Loxoprofen Sodium, a Non-Selective NSAID, Reduces Atherosclerosis in Mice by Reducing Inflammation

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Summary Recently, it is suggested that the use of nonsteroidal anti-inflammatory drugs (NSAID) may contribute to the occurrence of cardiovascular events, while the formation of atherosclerotic lesions is related to inflammation. Loxoprofen sodium, a non-selective NSAID, becomes active after metabolism in the body and inhibits the activation of cyclooxygenase. We fed apoE^{-/-} mice a western diet from 8 to 16 weeks of age and administered loxoprofen sodium. We measured atherosclerotic lesions at the aortic root. We examined serum levels of cholesterol and triglycerides with HPLC, platelet aggregation, and urinary prostaglandin metabolites with enzyme immune assay. Atherosclerotic lesion formation was reduced to 63.5% and 41.5% as compared to the control in male and female apoE^{-/-} mice treated with loxoprofen sodium respectively. Urinary metabolites of prostaglandin E₂, F_{1α}, and thromboxane B₂, and platelet aggregation were decreased in mice treated with loxoprofen sodium. Serum levels of cholesterol and triglycerides were not changed. We conclude that loxoprofen sodium reduced the formation of early to intermediate atherosclerotic lesions at the proximal aorta in mice mediated by an anti-inflammatory effect.

Key Words: non-selective nonsteroidal anti-inflammatory drugs, loxoprofen sodium, atherosclerosis

Introduction

As a result of progress in therapy for rheumatoid arthritis (RA), cardiovascular events have become the major cause of death in these patients. RA is associated with an increased and accelerated vascular risk that results in lifespan reduc-

tion [1–3]. There is an association between disease activity and cardiovascular risk in RA and other inflammatory disorders, such as systemic lupus erythematosus (SLE) [1, 4, 5]. Several factors may be associated with accelerated arteriosclerosis in patients with RA or SLE. One factor may be the disease itself, while others may include the pharmacological therapies that patients with RA or SLE receive.

Rofecoxib, a highly selective cyclooxygenase(COX)-2 inhibitor, has recently been voluntarily withdrawn from the market due to evidence from the APPROVe trial demonstrating increased cardiovascular events after 18 months

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of use. Combination therapy with the selective COX-2 inhibitors, valdecoxib and parecoxib increased the risk by 3.7-fold [6]. Concerns have been raised that a highly selective COX-2 inhibition may promote cardiovascular events by inhibiting prostacyclin and inducing a prothrombotic state [7]. In contrast, inhibition of COX-1 mediated production of platelet thromboxane (Tx) by aspirin reduces the risk of myocardial infarction and stroke [8].

Selective COX-2 inhibitors are the major anti-pain drugs used in western countries. Loxoprofen sodium is a pro-drug of phenyl mefenamic acid and a non-selective COX inhibitor. It is the dominant anti-pain drug in Japan and most frequently prescribed in East Asian countries [9]. In view of the reports related to COX-2 selective inhibitors in the USA, the adverse effects of nonsteroidal anti-inflammatory drugs (NSAIDs) associated with cardiovascular disease were surveyed in Japan in June 2005. However, no evidence was found of any adverse effects of loxoprofen sodium associated with cardiovascular events. The Pharmaceutical and Medical Devices Agency in Japan concluded that it was not necessary to perform safety measures for loxoprofen sodium against cardiovascular events [www.info.pmda.go.jp].

It has been reported that selective inhibition of COX-1 attenuates atherosclerosis in apoE^{-/-} mice [10]. However, reports on the effect of selective COX-2 inhibition on the development of atherosclerosis in murine models have been mixed with decreased, increased or unchanged atherosclerotic lesions all noted [11, 12]. The divergence in the results may be the consequence of differences in experimental design, including efficacy and selectivity of the inhibitors, gender of the mice and stage of atherosclerotic lesions.

