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Effect of E3040, an inhibitor of 5-lipoxygenase and thromboxane synthase, on rat bowel damage induced by lipopolysaccharide

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Abstract

Intravenous administration of lipopolysaccharide to rats that had been immunized with lipopolysaccharide induced hemorrhagic damage in the large intestine. We investigated the role of 5-lipoxygenase and thromboxane synthase products in the damage of the large intestine induced by lipopolysaccharide. In the large intestine of lipopolysaccharide-immunized rats, intravenous injection of lipopolysaccharide increased the vascular permeability, production of leukotriene B_4, leukotriene C_4/D_4, thromboxane B_2 and prostaglandin E_2, and also increased the activity of myeloperoxidase, a marker enzyme of neutrophils. Oral administration of E3040 (6-hydroxy-5,7-dimethyl-2-(methylamino)-4-(3-pyridylmethyl)benzothiazole), a novel dual inhibitor of 5-lipoxygenase and thromboxane synthase, at 30 and 100 mg/kg inhibited the increase in vascular permeability induced by lipopolysaccharide in the large intestine. E3040 inhibited the production of leukotriene B_4 and thromboxane B_2 and tended to increase the production of prostaglandin E_2 in the large intestine. Sulfasalazine (500 mg/kg) and prednisolone (10 mg/kg), drugs used for the treatment of inflammatory bowel disease, had no significant effect on eicosanoid production and vascular permeability. These results indicate that E3040 inhibits the production of both leukotriene B_4 and thromboxane B_2 and prevents lipopolysaccharide-induced damage in the large intestine of lipopolysaccharide-immunized rats.

Keywords: E3040; 5-Lipoxygenase; Thromboxane synthase; Lipopolysaccharide

1. Introduction

Although the cause of inflammatory bowel disease remains unknown (Flocchi, 1998), a possible etiologic role for bacterial cell-wall products has been postulated. Lipopolysaccharide, a cell-wall constituent of Gram-negative bacteria, is present in large quantities in the human gut and may enter through the intestinal mucosal barrier as a result of increased bowel permeability (Deventer et al., 1988). Patients with Crohn's disease are reported to show systemic endotoxemia (Wellman et al., 1986). This foreign antigen is capable of initiating immune responses by activating macrophages, neutrophils and lymphocytes in the bowel mucosa. The sequential immune responses might contribute to the bowel disease (James and Klapproth, 1996). While orally administered lipopolysaccharide does not have any harmful effects in animals, intravenous administration of lipopolysaccharide causes endotoxic shock. The pathogenesis includes numerous biological effects such as hypotension, respiratory dysfunction, activation of platelets and leukocytes, and modulation of cellular immune responses, as well as diarrhea and gastrointestinal damage accompanied by increases in vascular permeability (Wallace et al., 1987; Gonzalez-crussi and Hsueh, 1983).

A number of mediators, such as platelet-activating factor, arachidonic acid metabolites and cytokines, have been shown to be involved in the pathogenesis of endotoxic shock, etc. (Feuerstein and Hallenbeck, 1987; Parrillo, 1993). Thromboxane synthase inhibitors improve the survival ratio and cardiovascular response of rats treated with bolus injection of lipopolysaccharide (Fukumoto and Tanaka, 1983; Ebara et al., 1996). Cyclooxygenase inhibitors also attenuate the lipopolysaccharide-induced changes in cardiovascular response and colonic contraction in rats (Ebara et al., 1996; Pons et al., 1994). However, exogenously administered prostaglandin E_2 inhibits lipopolysaccharide-induced gastrointestinal damage (Pons et

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al., 1994; Ibbotson and Wallace, 1989). These findings suggest that thromboxanes play a role in the pathogenesis of disorders induced by lipopolysaccharide. Relatively little is known, however, about the role of lipoxygenase products in lipopolysaccharide-induced disorders. It has been shown that levels of leukotrienes are increased in the bile and plasma of animals treated with lipopolysaccharide (Hagmann et al., 1985; Jansen et al., 1991; Mozes et al., 1991), and that leukotriene inhibitors prevent endotoxic shock (Bonnet et al., 1983; Wallace and Whittle, 1986). Other investigators have reported that a 5-lipoxygenase inhibitor improves only partially platelet-activating factor-induced porcine hemodynamics (Olson et al., 1993) and that the inhibitors do not affect platelet-activating factor-induced increases in vascular permeability in rat airways (Sirois et al., 1990).

