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Short communication

Protective effect of DS-4574, a peptidoleukotriene receptor antagonist, against endotoxin-induced intestinal injury in rats

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Abstract

We evaluated the protective effect of DS-4574 (6-(2-cyclohexylethyl)-[1,3,4]thiadiazolo[3,2-*a*]-1,2,3-triazolo[4,5-*d*]pyrimidin-9(3*H*)-one), a peptidoleukotriene receptor antagonist, against intestinal mucosal injury evoked by endotoxin in rats by exploring changes in hematocrit and plasma leakage along with morphological features. Treatment with *Escherichia coli* endotoxin (5 mg/kg i.v.) alone elicited hemoconcentration, vasocongestion and a marked mucosal necrosis. DS-4574 (10–50 mg/kg) effectively prevented these changes on either oral or intraduodenal administration. These results demonstrate that peptidoleukotrienes may be key mediators in the intestinal injury induced by endotoxin in rats.

Keywords: DS-4574; Peptidoleukotriene receptor antagonist; Endotoxin; Small intestinal mucosal injury; Hemoconcentration; Plasma leakage

1. Introduction

DS-4574 (6-(2-cyclohexylethyl)-[1,3,4]thiadiazolo[3,2-*a*]-1,2,3-triazolo[4,5-*d*]pyrimidin-9(3*H*)-one), a peptidoleukotriene receptor antagonist, has been reported to antagonize the contraction induced by leukotriene C_4, leukotriene D_4 and leukotriene E_4 in isolated guinea pig ileum with IC_{50} values of 3.5×10^{-7}, 2.0×10^{-7} and 2.6×10^{-7} M, respectively (Aibara et al., 1991). In the in vivo studies, oral treatment with this compound inhibited bronchoconstriction induced by leukotriene C_4, leukotriene D_4 and leukotriene E_4 in guinea pigs, with ID_{50} values of 33, 16 and 44 mg/kg, respectively (Aibara et al., 1993). Moreover, orally administered DS-4574 prevented gastric mucosal injury induced by aspirin (ID_{50} = 20 mg/kg), acidified ethanol (0.4 mg/kg) and water-immersion restraint stress (25 mg/kg) in rats (Tabuchi and Kurebayashi, 1992; Tabuchi et al., 1993). Chemical mediators such as peptidoleukotrienes and platelet-activating factor were recently implicated as key mediators in the pathological and inflammatory actions of endotoxin, which induces hemorrhagic mucosal necrosis associated with vascular congestion in the small intestine (Cook et al., 1985; Hsueh et al., 1985; Teṛashita et al., 1985; Wallace and Whittle, 1986; Wallace et al., 1987; Whittle et al., 1987). These pathological changes resemble the intestinal injury of septic shock in humans (Parker and Parillo, 1983; Shumer, 1979). To better understand the role of peptidoleukotrienes against the pathological effects of endotoxin, we evaluated the effect of DS-4574 on intestinal mucosal injury induced by endotoxin in rats.

2. Materials and methods

Six-week-old male Sprague-Dawley rats weighing 160–180 g (Japan SLC, Shizuoka, Japan) were used. Before use, the animals were fasted overnight but allowed free access to water. DS-4574 was synthesized in our laboratory, and *Escherichia coli*-derived endotoxin was purchased from Difco Laboratories (Detroit, MI, USA). DS-4574 was suspended in 0.5% carboxymethylcellulose sodium solution, and endotoxin was dissolved in physiological saline. DS-4574 (10–50 mg/kg) was administered orally or intraduodenally 30 min before intravenous injection of endotoxin (5 mg/kg). Intraduodenal administration was selected as