The surveillance study of adverse effects of loxoprofen sodium in Japan and these studies of non-selective inhibition of COX inhibition on the development of atherosclerosis in animal models demonstrate the ability of non-selective COX inhibition with loxoprofen sodium to reduce early and intermediate atherosclerotic lesion formation in apoE^{-/-} mice, supporting the efficacy of anti-inflammatory approaches in the prevention of atherosclerosis.

Materials and Methods

Healthy volunteers

Three healthy men participated in this study. They received blood examinations and collected urine for 24 h before administration of loxoprofen sodium. After receiving loxoprofen sodium 60 mg/body three times a day for 4 days, they received blood examinations and collected urine again. The ethics committee of Kyoto Prefectural University of Medicine approved the study.

Animal procedures

ApoE^{-/-} mice (C57BL/6J-ApoE^{tm1Unc}) were originally purchased from Jackson Laboratories (Bar Harbor, ME). ApoE^{-/-} mice were at the 10th backcross into the C57BL/6 background. Mice were maintained on a rodent chow diet containing 4.5% fat (Oriental BioService, Kyoto, Japan) and autoclaved acidified (pH 2.8) water before 8 weeks of age. From 8 (day 57) to 16 (day 113) weeks of age, these mice were maintained on a “Western” high-fat diet (0.2% cholesterol, 21% saturated fat; Oriental BioService, Kyoto, Japan). The atherosclerosis studies were designed to examine the effect of administration of loxoprofen sodium during early atherosclerotic lesion formation in apoE^{-/-} mice. The mice were divided into 3 groups (vehicle group, 7 day loxoprofen sodium group, 56 day loxoprofen sodium group) at 8 weeks of age (day 57), randomly. Animal care and experimental procedures were carried out in accordance with the regulations and with the approval of Kyoto University’s Animal Care Committee. The mice were continued on this regimen until 16 weeks of age, when they were killed with an overdose of sodium pentobarbital.

Administration of loxoprofen sodium or indomethacin to apoE^{-/-} mice

Randomly selected mice were treated with loxoprofen sodium (4 mg/kg/day) added to the drinking water from 8 (day 57) to 16 (day 113) weeks of age or from 15 (day 106) to 16 (day 113) weeks of age. The dose of loxoprofen sodium used in our study was chosen based on oral dosing in acute studies of carageenan induced footpad edema plethysmometry in rats in which the oral ED50 for this assay in rats is 2 mg/kg, twice a day [13]. The dose of the drug was calculated on the basis of the average consumption of water and the body weight, determined weekly. Loxoprofen sodium was kindly provided by Daiichi-Sankyo, Co. (Tokyo, Japan).

Urinary thromboxane, prostaglandin I, and prostaglandin E₂ metabolite excretion

As platelet TxA₂ metabolite, prostaglandin (PG) I metabolite, and PGE₂ metabolites, we measured urinary 2,3 dinor TxB₂, urinary 2,3 dinor 6 keto PGF_{1α}, urinary PGE₂ metabolites. From 15 to 16 weeks of age, 24 h urine samples of mice were collected with metabolic cages (Tecniplast, Italy). Mouse and human samples were analyzed by a commercial assay according to the manufacturer’s procedure (Cayman Chemical, MI). Urine creatinine (CRE) was measured with dry chem system (SRL, Tokyo, Japan).

Determination of the platelet aggregation

ApoE^{-/-} mice were maintained on the “Western” high-fat diet from 8 to 16 weeks of age and were treated with vehicle or 4 mg/kg/day of loxoprofen sodium from 15 to 16 weeks

of age. At 90 min after final dose administration, blood was collected from five mice in each group. Blood was collected in 12.9 mM sodium citrate and gently agitated at 37°C for 30 min, and centrifuged at 250 g for 15 min to obtain plasma [14]. Blood samples were also collected from healthy volunteers in 12.9 mM sodium citrate and centrifuged at 150 g for 15 min to obtain platelet rich plasma (PRP) before loxoprofen sodium administration and after receiving loxoprofen sodium 60 mg/body three times a day for 4 days. After separation of PRP, tubes were centrifuged again at 1,200 g for 15 min to obtain platelet poor plasma.