The hemorrhagic damage to the gastrointestinal tract of rats induced by intravenous administration of lipopolysaccharide is known to be localized to the stomach and small intestine and not to occur in the colon (Wallace et al., 1987). In the present study, we induced hemorrhagic damage to the large and small intestine, by intravenous administration of lipopolysaccharide to rats which had been immunized with lipopolysaccharide. In this model, we investigated the changes in vascular permeability and eicosanoid production in the large intestine. We also examined the effects of E3040 (6-hydroxy-5,7-dimethyl-2-(methylamino)-4-(3-pyridylmethyl)benzothiazole), a novel dual inhibitor of 5-lipoxygenase and thromboxane synthase (Hibi et al., 1994), on the damage to the large intestine induced by lipopolysaccharide, comparing them to those of sulfasalazine and prednisolone.

2. Materials and methods

2.1. Animals

Four- to five-week-old male Fisher rats (approx. 150 g) were purchased from Charles River Japan (Kanagawa). Animals were housed in a cage with a wire-mesh bottom in a room at 23 °C with a 12-h light/dark cycle. Standard laboratory rat chow and tap water were available ad libitum. Experiments were performed according to our company's guidelines for animal experimentation (Eisai Research Laboratories, Ibaraki, Japan).

2.2. Drugs and chemicals

E3040 (Fig. 1) was synthesized at Eisai. The other drugs and chemicals were purchased from commercial sources: lipopolysaccharide (*E. coli* 0111:B4), sulfasalazine, prednisolone, hexadecyltrimethylammonium bromide and *o*-dianisidine (Sigma, USA); Evans blue (Tokyo Kasei Kogyo, Tokyo); Freund complete adjuvant (Difco, USA); calcium ionophore A23187 (Cabiochem, USA); enzyme immunoassay (EIA) kit for leukotriene B_4, thromboxane B_2 and prostaglandin E_2 (Cayman Chemical, USA).

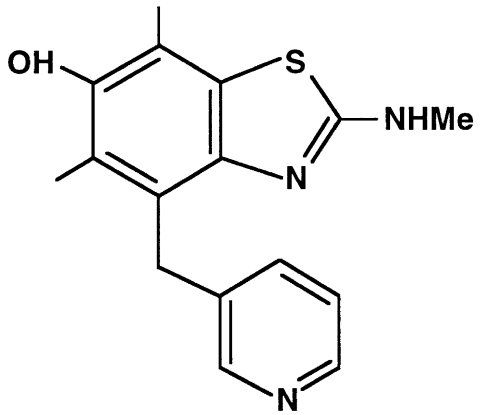

Fig. 1. Chemical structure of E3040.

2.3. Induction of bowel damage by lipopolysaccharide

Rats were immunized with 0.01–1000 μg of lipopolysaccharide-containing Freund complete adjuvant (1:1, v/v) by injection into the skin of the intrascapular area (1 ml of emulsion/rat). Seven days later, rats were deprived of food for 18–24 h before an intravenous injection of lipopolysaccharide (0.1, 1 and 10 mg/kg). Lipopolysaccharide solution (lipopolysaccharide/2% Evans blue/saline, 0.5 ml/rat) was administered into the tail vein while the animals were conscious. Rats were killed by exsanguination 5, 15, 30 and 60 min after the injection of lipopolysaccharide (time-course study) or 30 min later (efficacy study for test drugs), and the damage to the small and large intestines was evaluated by determining the vascular permeability. Rats were pretreated with E3040 orally 30 min, 3 h or 6 h before the intravenous administration of lipopolysaccharide. The reference compounds were administered orally 6 h before the injection of lipopolysaccharide. The control group received 0.5% methylcellulose (solvent of test drugs) instead of test drugs.

2.4. Bowel vascular permeability

Bowel vascular permeability was evaluated by measuring the extravasation of Evans blue dye according to the method of Ibbotson and Wallace (1989). For determination of the Evans blue content of the small intestine lumen, the small intestine was removed and its lumen was washed with 10 ml of saline. Acetone was added to the washing solution (1:1, v/v). The solution was mixed on a recipro shaker and centrifuged at 3000 rpm for 20 min. The amount of Evans blue in the supernatant was determined with a spectrophotometer at 620 nm (Hitachi, U3200). The Evans blue content of the small intestine lumen is expressed as Evans blue μg/ml of the intestine washing solution (μg/ml of solution). For determination of the

Evans blue content of the large intestine, the tissue (approx. 7 cm long from the end of the cecum) was removed and weighed. It was placed into a test tube containing 1 ml of 1 N NaOH and incubated at 37 °C for 24 h in a water bath. At the end of the incubation, 9 ml of an acetone/0.6 N phosphoric acid solution (13:5, v/v) was added to the test tube. The solution was mixed on a recipro shaker and centrifuged at 3000 rpm for 20 min. The amount of Evans blue in the supernatant was determined with a spectrophotometer at 620 nm. The Evans blue content of the large intestine is expressed as Evans blue μg/g wet wt. of large intestine (μg/g of tissue).