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an appropriate route for anesthetized animals (plasma leakage study) because of depression in gastric motility. Three separate experiments were performed since there was a difference in optimum conditions as based on preliminary studies. In the first, 1 h after endotoxin treatment, blood was withdrawn under light ether anesthesia from the abdominal vena cava using a syringe containing citrate and the hematocrit level was measured by the centrifuge method. In the second, under Inactin (120 mg/kg i.p., Byk Gulden Co., Konstanz, Germany) anesthesia DS-4574 was administered intraduodenally and 30 min later both the pylorus and ileocecum were ligated. Immediately after ligation, the endotoxin (5 mg/kg) and [^{125}I]bovine serum albumin (100 μCi/kg, Daiichi Pure Chemicals Co., Tokyo, Japan) were administered simultaneously to the cervical vein. One hour later, the blood sample was collected and the intestinal loop was resected for measurement of the amount of the labelled albumin leaked into the lumen of the small intestine (Wallace et al., 1987). Radioactivity in the plasma and luminal fluid was counted with a gamma spectrometer. The amount of plasma leakage was calculated as described elsewhere (Wallace et al., 1987) and expressed in terms of μl/100 g body weight. In the third experiment, 4 h after the endotoxin treatment, under ether anesthesia the small intestine from the proximal part of the duodenum to the distal end of the ileum was resected for histological examination. The tissue was fixed in 10% buffered formaldehyde, routinely processed, and stained with hematoxylin and eosine. The severity of vasocongestion and mucosal necrosis was graded microscopically as follows: no change (−), minimal (±), slight (+), and moderate (++).

Statistical analysis was performed using Dunnett's multiple comparison test. A P value of less than 0.05 was considered significant.

3. Results

Intravenous administration of endotoxin alone to rats induced significant hemoconcentration, as evidenced by a rise in hematocrit (vehicle control: 44.1% vs. endotoxin alone: 51.7%; Fig. 1a). Oral treatment with DS-4574 significantly inhibited this change at each dose (46.6, 44.4 and 44.3% at 10, 25 and 50 mg/kg, respectively; Fig. 1a). Endotoxin administration also induced an increase in plasma leakage into the small intestinal lumen (vehicle control: 62 vs. endotoxin alone: 154 μl/100 g body weight). Intraduodenal treatment with DS-4574 markedly inhibited this increase in plasma leakage level in a dose-related fashion (95.4, 66.1 and 60.1 μl/100 g body weight at 10, 25 and 50 mg/kg, respectively; Fig. 1b).

Histopathological examination of rats treated with the endotoxin revealed marked hemorrhagic necrosis with vasocongestion in the small intestinal mucosa including the duodenum, jejunum and ileum. Oral treatment with DS-4574 effectively prevented these lesions in a dose-dependent manner. Notably, a high dose (50 mg/kg) of this compound completely inhibited these pathological changes (Table 1).

4. Discussion

Endotoxin shock is characterized by hypotension, intravascular coagulation, hemoconcentration, and an increase in vascular permeability, and further diverse pathological changes are found in almost all main organs (Parker and Parillo, 1983; Shumer, 1979). These actions of endotoxin may result from a direct response of the vascular endothelium to this lipopolysaccharide component of bacterial cell walls (Mayrick et al., 1986) or be caused via the release of secondary mediators

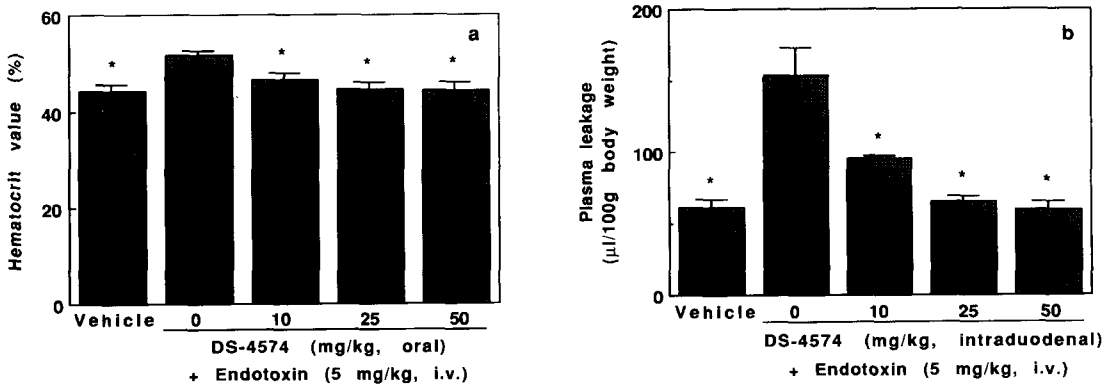

Fig. 1. Protective effects of DS-4574 on hemoconcentration (a) and plasma leakage into the small intestine loop (b) in rats. DS-4574 (10, 25 and 50 mg/kg) was administered orally or intraduodenally 30 min before an intravenous injection of endotoxin (5 mg/kg). Each column and vertical bar represents the means ±S.E.M. ($n = 6$). *$P < 0.01$ vs. endotoxin alone.