To measure changes in the light transmission rate, PRP samples (200 μ l) were incubated for 2 min at 37°C, then with 22.2 μ l of adenosine diphosphate (ADP) or collagen solution for 5 min at 37°C. The intensity of light transmitted over 5 min was measured using MCM hematoracer 313 (MC medical, Tokyo, Japan). ADP and collagen solution were purchased from MC medical (MC medical, Tokyo, Japan). ADP added at 1, 2, 4, 8 μ M in humans and at 0.5, 1, 2, 4 μ M in mice. Similarly, collagen was added at 0.25, 0.5, 1, 2 μ g/mL in humans and 0.5, 1, 2, 4 μ g/mL in mice. The pressure rate was standardized using a grading curve produced by plotting data with four concentrations of agonist on the x-axis and pressure rate (%) on the y-axis. The concentration of agonist causing a 50% maximum aggregation rate was calculated and applied as the platelet aggregatory threshold index (PATI) [14].

Serum lipids and lipoprotein distribution analysis

ApoE^{-/-} mice were maintained on a “Western” high-fat diet from 8 to 16 weeks of age and were treated with vehicle or 4 mg/kg/day of loxoprofen sodium from 8 to 16 weeks of age. The sera were obtained from mice fasted overnight (11 animals from each group). Serum albumin, CRE, and free fatty acids (FFA) were analyzed with dry chem system (SRL, Tokyo, Japan). A high performance liquid chromatograph (HPLC) system with two tandem gel permeation columns was used to evaluate the size distribution of plasma lipoprotein particles (Skylight Biotech, Akita, Japan) [15, 16]. Fast performance liquid chromatography was performed on an HPLC system model 600 (Waters) with a Sepharose 6 column (Skylight Biotech, Akita, Japan). Samples were diluted 20 times and analyzed at a flow rate of 350 μ l/min by monitoring the concentrations of total cholesterol, and triglycerides with absorbance at 550 nm.

Analysis of aortic lesions

The hearts were flushed through the left ventricle, and the aortic root was obtained for analysis. The hearts were removed immediately after the mice were killed, rinsed in cold phosphate buffered saline to remove traces of blood, and placed in 4% paraformaldehyde overnight. The hearts

were sliced with a scalpel on a plane parallel to the tips of the atria at the base of the aortic root, according to a procedure described by Paigen *et al.* [17]. The tissue was processed and embedded in OTC compound for histological sectioning by conventional methods. Fifteen alternate cryosections of 10-mm thickness were collected from the proximal aorta starting from the beginning of the aortic sinus and extending 300 μ m distally. The sections were stained with Oil-Red-O and photographed at a magnification of 400 with an Olympus U-CMAD3 camera connected to an Olympus BX50 microscope and DXC-S500/OL digital capture (Olympus, Tokyo, Japan). The vessel structures were traced and areas calculated in the computer-digitized images with the Image J program (National Institutes of Health, MD). Sections from the aortic arch were used to quantify atherosclerosis (8 animals from each group). An observer who was blinded to all other data analyzed the lesions.

Histological examination of lesion morphology

Sections (10 μ m) of OCT embedded tissue were acetone fixed, and endogenous peroxidase activity was quenched with 0.3% hydrogen peroxide. Serial sections were subjected to immunostaining with 2 μ g/mL goat anti-COX-1 antibody (Santa Cruz Biotechnology, CA), 2 μ g/mL goat anti-COX-2 antibody (Santa Cruz Biotechnology, CA), 5 μ g/mL goat monoclonal antibodies to macrophages (MOMA2) antibody (AbD Serotec, Oxford, UK), followed by avidin-biotin rabbit anti-goat antibody (Nichirei, Tokyo, Japan). The slides were then incubated with 3,3-Diaminobenzidine tetrahydrochloride (Sigma, MO) and counterstained with hematoxylin (Wako Pure Chemical Industries, Osaka, Japan). Isotype-matched controls were stained in parallel and in all cases showed no significant reactivity (data not shown). Intensity of COX-1, COX-2, and MOMA2 staining was measured by determining the maximum density of DAB stained cells using the Image J program (National Institutes of Health, MD). Sections from the aortic arch were used to quantify atherosclerosis (8 animals from each group). A blinded observer analyzed the lesions.