2.5. Ex vivo eicosanoid production in the large intestine

Eicosanoid production in the large intestine was determined as described by Dreyling et al. (1986). The large intestine was incubated in 3 ml of modified Tyrode solution (NaCl 8.0, KCl 0.2, $MgCl_2 \cdot 6H_2O$ 0.21, $NaHCO_3$ 1.0, NaH_2PO4 0.058, $CaCl_2 \cdot 2H_2O$ 0.13, glucose 1.1 g/l) containing 5 μg/ml of calcium ionophore A23187. Incubation was carried out at 37 °C for 20 min and terminated by removal of the tissue. The incubation medium was centrifuged at 3000 rpm for 10 min at 4 °C and the supernatant was stored at −80 °C until assay. Leukotriene B_4, thromboxane B_2 and prostaglandin E_2 levels in an aliquot of the supernatant were determined by enzyme immunoassay. Eicosanoid production in the large intestine is expressed as eicosanoid ng/g wet wt. of large intestine/20-min incubation (ng/g/20 min).

2.6. Assay for myeloperoxidase activity

Myeloperoxidase activity in the large intestine was measured by the method of Bradley et al. (1982). After the incubation for determining eicosanoid production, the large intestine was homogenized in 50 mM phosphate buffer, pH 6.0, containing 0.5% hexadecyltrimethylammonium bromide using a Physcotron homogenizer (Nichion Irika Seisakusho, Tokyo) and then sonicated (Brabson Sonifier 185, Branson Sonic, USA). The homogenate was frozen and thawed three times and centrifuged at 3000 rpm for 10 min. An aliquot of the supernatant was assayed for myeloperoxidase activity using 0.0005% of hydrogen peroxide as a substrate and 0.167 mg/ml of *o*-dianisidine. One unit of myeloperoxidase activity was defined as the amount degrading 1 μmol of peroxide/min at 25 °C. Myeloperoxidase activity in the large intestine is expressed as units/g of wet wt. of large intestine (U/g of tissue).

2.7. Statistics

Results are expressed as means ± standard error of the mean (S.E.M). The statistical differences in the data between control rats and the compound-treated rats were analyzed using Dunnett's multiple comparison tests.

3. Results

3.1. Bowel damage induced by lipopolysaccharide in lipopolysaccharide-immunized rats

When 0.1, 1 or 10 mg/kg of lipopolysaccharide was administered intravenously to rats which had been immunized with lipopolysaccharide at 0.1 or 1 mg/rat, damage to the small and large intestine was detected (Fig. 2). In non-immunized rats, intravenous administration of lipopolysaccharide resulted in hemorrhagic damage and an increase in the vascular permeability of the small intestine, but there were no changes in the large intestine. The rats immunized with lipopolysaccharide showed mucosal damage and an increase in the vascular permeability of the large intestine following the injection of lipopolysaccharide (Fig. 2B). The damage to the large intestine was characterized by marked parenchymal hemorrhage mainly