Table 1
Protective effects of DS-4574 against endotoxin-induced small intestinal injury in rats

Treatment	n	Vascongestion				Mucosal necrosis			
		– [a]	±	+	++	–	±	+	++
Duodenum									
Endotoxin alone	6	1 [b]	1	3	1	5	0	1	0
Endotoxin +									
DS-4574 10 mg/kg	6	3	1	1	1	5	0	1	0
DS-4574 25 mg/kg	6	5	1	0	0	6	0	0	0
DS-4574 50 mg/kg	6	6	0	0	0	6	0	0	0
Jejunum									
Endotoxin alone	6	1	1	2	2	2	1	3	0
Endotoxin +									
DS-4574 10 mg/kg	6	5	0	1	0	4	1	1	0
DS-4574 25 mg/kg	6	6	0	0	0	6	0	0	0
DS-4574 50 mg/kg	6	6	0	0	0	6	0	0	0
Ileum									
Endotoxin alone	6	2	0	3	1	4	0	2	0
Endotoxin +									
DS-4574 10 mg/kg	6	2	1	2	1	3	1	1	1
DS-4574 25 mg/kg	6	4	0	1	1	3	2	0	1
DS-4574 50 mg/kg	6	6	0	0	0	6	0	0	0

DS-4574 (10, 25 and 50 mg/kg) was administered orally to rats 30 min before intravenous injection of endotoxin (5 mg/kg). No changes in vehicle control animals treated with physiological saline (5 ml/kg i.v.) and 0.5% carboxymethylcellulose sodium solution (5 ml/kg, oral) were noted in any portion.
[a] Grading of histophatological findings was as follows: no change (–), minimal (±), slight (+) and moderate (++). [b] Number of rats with the grading.

(Parker and Parillo, 1983). Concerning the intestinal mucosal injury evoked by endotoxin, certain chemical mediators such as peptidoleukotrienes and platelet-activating factor were recently reported to play a pivotal role in the onset of this injury (Cook et al., 1985; Hsueh et al., 1985; Terashita et al., 1985; Wallace and Whittle, 1986; Wallace et al., 1987; Whittle et al., 1987). Accordingly, although intravenous injection of peptidoleukotriene alone did not produce endotoxin-like gastrointestinal mucosal injury, peptidoleukotriene receptor antagonists prevented the hemoconcentration induced by endotoxin (Cook et al., 1985) and ischemic bowel necrosis induced by a combination of platelet-activating factor and endotoxin (Hsueh et al., 1986). In contrast, intravenous administration of platelet-activating factor produced many of the symptoms of endotoxin shock (Wallace et al., 1987) and further intravenous injection of endotoxin led to a time-dependent increase in the jejunal formation of platelet-activating factor (Whittle et al., 1987). Moreover, platelet-activating factor receptor antagonist prevented endotoxin-induced gastrointestinal mucosal necrosis (Braquet et al., 1988; Wallace et al., 1987). In the present study, we focused on the role of peptidoleukotrienes in endotoxin-induced small intestinal injury using DS-4574, a potent peptidoleukotriene receptor antagonist, under development in our laboratory (Aibara et al., 1991, 1993; Tabuchi and Kurebayashi, 1992; Tabuchi et al., 1993).

Wallace and Whittle (1986) and Wallace et al. (1987) have shown that endotoxin induces hemorrhagic necrosis associated with vascular congestion in the small intestine but not in the distal colon. As in previous reports, the endotoxin used in the present study also caused hemoconcentration and elevated plasma leakage into the small intestinal lumen (Cook et al., 1985; Wallace et al., 1987) and produced a marked hemorrhagic necrosis with vasocongestion only in the small intestine. Like FPL55712 (7-(3-(4-acetyl-3-hydroxy-2-propylphenoxy)-2-hydroxypropoxy)-4-oxo-8-propyl-4*H*-1-benzopyran-2-carboxylate), another peptidoleukotriene antagonist (Hsueh et al., 1986), DS-4574, prevented these changes both functionally and morphologically, suggesting that peptidoleukotrienes may be key mediators in the intestinal injury induced by endotoxin in rats and that the pathogenesis of this model may involve the enhanced release of peptidoleukotrienes.

In conclusion, the present results clearly demonstrate that DS-4574, through its peptidoleukotriene receptor antagonistic action, exerts a protective effect against intestinal mucosal damage induced by endotoxin.

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