Statistical analysis

Data are expressed as mean $\pm$ SEM. Statistical significance was calculated in the SPSS statistical package, version 11.0.1 J (SPSS, IL) for Windows. Student's *t* test was used for comparisons between two groups and Dunnett test was used to for comparisons among more than three groups. Probability values <0.05 were regarded as significant.

Fig. 1. Inhibition of prostaglandin production in healthy volunteers and apoE^{-/-} mice by loxoprofen sodium. A, B, and C; Urinary prostaglandin metabolites in apoE^{-/-} mice reconstituted with vehicle (open bars), with loxoprofen sodium (solid bars). From mice (15 to 16 weeks) given vehicle or loxoprofen sodium, urine samples for 24 h were collected in metabolic cages. Urinary metabolites of TxB_2 (A), $\text{PGF}_{1\alpha}$ (B), and PGE_2 (C) were analyzed by enzyme immune assay. D, E, and F, Urine was gathered from 3 healthy men for 24 h before and after receiving loxoprofen sodium 60 mg/body three times a day for 4 days. Urinary metabolites of TxB_2 (D), $\text{PGF}_{1\alpha}$ (E), and PGE_2 (F) were analyzed by enzyme immune assay. Data were expressed as the average (mean $\pm$ SEM) of the individual ($n = 8$) assays in each group. CRE indicates creatinine. Student *t* test was used for comparisons between two groups and Dunnett test was used for comparisons between two groups.

Fig. 2. Inhibition of platelet aggregation by loxoprofen sodium. Figures show representative % light transmission curves at each concentration of platelet aggregation stimulative agents. Platelet aggregation was stimulated by adenosine diphosphate (ADP) and collagen and was quantified with an MCM hematracr 313. Y-axis means % light transmission and X-axis means time. Black bar on X-axis indicates 5 min. A and B; mouse platelet aggregation with vehicle at day 7, C and D; mouse platelet aggregation with loxoprofen sodium at day 7, E and F; human platelet aggregation with vehicle at day 4, G and H; healthy human platelet aggregation with loxoprofen sodium at day 4.

Table 1. Serum lipid levels in apoE^{-/-} mice treated with vehicle or 4 mg/kg/day loxoprofen sodium from 8 to 16 weeks of age

	Male			Female		
	Vehicle	Loxoprofen Na 56 days	<i>p</i>	Vehicle	Loxoprofen Na 56 days	<i>p</i>
N	11	11		11	11	
Body weight [g]	27.3 ± 0.48	27.17 ± 0.34	0.73	21.80 ± 0.91	20.04 ± 0.57	0.12
Albumin [mg/dL]	3.01 ± 0.03	2.94 ± 0.03	0.07	3.12 ± 0.03	3.12 ± 0.04	0.98
Creatinine [mg/dL]	0.07 ± 0.01	0.09 ± 0.01	0.15	0.08 ± 0.01	0.08 ± 0.01	0.98
Free fatty acids [μEQ/L]	662.64 ± 110.4	553.17 ± 117.59	0.51	413.02 ± 58.2	383.19 ± 107.33	0.81
Total cholesterol [mg/dL]	1253.26 ± 51.51	1162.89 ± 52.74	0.23	1045.65 ± 39.81	1091.43 ± 103.33	0.69
Triglycerides [mg/dL]	39.60 ± 6.44	34.95 ± 3.39	0.53	21.11 ± 1.93	21.26 ± 2.15	0.96

From the 8th week to 16th week, mice were randomized to receive a western diet and vehicle or 4 mg/kg/day of loxoprofen sodium ($n = 11$ animals for each group). Mice were fasted for 4 h, and lipoprotein distribution was determined by HPLC. Serum albumin, creatinine, and free fatty acids were analyzed with dry chem system. Results are expressed as mean ± SEM. Student *t* test was used for comparisons between two groups.