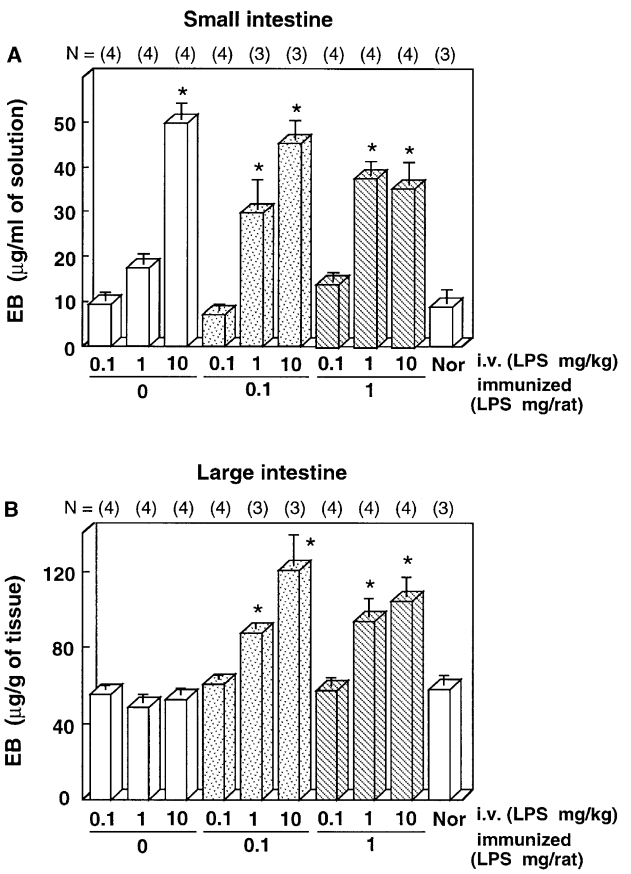

Fig. 2. The damage to small and large intestines induced by intravenous administration of lipopolysaccharide (LPS) in lipopolysaccharide-immunized rats. Rats were immunized with 0.1 and 1 mg of lipopolysaccharide (0: immunized with only Freund complete adjuvant). Seven days later, rats received lipopolysaccharide intravenously at 0.1, 1 and 10 mg/kg containing 2% Evans blue/saline. Thirty minutes after injection, the amount of Evans blue (EB) in the lumen of the small intestine and in the large intestine was determined, as a marker of the vascular permeability. Nor, Normal (saline control); (A) small intestine; (B) large intestine. * $P < 0.05$ vs. Normal.

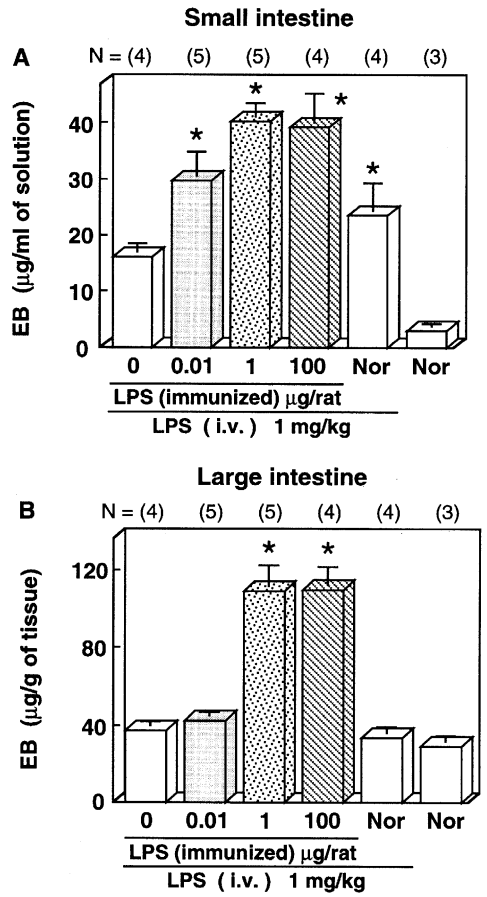

Fig. 3. The damage to small and large intestines induced by intravenous administration of lipopolysaccharide (LPS) in low doses in lipopolysaccharide-immunized rats. Rats were immunized with 0.01, 1 and 100 μg of lipopolysaccharide (0: immunized with only Freund complete adjuvant). Seven days later, rats received lipopolysaccharide intravenously at 1 mg/kg containing 2% Evans blue/saline. Thirty minutes after injection, the amount of Evans blue (EB) in the lumen of the small intestine and in the large intestine was determined, as a marker of vascular permeability. Nor, Normal (non-immunized rats); (A) small intestine; (B) large intestine. * $P < 0.05$ vs. Normal (non-immunized, saline control).

located in the upper region of the colon with little damage in the rectum. The damage to the small intestine induced by lipopolysaccharide at 1 mg/kg in lipopolysaccharide-immunized rats was severer than that in non-immunized rats; however, there was no difference in the damage to the small intestine induced by lipopolysaccharide at 10 mg/kg between non-immunized rats and lipopolysaccharide-immunized rats (Fig. 2A).

After rats had been immunized with lower concentrations of lipopolysaccharide (0.01 and 1 μg/rat), bowel damage was examined by intravenous administration of lipopolysaccharide (1 mg/kg). As shown in Fig. 3B, injection of lipopolysaccharide caused damage to the large intestine in rats immunized with lipopolysaccharide at 1 μg/rat, but not in rats immunized with 0.01 μg/rat. Damage to the small intestine induced by lipopolysaccharide injection in lipopolysaccharide-immunized rats was severer than that in non-immunized rats (Fig. 3A).

