Administration of FR167653, a New Anti-Inflammatory Compound, Inhibits Aspirin-Induced Gastric Mucosal Injury in Rats

Hiroshi Ichikawa1, Norimasa Yoshida2,*, Tomohisa Takagi1, Osamu Handa1, Yuji Naito1, Takeshi Okanoue2, and Toshikazu Yoshikawa1

1Inflammation and Immunology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto 602-8566, Japan
2Molecular Gastroenterology and Hepatology, Graduate School of Medical Science, Kyoto Prefectural University of Medicine, Kyoto 602-8566, Japan

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Summary Neutrophils activation and inflammatory cytokines play a critical role in aspirin-induced gastric mucosal injury. FR167653 was first discovered to be a potent inhibitor of interleukin (IL)-1β and tumor necrosis factor-α (TNF-α) production. The purpose of this study is to investigate the anti-inflammatory effects against aspirin-induced gastric mucosal injury in rats. The intragastric administration of acidified aspirin induced hyperemia and hemorrhagic erosions in rat stomachs. The increase in the total gastric erosive area after aspirin administration was significantly inhibited by treatment with FR167653 in a dose-dependent manner. The increases in thiobarbituric acid-reactive substances, myeloperoxidase activity and the contents of TNF-α and IL-1β in gastric mucosa after aspirin administration were both significantly inhibited by pre-treatment with FR167653. Based on these data, the beneficial effects of FR167653 on aspirin-induced gastric mucosal injury may be attributed to its anti-inflammatory properties.

Key Words: FR167653, Aspirin, NSAID, TNF-α, IL-1β

Introduction

Nonsteroidal anti-inflammatory drugs (NSAIDs) such as aspirin are among the most widely used drugs worldwide, and they are used extensively to treat a wide variety of medical conditions. Although NSAID have many beneficial effects, each dose is known to cause measurable damage to the gastric mucosa [7]. It has been demonstrated in various researchers that development of endothelial cell injury and leukocyte margination within the gastric microcirculation are early events in the pathogenesis of NSAID-induced gastropathy [2–4]. We previously reported that aspirin promoted neutrophil adherence to endothelium via CD11/CD18-dependent interactions, with intercellular adhesion molecule-1 (ICAM-1) expressed on endothelial cells [4]. Furthermore, other studies have concluded that NSAID administration resulted in an early increase in TNF-α plasma levels and suggested that this cytokine may up-regulate adhesion molecule expression on leukocytes and endothelial cells, leading to intravascular thrombi formation and free radical-mediated damage to the endothelium and epithelium [5–7].

FR167653, 1-[(7-(4-fluorophenyl)-1, 2, 3, 4-tetrahydro-8-(4-pyridyl) pyrazolo [5, 1-c][1, 2, 4]triazin-2-yl]-2-phenylethanedione sulfate monohydrate (Fig. 1), was first discovered to be a potent inhibitor of IL-1β and TNF-α production in lipopolysaccharide (LPS)-stimulated human monocytes and
phytohemagglutinin-M-stimulated human lymphocytes [8]. Takahashi et al. [9] and Kawano et al. [10] also confirmed the inhibitory effect of FR167653 on cytokine production.

Recently it was demonstrated that FR167653 ameliorates endotoxin shock in rabbits and intravascular coagulation in rats [8], and that FR167653 ameliorates cardiac dysfunction caused by chronic infusion of LPS in rats [11]. In addition, FR167653 protected lung, liver, and heart against ischemia-reperfusion injury in dogs [12–14], and the protection against liver ischemia-reperfusion injury was associated with inhibition of proinflammatory cytokines from cytokine-induced neutrophil chemoattractant [15, 16]. Furthermore, it has been reported that FR167653 markedly reduced the *H. pylori*-induced increase in endogenous p38 kinase activity in the gastric mucosa, and also significantly inhibited neutrophil chemokine production [17].

The purpose of this paper is to determine whether FR167653 can ameliorate aspirin-induced gastric mucosal injury in rats, and whether the agent can inhibit the increase in neutrophil accumulation associated with inflammatory cytokines.

Materials and Methods

Reagents

Aspirin (Sigma Chemical, St. Louis, MO, U.S.A.) was prepared as a suspension in a vehicle consisting of 0.25% carboxymethylcellulose and was added to 0.15 N HCl. FR167653 was kindly provided by Fujisawa Pharmaceutical Company (Osaka, Japan).