Results

Loxoprofen sodium inhibits platelet thromboxane production in apoE^{-/-} mice and healthy humans

To test the effect and the COX-2 selectivity of loxoprofen sodium, we measured platelet Tx, PGI₂, and PGE₂ production in apoE^{-/-} mice and healthy men. Loxoprofen sodium inhibited the platelet production of TxA₂ metabolite, urinary TxB₂, by 29.0% compared to vehicle in apoE^{-/-} mice (269.55 ± 77.48 ng/mg CRE vs 929.17 ± 196.29 ng/mg CRE, $p = 0.012$), and inhibited the production of urinary TxB₂ by 26.2% compared to pre medication in men (0.53 ± 0.10 ng/mg CRE vs 2.04 ± 0.12 ng/mg CRE, $p = 0.001$) (Fig. 1A and D). Loxoprofen sodium inhibited the production of PGE₂ metabolite in apoE^{-/-} mice by 9.0% compared to vehicle (1.29 ± 0.29 ng/mg CRE vs 14.49 ± 3.60 ng/mg CRE, $p = 0.003$, respectively), and inhibited the production of urinary PGE₂ metabolite by 29.7% compared to pre medication in men (0.06 ± 0.02 ng/mg CRE vs 0.20 ± 0.03 ng/mg CRE, $p = 0.028$) (Fig. 1C and F). Loxoprofen sodium also inhibited the production of PGI₂ metabolite, urinary 2,3 dinor 6 keto PGF_{1α}, in apoE^{-/-} mice by 26.9% compared to vehicle (107.0 ± 17.83 ng/mg CRE vs 397.2 ± 122.1 ng/mg CRE, $p < 0.001$), and inhibited the production of urinary 2,3 dinor 6 keto PGF_{1α} by 44.0% compared to pre medication in men (1.11 ± 0.04 ng/mg CRE vs 2.52 ± 0.09 ng/mg CRE, $p < 0.001$) (Fig. 1B and E).

Loxoprofen sodium inhibits platelet aggregation in apoE^{-/-} mice and healthy humans

Fig. 2 A, B, C and D shows representative results of mouse platelet aggregation stimulated by ADP or collagen solutions. Although PATI_{ADP} was 1.96 ± 0.66 μM and PATI_{collagen} was 2.17 ± 0.44 μg/ml in mice with vehicle, they were suppressed to >4 μM ($n = 15$, $p = 0.033$), and >4 μg/ml ($n = 15$, $p < 0.001$) after administration of loxoprofen

sodium. Fig. 2 E, F, G and H show representative results of healthy human platelet aggregation stimulated by ADP or collagen solutions, respectively. After administration of loxoprofen sodium, platelet aggregation was suppressed. Although PATI_{ADP} was 1.52 ± 0.39 μM and PATI_{collagen} was 0.41 ± 0.03 μg/ml in humans before administration of loxoprofen sodium, they were suppressed to 3.18 ± 1.50 μM ($n = 5$, $p = 0.004$), and 1.28 ± 0.50 μg/ml ($n = 5$, $p = 0.034$) after its administration.

Loxoprofen sodium does not affect serum levels of total cholesterol, triglycerides, or free fatty acids in apoE^{-/-} mice

Body weights were unchanged in mice treated with both loxoprofen sodium and vehicle (Table 1). Dry chem system demonstrated that the serum levels of albumin, CRE, and FFA were unchanged in mice treated with loxoprofen sodium and vehicle. HPLC analysis demonstrated that the serum levels of total cholesterol and serum triglycerides were unchanged (Table 1).