3.2. Eicosanoid production induced by lipopolysaccharide in the large intestine of lipopolysaccharide-immunized rats

In lipopolysaccharide-immunized rats (1 μg/rat), vascular permeability in the large intestine did not change 5 and 15 min after the intravenous injection of lipopolysaccharide at 1 mg/kg, but significantly increased 30 min

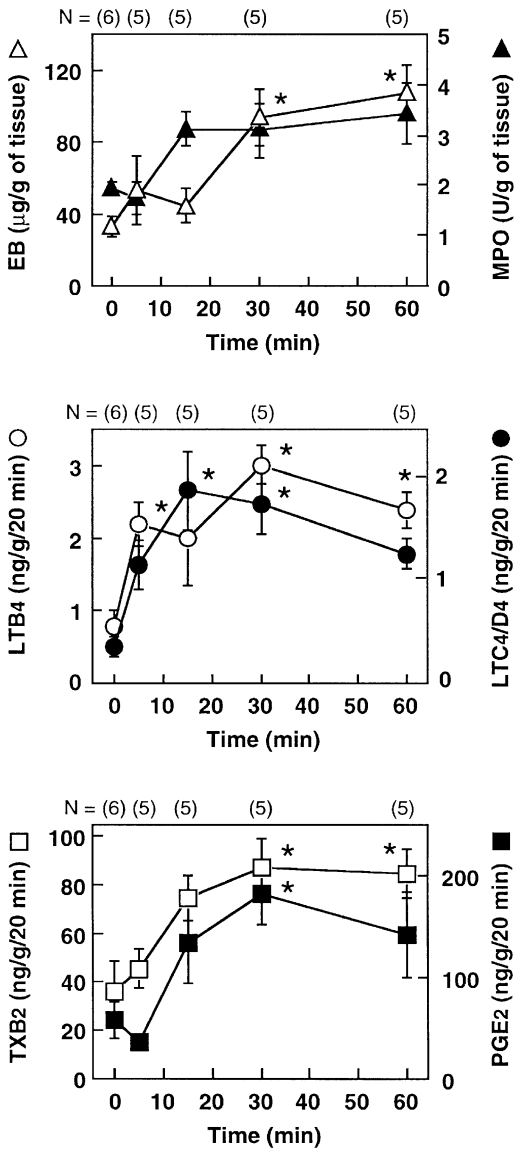

Fig. 4. Enhanced vascular permeability, eicosanoid production and myeloperoxidase activity in the large intestine induced by lipopolysaccharide in lipopolysaccharide-immunized rats. Rats were immunized with 1 μg of lipopolysaccharide. Seven days later, rats received lipopolysaccharide intravenously at 1 mg/kg containing 2% Evans blue or saline. At various times after the injection of lipopolysaccharide, the large intestine was assayed for eicosanoid production, myeloperoxidase activity (MPO) and Evans blue (EB) content as a marker of vascular permeability. LTB_4, leukotriene B_4; LTC_4/D_4, leukotriene C_4/D_4; TXB_2, thromboxane B_2; PGE_2, prostaglandin E_2. Evans blue, $N = 4$ in a separate experiment. * $P < 0.05$ vs. Values at 0 min.

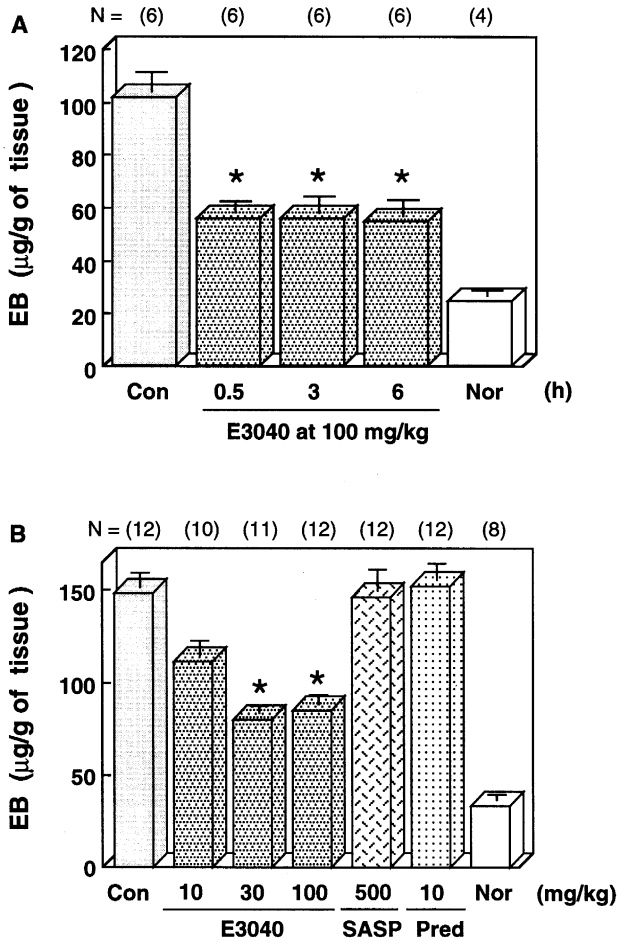

Fig. 5. Effect of E3040, sulfasalazine (SASP) and prednisolone (Pred) on the lipopolysaccharide-induced damage to the large intestine in lipopolysaccharide-immunized rats. Rats were immunized with 1 μg of lipopolysaccharide. Seven days later, rats received lipopolysaccharide intravenously at 1 mg/kg containing 2% Evans blue/saline. Thirty minutes after injection, the amount of Evans blue (EB) in the large intestine was determined, as a marker of the vascular permeability. (A) E3040 at 100 mg/kg was administered orally 30 min, 3 h or 6 h before i.v. Injection of lipopolysaccharide; (B) Test drugs were administered orally 6 h before i.v. injection of lipopolysaccharide. Con, Control (lipopolysaccharide control); Nor, Normal (saline control). * $P < 0.05$ vs. Control.