Experimental Animals

Male Wistar rats weighing 190–210 grams were obtained from Keari Co. Ltd. (Osaka, Japan). They were housed in stainless steel cages with wire bottoms and maintained on a 12-h light and 12-h dark cycle with the temperature and relative humidity of the animal room controlled at 21–23°C and 55–65%, respectively. They were not fed for 18 h prior to the experiments, but were allowed free access to water. All experimental procedures described below were approved by the Animal Care Committee of the Kyoto Prefectural University of Medicine (Kyoto, Japan).

Acute gastric damage induced by aspirin/HCl

Gastric hemorrhagic lesions were induced by intragastric administration of aspirin (200 mg/kg) and 0.15 N HCl in a volume of 0.5 ml/100 g body weight [18]. In the sham groups, rats were received an equivalent volume of the vehicle. At 3 h after administration of aspirin the animals were killed by exsanguinations via the abdominal aorta under urethane anesthesia (1,000 mg/kg). The stomach was dissected, removed, and cut along the greater curvature and rinsed with physiological saline.

FR167653 was given to the rats by subcutaneous injection 2 h before the aspirin administration. In the control (aspirin/HCl) groups and in the sham groups, rats were received an equivalent volume of the vehicle (physiological saline).

Evaluation of gastric mucosal injury induced by aspirin/HCl

Macroscopic gastric damage was examined under a dissecting microscope with a square grid and was expressed as the total area (square millimeters) of hemorrhagic erosions (erosion index) [19]. The gastric mucosa was scraped off with two glass slides and homogenized with 1.5 ml of 10 mM potassium phosphate buffer (pH 7.8) in a Teflon-Elvehjem homogenizer to measure concentration of thiobarbituric acid-reactive substances (TBA-RS) and myeloperoxidase (MPO) activity. The concentration of TBA-RS in the gastric mucosa, an index of lipid peroxidation, was measured by the method of Ohkawa et al. [20] and was expressed as nmol of malondialdehyde. TBA (BDH Chemicals, Poole, UK) and 1,1,3,3-tetramethoxy propane (Tokyo Kasei Co., Tokyo, Japan) were used for TBA assay and all other chemicals were of reagent grade. MPO activity in the gastric mucosa, an index of polymorphonuclear leukocytes accumulation, was determined by a modification of the method of Krawisz et al. [22]. Briefly, homogenized gastric mucosal samples were sonicated on ice for 10 s and centrifuged at 40,000 g for 15 min. The supernatant was then assayed for MPO activity. MPO activity was measured spectrophotometrically and one unit of MPO activity was defined at that degrading 1 micromol of peroxide per minute at 25°C.

Inflammatory cytokines in the gastric mucosa after administration of aspirin/HCl

The content of TNF-α and of IL-1β in the gastric mucosal homogenates were determined by ELISA using a rat TNF-α and rat IL-1β ELISA kits (Bio Source International, Inc., California, USA) according to the manufacturer’s instructions.
Statistical Analysis

All values were expressed as means ± SEM. Data were compared using an analysis of variance (ANOVA) followed by Scheffer’s test. A value of $p<0.05$ was considered significant.

Results

Macroscopic findings of gastric mucosa

The intragastric administration of acidified aspirin induced hyperemia and hemorrhagic erosions in rat stomachs. The increase in the total gastric erosive area after aspirin administration was significantly inhibited by treatment with FR167653 in a dose-dependent manner (Fig. 2, 3).

TBA-RS in gastric mucosa

TBA-RS in the gastric mucosa significantly increased from a basal mean concentration of $0.77 ± 0.022$ nmol/mg protein to $1.011 ± 0.072$ nmol/mg protein 3 h after administration of aspirin. This increase was significantly inhibited by treatment with FR167653 at a dose of 20 mg/kg (Fig. 4).

MPO activities in gastric mucosa

Neutrophil accumulation was also evaluated by measure-
ment of MPO activity in the gastric mucosal homogenates. Tissue-associated MPO activity in the gastric mucosa significantly increased from a basal mean concentration of 1.14 ± 0.287 mU/mg protein to 2.93 ± 0.423 mU/mg protein 3 h after administration of aspirin (Fig. 5). The increase in MPO activity in gastric mucosa after aspirin administration was significantly reduced by treatment with FR167653 at a dose of 20 mg/kg.

Inflammatory cytokines in the gastric mucosa after administration of aspirin/HCl

The contents of both mucosal TNF-α and IL-1β in the asp/HCl with vehicle group were significantly increased compared with the levels of those in the sham group (Table 1). These increases in the levels of TNF-α and IL-1β were significantly inhibited by the treatment with FR167653 at a dose of 20 mg/kg.