Loxoprofen sodium reduces extent of atherosclerosis in apoE^{-/-} mice

Atherosclerotic lesion area of the aortic root was significantly decreased to 41.5% (308,545 ± 36,892 μm², $p < 0.001$) in female mice receiving 4 mg/kg/day of loxoprofen sodium for 56 days and was decreased to 75.6% (487,455 ± 29,623 μm², $p = 0.001$) in female mice receiving 4 mg/kg/day of loxoprofen sodium for 7 days compared to mice treated with vehicle (644,372 ± 28,314 μm²) (Fig. 3D). Similarly, atherosclerotic lesion area was significantly decreased to 63.5% (372,145 ± 38,149 μm², $p = 0.015$) in male mice receiving loxoprofen sodium for 56 days and 84.8% (432,789 ± 39,236 μm², $p = 0.015$) in male mice receiving it for 7 days compared to mice treated with vehicle (vs 510,117 ± 34,409 μm²) (Fig. 3E).

Fig. 3. Representative Oil-Red-O stained sections from the proximal aorta of female apoE^{-/-} mice, and quantification of atherosclerotic lesion area. A, B, and C were representative Oil-Red-O stained sections from the proximal aorta of female apoE^{-/-} mice. (A), female mice treated with vehicle, (B) female mice with 4 mg/kg/day loxoprofen sodium for 7 days, and (C) female mice with it for 56 days. Scale bar = 200 μm . E indicated the area of atherosclerotic lesions in male mice receiving vehicle ($510,117 \pm 34,409 \mu\text{m}^2$), loxoprofen sodium (4 mg/kg/day) for 7 days ($432,789 \pm 39,236 \mu\text{m}^2$), loxoprofen sodium for 56 days ($372,145 \pm 38,149 \mu\text{m}^2$). Similarly, D indicated those in female mice, with the mean and SEM being $644,372 \pm 28,314 \mu\text{m}^2$, $487,455 \pm 29,623 \mu\text{m}^2$, and $308,545 \pm 36,892 \mu\text{m}^2$, respectively. Individual data were expressed as dots in each group, and the average was expressed as mean $\pm$ SEM. Dunnett test was used to for comparisons with mice receiving vehicle. Horizontal bar indicated the mean in each group.

Fig. 4. The extent of Oil-Red-O stained lesions, and MOMA2, COX-1 and COX-2 positive lesions in atherosclerotic lesions of female apoE^{-/-} mice. We applied Oil-Red-O stain and immunocytochemical detection of macrophages or COX in female apoE^{-/-} mice treated with vehicle (open bars), with 4 mg/kg/day loxoprofen sodium for 7 days (gray bars), and for 56 days (solid bars). We compared the prevalence of Oil-Red-O stained lesions (A). Sections from proximal aorta were stained with monoclonal antibodies to macrophages (MOMA-2) (B), COX-1 (C) or COX-2 (D). Data were expressed as the average (mean $\pm$ SEM). Dunnett test was used for comparisons among the three groups.

Representative Oil-Red-O and hematoxylin stained sections from the proximal aorta of female mice treated with vehicle (Fig. 3A), female mice receiving 4 mg/kg/day loxoprofen sodium for 7 days (Fig. 3B), and female mice receiving 4 mg/kg/day loxoprofen sodium for 56 days (Fig. 3C), indicate fatty streak lesions consisting predominantly of foam cells. The prevalence of Oil-Red-O stained lesions among arteriosclerotic lesions in male and female mice with loxoprofen sodium was equal to that in

male and female mice receiving vehicle (Fig. 4A).

Immunohistochemical analysis of MOMA2, COX-1 and COX-2 in atherosclerotic lesions of apoE^{-/-} mice

The prevalence of positive staining lesions of MOMA2, COX-1 and COX-2 in atherosclerotic lesions in female mice treated with loxoprofen sodium for both 7 days and 56 days was similar to that in female mice treated with vehicle (Fig. 4B, C and D).