later (Fig. 4). Myeloperoxidase activity in the large intestine increased 15 min after the injection of lipopolysaccharide. The production of leukotriene B_4 and leukotriene C_4/D_4 in the large intestine increased significantly 5 and 15 min after the injection, respectively, but both were maximal 30 min later. The production of thromboxane B_2 and prostaglandin E_2 significantly increased 30 min after the injection of lipopolysaccharide.

3.3. Effects of E3040, sulfasalazine and prednisolone on lipopolysaccharide-induced mucosal damage and eicosanoid production in the large intestine

The effects of E3040 on the damage to the large intestine induced by lipopolysaccharide (1 mg/kg, i.v.)

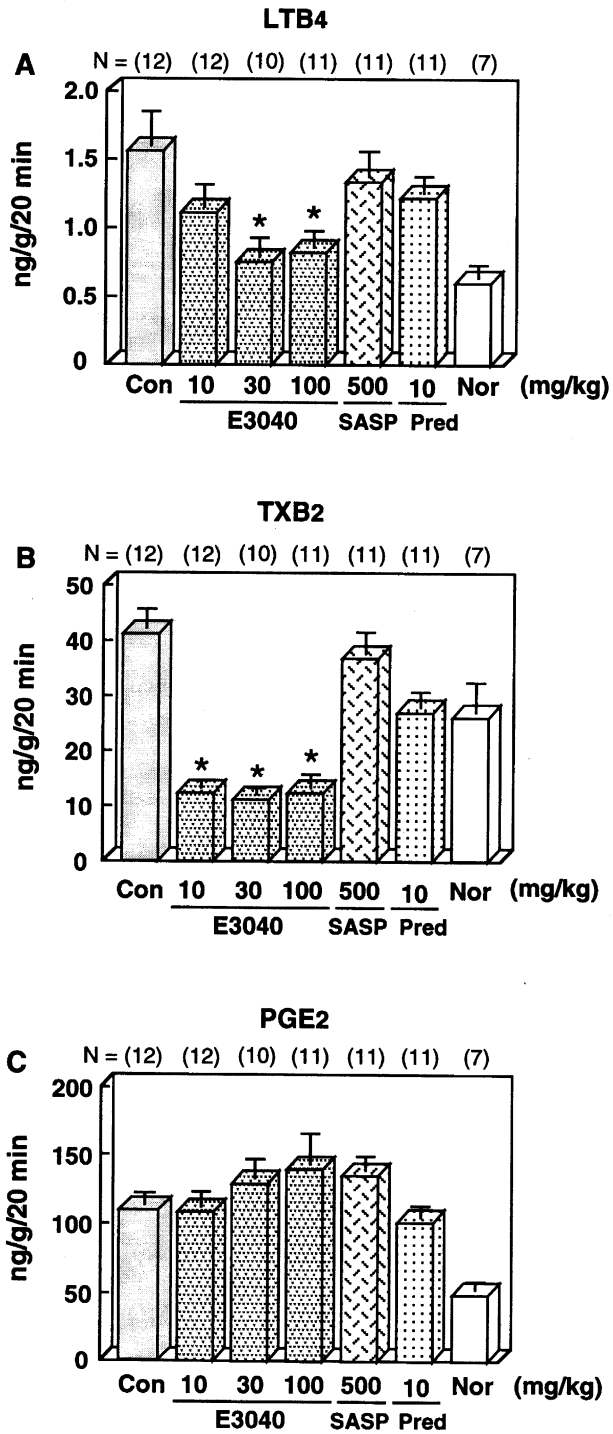

Fig. 6. Effect of E3040, sulfasalazine (SASP) and prednisolone (Pred) on lipopolysaccharide-induced eicosanoid production in the large intestine of lipopolysaccharide-immunized rats. Rats were immunized with 1 μg of lipopolysaccharide. Seven days later, rats received lipopolysaccharide intravenously at 1 mg/kg. Thirty minutes after injection, the large intestine was assayed for eicosanoid production. Test drugs were administered orally 6 h before i.v. injection of lipopolysaccharide. (A) Leukotriene B_4 (LTB_4); (B) thromboxane B_2 (TXB_2); (C) prostaglandin E_2 (PGE_2); Con, Control (lipopolysaccharide control); Nor, Normal (saline control). * $P < 0.05$ vs. Control.