Discussion

Nonsteroidal anti-inflammatory drugs such as aspirin and indomethacin cause acute erosions in the gastric mucosa of rats if administered orally in high doses [2, 23]. Although this injury results from complex interaction of luminal and mucosal factors, a critical event is the enhanced adhesion of neutrophils to endothelial cells in the gastric mucosal microcirculation [2, 4, 23]. It has been reported that both indomethacin and aspirin act on the postvenular capillary endothelial cells to increase their neutrophil adhesiveness [4, 24]. In addition to accumulation of neutrophils, active oxygen species and inflammatory cytokines, which were produced by adhered neutrophils or endothelial cells, play an important role in the pathogenesis of aspirin-induced mucosal injury [4, 25].

We have already demonstrated that various antioxidants or drugs were effective against aspirin-induced gastric mucosal injury in rats in vivo [26–29]. In each case, inflammatory cytokines are closely involved in these pathologic conditions. Especially both IL-1β and TNF-α which are formed at the early stage of inflammation attributes great importance to aspirin-induced gastric mucosal injury [29].

In these experiments, we have reported for the first time that a new anti-inflammatory compound, FR167653, dramatically reduced aspirin-induced gastric mucosal injury in rats. Inflammatory cytokines, such as TNF-α and IL-1β, in gastric mucosa was significantly increased by the aspirin administration and this increase of cytokines was inhibited by the treatment of FR167653. Furthermore, both the increase of mucosal MPO activities, the indices of neutrophil infiltration, and the increase of mucosal TBA-RS, the indices of lipid peroxidation, induced by aspirin administration were significantly reduced by the treatment of FR167653.

FR167653 is a low-molecular pyrazolotriazine derivative and has been characterized as a potent suppressant of TNF-α and IL-1β production at the transcriptional and translational levels [12, 17, 30–32]. It was suggested that FR167653 can reduce the gastric mucosal injury induced by aspirin via the mechanisms of inhibition of inflammatory cytokines. Recently, this compound is characterized by the presence of the pyridine and fluorophenyl rings that are essential for binding to p38 MAPK. It has been reported that FR167653 competes with ATP for binding to p38 MAPK. In addition, a recent study revealed that FR167653 is a p38 MAPK-selective inhibitor without affecting the activities of other protein kinases, such as ERK-1, JNK-2, protein kinase A, protein kinase C, protein kinase G or epidermal growth factor receptor kinase [17]. It has been also reported that the activation of p38 MAPK is involved in intracellular signaling pathways that regulate the production of several cytokines or chemokines, such as IL-1β, IL-6, IL-8, TNF-α and MCP-1 [33–35]. Especially IL-1 is known to be a potent activator of MAPK pathways [36, 37].

On another front, various types of mediators, including IL-1β and TNF-α, have been reported to participate in the pathogenesis of aspirin-induced gastric mucosal injury. Ordan et al. reported that aspirin likewise enhanced expression of proinflammatory cytokines and chemokines (IL-1β, IL-6, and TNF-α) that are likewise regulated at the level of message stability via p38 activation [38]. Furthermore, they also reported that aspirin induced the activation of p38 and c-Jun N-terminal kinase (JNK) mitogen-activated protein kinases [38]. It is necessary to clarify the involvement with aspirin-induced gastric mucosal disorder and p38 MAPK from now on.

As mentioned above, it has been suggested that protective effect with FR167653 on aspirin-induced gastric mucosal

Table 1. Effect of FR167653 on inflammatory cytokines in the gastric mucosa after administration of aspirin in rats

<table>
<thead>
<tr>
<th></th>
<th>Normal (Sham) (n = 6)</th>
<th>Control (Asp/HCl) (n = 6)</th>
<th>Asp/HCl + FR167653 (5 mg/kg) (n = 6)</th>
<th>Asp/HCl + FR167653 (20 mg/kg) (n = 6)</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF-α (pg/mg prot)</td>
<td>1742 ± 124</td>
<td>3730 ± 328*</td>
<td>3100 ± 300</td>
<td>2399 ± 219*</td>
</tr>
<tr>
<td>IL-1β (pg/mg prot)</td>
<td>310 ± 22.0</td>
<td>933 ± 33.0*</td>
<td>831 ± 34.9</td>
<td>741 ± 36.0*</td>
</tr>
</tbody>
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Each value indicates the mean ± SE. *p<0.05 compared with the value of sham groups, †p<0.05 compared with the value of control (Asp/HCl) groups.
FR167653 over the medical treatment against the NSAID-production in early stage of inflammation. The usefulness of injury may be associated with inhibition of cytokine production in early stage of inflammation. The usefulness of FR167653 over the medical treatment against the NSAID-induced gastric ulcer is sufficiently promising.

References