Side effects of loxoprofen sodium in apoE^{-/-} mice

Treatment of apoE^{-/-} mice with 4 mg/kg/day loxoprofen sodium was well tolerated. The histological examination of liver, kidney and stomach showed no abnormalities even at a dose of 8 mg/kg of loxoprofen sodium.

Discussion

Atherosclerotic lesion formation was reduced to 63.5% and 41.5%, in male and female apoE^{-/-} mice with loxoprofen sodium compared to control, respectively. However, the composition of atherosclerotic lesions in mice treated with loxoprofen sodium differed from that in the mice receiving vehicle. The extent of Oil-Red-O, COX-1 and COX-2 expression was the same in mice receiving loxoprofen sodium and vehicle. Loxoprofen sodium reduced the production of TxB₂ and PGI₂, and also reduced platelet aggregation *in vivo*. Loxoprofen sodium has anti-inflammatory effects and reduced the production of PGE₂. Thus, loxoprofen sodium, a non-selective NSAID, may reduce atherosclerotic lesion formation in apoE^{-/-} mice by an anti-inflammatory effect. It is widely accepted that aspirin prevent the occurrence of myocardial infarction and/or ischemic stroke primarily through the inhibition of platelet aggregation, although their role in the development of fatty streak lesions remains a matter of debate.

Belton *et al.* demonstrated that the profile of PG generation and expression of COX were remarkably similar in the apoE^{-/-} murine model and human atherosclerosis. In the apoE^{-/-} murine model and human atherosclerosis, there is an increase in the biosynthesis of TXA₂ and PGI₂, with induction of COX-1 and COX-2 [18]. The COX-2 selectivity of loxoprofen sodium active metabolite (loxoprofen-SRS) *in vitro* (the ratio of COX-1 IC₅₀ /COX-2 IC₅₀) was 0.35 [19]. Our study indicated that administration of loxoprofen sodium for apoE^{-/-} mice (4 mg/kg/day) reduced biosynthesis of PEG₂ metabolite to 9.0%, TXA₂ metabolite to 29.0%, and PGI₂ metabolite to 26.9%. Moreover, administration of loxoprofen sodium to healthy men (60 mg/body, three times a day) reduced the biosynthesis of PGE₂ metabolite to 29.7%, TXA₂ metabolite to 26.2%, and PGI₂ metabolite to 44.0%. Thus, loxoprofen sodium may be a well-balanced COX-1 and COX-2 inhibitor, which contributes to a reduction of atherosclerotic lesion formation.

On the other hand, loxoprofen sodium did not change the serum levels of total cholesterol or serum triglycerides. Some COX inhibitors might affect lipid metabolism. Rofecoxib has been reported to increase the susceptibility of human LDL-c and cell membrane lipids to oxidative modification, a hallmark feature of atherosclerosis. Rofecoxib promoted the non-enzymatic formation of isoprostanes from biological lipids, which act as important mediators of inflammation in the atherosclerotic plaque [20]. Celecoxib

does not have these effects. Thus, each NSAID has different pharmacologic actions [20–22].

In conclusion, loxoprofen sodium, a non-selective NSAID, reduces atherosclerotic lesion formation in apoE^{-/-} mice by suppression of PGE₂, TxB₂ and PGI₂. The influence of COX-2 selective inhibition in atherosclerosis has been controversial, but non-selective NSAID could reduce atherosclerotic lesion formation in a murine model. Our data may provide insights into the mechanisms underlying the cardiovascular safety of NSAIDs.

Abbreviations

RA, rheumatoid arthritis; SLE, systemic lupus erythematosus; COX-2, cyclooxygenase-2; NSAIDs, nonsteroidal anti-inflammatory drugs; Tx, thromboxane; PG, prostaglandin; PRP, platelet rich plasma; ADP, adenosine diphosphate; PATI, platelet aggregatory threshold index; HPLC, high performance liquid chromatograph.

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