were investigated in lipopolysaccharide (1 μg/rat)-immunized rats. At first, E3040 at 100 mg/kg was given to the immunized rats orally 30 min, 3 h or 6 h before i.v. injection of lipopolysaccharide. In all cases, E3040 attenuated the damage in the large intestine to a similar extent (Fig. 5A). Next, the inhibitory effects of E3040 at several doses administered 6 h before the injection of lipopolysaccharide were compared with those of sulfasalazine and prednisolone. As shown in Fig. 5B, E3040 at 30 and 100 mg/kg inhibited the increases in vascular permeability. Neither sulfasalazine at 500 mg/kg nor prednisolone at 10 mg/kg had an effect.

The effects of E3040, sulfasalazine and prednisolone on eicosanoid production in the large intestine were examined under the same experimental conditions as indicated in the legend of Fig. 5B. E3040 significantly inhibited the production of leukotriene B_4 at 30 and 100 mg/kg (Fig. 6A) and thromboxane B_2 at 10, 30 and 100 mg/kg (Fig. 6B), and tended to increase the production of prostaglandin E_2 (Fig. 6C). Sulfasalazine at 500 mg/kg had no significant effect on eicosanoid production. Prednisolone at 10 mg/kg showed a tendency to inhibit eicosanoid production, but its effect was not significant.

4. Discussion

Intravenous administration of lipopolysaccharide to normal rats produced hemorrhagic damage and an increase in vascular permeability in the small intestine, but did not cause any disorder in the large intestine at the maximal dose of 10 mg/kg. After the immunization of rats with lipopolysaccharide, intravenous administration of a low dose of lipopolysaccharide (1 mg/kg) produced parenchymal hemorrhage in the small and large intestines, accompanied by an increase in vascular permeability. The damage to the small intestine induced by lipopolysaccharide in lipopolysaccharide-immunized rats was slightly severer than that in normal rats. The mechanisms underlying the damage to the large intestine have not been elucidated; however, similar results have been reported in other colitis models: immune complexes (serum albumin and anti-serum albumin complex) (Axelsson and Ahlstedt, 1990) and peptidoglycan-polysaccharide polymers (cell-wall polymers from Group A streptococci) (Woolverton et al., 1989) induce damage in the small intestine and colon.

Injection of lipopolysaccharide to lipopolysaccharide-immunized rats resulted in an increase in eicosanoid production in the large intestine along with an elevation of myeloperoxidase activity, a marker enzyme of neutrophils. The production of leukotriene B_4 and leukotriene C_4/D_4 was rapidly increased (5 min after the injection) and levels of cyclooxygenase products (thromboxane B_2 and prostaglandin E_2) were also increased in the large intestine. The damage to the large intestine (an increase in vascular permeability) was produced relatively late (30 min after injection of lipopolysaccharide). The time course of the development of damage to the large intestine was similar to that to the duodenum and stomach, as reported by Ibbotson and Wallace (1989). Although the cellular origins of the increased eicosanoid production are not clear, the above results suggest that the early enhancement of leukotrienes was related to the cellular infiltration of neutrophils (Ford-Hutchinson et al., 1980), and the enhanced production of thromboxane B_2 and prostaglandin E_2 was probably derived from platelets (Mejerus, 1983) and bowel epithelium (Zifroni et al., 1983) and was associated with the destruction of microvessels. Thus, lipopolysaccharide may induce an acute inflammation in the large intestine, accompanied by the infiltration of inflammatory cells such as neutrophils.

Thromboxane synthase inhibitors have been shown to attenuate the lipopolysaccharide-induced damage (Fukumoto and Tanaka, 1983; Ebara et al., 1996). However, the results on the effect of 5-lipoxygenase inhibitors on endotoxic shock are inconsistent (Bonnet et al., 1983; Wallace and Whittle, 1986; Olson et al., 1993; Sirois et al., 1990). Woolverton et al. (1989) indicated that both inhibitors of 5-lipoxygenase and thromboxane synthase prevented the intestinal vascular permeability induced by peptidoglycan-polysaccharide polymers in rats. The authors also reported that indomethacin, a cyclooxygenase inhibitor, exaggerated the intestinal vascular permeability. Indomethacin potentiates the endotoxin-induced gastric mucosal injury (Pique et al., 1988) and is well known to cause gastric and intestinal ulceration (Fang et al., 1977). These results suggest that the products of thromboxane synthase and 5-lipoxygenase contribute, at least in part, to the lipopolysaccharide-induced bowel disorder.

