out ear tissue.

2.6. Measurement of myeloperoxidase activity

Myeloperoxidase activity was measured according to the method reported previously (Tsuji et al., 1998). The samples were homogenized and subjected to two freeze–thaw cycles, and then centrifuged at 12 000 × *g* for 15 min. Aliquots of 30 μl of the supernatant were diluted with 100 μl of phosphate buffered saline and 75 μl of 0.22 M phosphate buffer in 96-well plates, then mixed with 35 μl of enzyme substrate containing tetramethylbenzidine/hydrogen peroxide (Amersham, Little Chalfont, UK) for 5 min at 37°C. The reaction was terminated by adding 30 μl of 1.0 M sulfuric acid, and the absorbance of each sample was measured with a microtiter plate reader at 450 nm. The activity was expressed as units of OD/min per cm^2 by conversion using the area of the punched out ear tissue (0.2826 cm^2).

2.7. Measurement of leukotriene B_4 or prostaglandin E_2

The measurement of leukotriene B_4 or prostaglandin E_2 was performed according to the method reported previously (Tsuji et al., 1998). The samples were homogenized and centrifuged at 12 000 × *g* for 15 min. The supernatants were evaporated to dryness and dissolved in 3 ml of 0.1 M citrate buffer (pH 3.0). Solutions were loaded onto C18 SepPak (Waters, Milford, MA, USA) columns, which were then washed with 25% methanol in water containing 1% acetic acid. Leukotriene B_4 and prostaglandin E_2 were

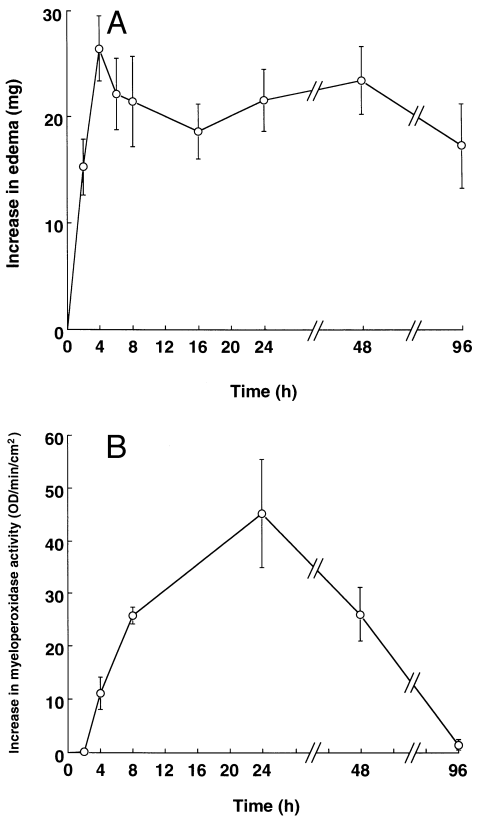

Fig. 1. Carrageenan-induced mouse ear dermatitis (a: edema formation, b: neutrophil accumulation). The lines with vertical lines represent the means ± SE of four animals. (a) Evaluation of edema formation was performed at various time points after carrageenan injection. (b) Myeloperoxidase activity was measured at various time points after carrageenan injection.

eluted from these columns by 80% methanol in water containing 1% acetic acid, and subsequently lyophilized to remove the organic solvent. The recoveries of leukotriene B_4 and prostaglandin E_2 in this extraction procedure were 89% and 96%, respectively. Leukotriene B_4 and prostaglandin E_2 were quantified using appropriate enzyme immunoassay kits (Amersham). The amounts of leukotriene B_4 or prostaglandin E_2 were expressed as units of pg/cm^2 by conversion using the area of the punched out ear tissue (0.2826 cm^2).

2.8. Statistical analysis

Dunnett's multiple comparison test and Student's *t*-test (Statistical Library, Yukms, Tokyo, Japan) were used for the statistical analysis of the results.

3. Results

3.1. Carrageenan-induced inflammation

The time-course of edema formation in the mouse ear following intradermal injection of carrageenan is shown in Fig. 1a. There was an immediate increase of edema which peaked at 4 h and about 48 h after carrageenan injection. The time-course of neutrophil accumulation in the mouse ear following intradermal injection of carrageenan is shown in Fig. 1b. Neutrophil accumulation gradually increased reaching a peak at about 24 h then returned to the low level 96 h after carrageenan injection. In a preliminary study, there was an immediate increase in leukotriene B_4 or prostaglandin E_2 concentration which peaked at about 30 min. Then, leukotriene B_4 or prostaglandin E_2 concentration gradually decreased (data not shown).