The present study showed that E3040, a novel dual inhibitor of 5-lipoxygenase and thromboxane synthase, inhibited the production of leukotriene B_4 and thromboxane B_2 in the large intestine induced by lipopolysaccharide. The compound also prevented the increase in bowel vascular permeability. These data suggest that E3040 prevented lipopolysaccharide-induced bowel damage by decreasing the amount of 5-lipoxygenase and thromboxane synthase products. E3040 slightly increased the production of prostaglandin E_2 in the large intestine. This increase in prostaglandin E_2 production may be explained by shunting of the substrate of both thromboxane synthase and 5-lipoxygenase toward the cyclooxygenase pathway.

Sulfasalazine and prednisolone are widely used for the treatment of inflammatory bowel disease. Several reports have indicated that the drugs improve colonic inflammation on repeated administration (Axelsson and Ahlstedt, 1990; Fitzpatrick et al., 1990), and a limited number of reports have shown the efficacy of these drugs in preventing acute colonic inflammation in rodent models. In the present study, we investigated the effect of a single administration of sulfasalazine and prednisolone on the damage

to the large intestine induced by lipopolysaccharide. In this model, sulfasalazine showed no significant effect on the increase in bowel vascular permeability. The dose of sulfasalazine used in the study is similar to that used in other colitis models. Sulfasalazine was administered 6 h before the injection of lipopolysaccharide, because the drug is known to exert its effect via an active metabolite that acts directly on the colonic mucosa from the luminal side (Greenfield et al., 1993). 5-Aminosalicylic acid, an active metabolite of sulfasalazine, has been shown to possess several actions including inhibition of eicosanoid production (Greenfield et al., 1993; Mori et al., 1999). However, the in vitro inhibitory effects of sulfasalazine and 5-aminosalicylic acid on eicosanoid production are relatively weak (Tornhamre et al., 1989). It is suggested that sulfasalazine has no preventive effect on the acute and severe bowel damage induced by lipopolysaccharide because the compound does not affect eicosanoid production, as shown by the present ex vivo data.

Steroid is known to block eicosanoid synthesis in inflamed tissue by affecting phospholipase A_2 (Rask-Madsen et al., 1992). In the present study, prednisolone at a dose of 10 mg/kg showed a tendency to inhibit eicosanoid production but had no significant effect on the bowel damage induced by lipopolysaccharide. The efficacy of steroid on bowel inflammation is controversial: Wallace and MacNaughton (1988) have reported that dexamethasone inhibits platelet-activating factor-induced gastrointestinal damage, while Woolverton et al. (1989) have shown no inhibition by prednisolone of peptidoglycan-polysaccharide-induced intestinal vascular permeability. These differences in the effects of steroids on bowel damage might be related to the time-schedule of dosing, as reported by Wallace and Whittle (1986). The authors have demonstrated that pretreatment (2 h before administration of platelet-activating factor) with dexamethasone inhibits the gastric damage induced by platelet-activating factor, while acute pretreatment (15 min before) with the drug has no effect.

It has been reported that "intestinal endotoxemia" occurs in patients with inflammatory bowel disease (Deventer et al., 1988); intestinal endotoxins are absorbed from the damaged gut mucosa and directly enter the systemic circulation. Besides the several systemic effects of endotoxins, bacterial cell-wall products such as lipopolysaccharide have been postulated to play a role in the pathogenesis of bowel disorders. 5-Lipoxygenase inhibitor (Bonnet et al., 1983; Wallace and Whittle, 1986) and thromboxane synthase inhibitor (Woolverton et al., 1989) have been reported to prevent the gastrointestinal damage induced by lipopolysaccharide or platelet-activating factor. The therapeutic potential of these inhibitors seems promising and their efficacy has been proved to some extent in clinical trials with patients with inflammatory bowel disease (Laursen et al., 1990; Roberts et al., 1997; Casellas et al., 1995).

In conclusion, intravenous administration of lipopolysaccharide to lipopolysaccharide-immunized rats produced hemorrhagic damage in the large intestine, accompanied by an increase in vascular permeability. Myeloperoxidase activity, a marker enzyme of neutrophils, and eicosanoid production were elevated in the damaged large intestine. E3040 inhibited the production of both leukotriene B_4 and thromboxane B_2 and also prevented the damage to the large intestine induced by lipopolysaccharide. These results suggest that E3040, a novel dual inhibitor of 5-lipoxygenase and thromboxane synthase, may be clinically useful for the management of the endotoxin-related response in inflammatory bowel disease.

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