3.2. Effects of SA6541 or indomethacin on carrageenan-induced inflammation

The effects of SA6541 on carrageenan-induced edema formation, neutrophil accumulation and the increase in leukotriene B_4 or prostaglandin E_2 concentration in the mouse ear are shown in Fig. 2. SA6541 (100 mg/kg) significantly inhibited edema formation 4 h after carrageenan injection (about 20% inhibition) (Fig. 2a) and neutrophil accumulation 8 h after carrageenan injection (about 53% inhibition) (Fig. 2b). SA6541 also inhibited

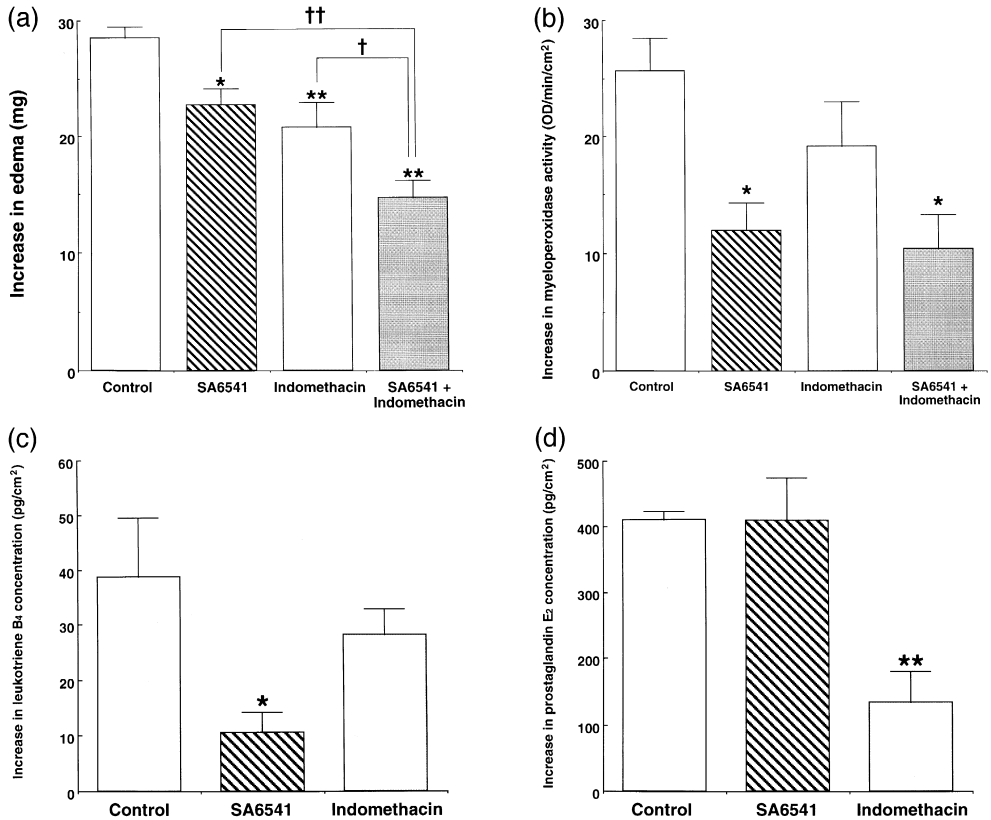

Fig. 2. Effects of SA6541 (100 mg/kg) and indomethacin (5 mg/kg) on carrageenan-induced mouse ear dermatitis (a: edema formation, b: neutrophil accumulation, c: leukotriene B_4 concentration, d: prostaglandin E_2 concentration). The bars with vertical lines represent the means ± SE of four to 14 animals. (a) The samples used for evaluation of edema formation were collected 4 h after carrageenan injection. (b) The samples used for measurement of myeloperoxidase activity were collected 8 h after carrageenan injection. (c, d) The samples used for measurement of leukotriene B_4 or prostaglandin E_2 were collected 30 min after carrageenan injection. $*P < 0.05$, $**P < 0.01$ vs. control group (by Dunnett's multiple comparison test). $\dagger P < 0.05$, $\dagger\dagger P < 0.01$ between two groups (by Student's *t*-test).

the increase in leukotriene B_4 concentration 30 min after carrageenan injection (about 73% inhibition) (Fig. 2c), whereas it had no effect on the increase in prostaglandin E_2 concentration (Fig. 2d). On the other hand, indomethacin (5 mg/kg) inhibited edema formation (about 27% inhibition) (Fig. 2a) but not neutrophil accumulation (Fig. 2b). Indomethacin also inhibited the increase in prostaglandin E_2 concentration (about 67% inhibition) (Fig. 2d), whereas it had no effect on the increase in leukotriene B_4 concentration (Fig. 2c). Since both SA6541 and indomethacin inhibited edema formation, we investigated the effects of combination therapy with both drugs on carrageenan-induced edema formation. Combination therapy strongly inhibited edema formation (about 48% inhibition) more than either drug alone (Fig. 2a). Combination therapy also inhibited neutrophil accumulation (about 59% inhibition), however its efficacy was almost the same as that of SA6541 alone (Fig. 2b).

4. Discussion

We showed previously that the newly synthesized agent SA6541 is a potent leukotriene A_4 hydrolase inhibitor (Tsuji et al., 1998). Moreover, in a previous study, we showed that orally administered SA6541 was efficacious in two models of epidermal inflammation in mice. In the present study, we investigated the effects of SA6541 on another epidermal inflammatory model in mice. Intradermal injection of carrageenan induced edema formation and neutrophil accumulation following prostaglandin E_2 and leukotriene B_4 production. SA6541 inhibited leukotriene B_4 production, edema formation and neutrophil accumulation. On the other hand, indomethacin inhibited prostaglandin E_2 production and edema formation but not neutrophil accumulation. These results suggest that leukotriene B_4 may be important not only for neutrophil accumulation but also for edema formation in early phase of carrageenan-induced dermatitis. In this model, cyclooxygenase products may be important for edema formation only. In addition, combination therapy with SA6541 and indomethacin strongly inhibited edema formation more than treatment with either drug alone. These results suggest that edema formation may be due, at least in part, to the additive effects of cyclooxygenase products and leukotriene B_4.

Some investigators have reported that leukotriene B_4 has important roles in edema formation. Topical application of a single dose of leukotriene B_4 (50 ng) under occlusion to the skin of normal volunteers and to the clinically normal skin of patients with untreated stable plaque psoriasis caused an inflammatory reaction including erythema and edema (Wong et al., 1985). Raychaudhuri et al. (1995) reported that the potent leukotriene B_4 receptor antagonist CGS25019C inhibited arachidonic acid-induced ear edema in mice and leukotriene B_4-induced neutropenia in rats. Fretland et al. (1990, 1995) reported that orally active leukotriene B_4 antagonists SC-41930 and SC-53228 reduced edema and inflammatory cell infiltration in the phorbol-12-myristate-13-acetate-induced skin inflammation model in guinea pigs. Rao et al. (1994) also reported that selective inhibitors of 5-lipoxygenase and leukotriene B_4 receptor antagonists attenuated phorbol ester-induced dermal inflammation in mice. Our results are in agreement with those of these other studies.

The dose of SA6541 or indomethacin used in this study may be enough for each enzyme inhibition (Utsunomiya et al., 1994; Tsuji et al., 1998). In the present study, SA6541 and indomethacin significantly inhibited leukotriene B_4 and prostaglandin E_2 production, respectively. However, about 52% of the edema was resistant to inhibition by these agents. These results suggest that other mediators may be important for edema formation. Bradykinin may be another candidate for the edema formation in carrageenan-induced dermatitis. In carrageenan-induced edema in rats, an involvement of the kallikrein–kinin system has been reported (Dozen et al., 1989). In addition, about 41% of the neutrophil accumulation was resistant to inhibition by these agents. Utsunomiya et al. (1991, 1996) reported that tumor necrosis factor, interleukin-1, interleukin-6 and cytokine-induced neutrophil chemoattractant were detected in rat carrageenin-induced pleurisy, and these cytokines caused neutrophil infiltration by intrapleural injection. Therefore, these cytokines may be the other candidates for the neutrophil infiltration in carrageenan-induced dermatitis.

5. Conclusion

In conclusion, we have shown that leukotriene B_4 may be important for neutrophil accumulation and edema formation in early phase of mouse ear inflammation.

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