Effect of the Oral Absorption of Benzenesulfonanilide-Type Cyclooxygenase-1 Inhibitors on Analgesic Action and Gastric Ulcer Formation

XIAOXIA ZHENG, HIROYUKI ODA, SHUN HARADA, YUKIO SUGIMOTO, AKIHIRO TAI, KENJI SASAKI, HIROKI KAKUTA

Division of Pharmaceutical Sciences, Okayama University Graduate School of Medicine, Dentistry and Pharmaceutical Sciences, 1-1-1, Tsushima-Naka, Okayama 700-8530, Japan

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ABSTRACT: A benzensulfonanilide-type cyclooxygenase-1 (COX-1)-selective inhibitor, ZXX2-77: 4-amino-4′-chloro-*N*-methylbenzenesulfonanilide (**4a**), has been reported as a novel analgesic that does not cause gastric damage. This compound has a weak analgesic effect but has potent *in vitro* COX-1 inhibitory activity. Since the reason for the weak analgesic effect *in vivo* was thought to be the low rate of oral absorption, the blood concentration of ZXX2-77 (**4a**) was measured in rats. It was found that the C_{max} value (1.2 μM) of ZXX2-77 (**4a**) at a dose of 30 mg/kg did not reach the COX-1 IC_{50} value (3.2 μM). On the other hand, ZXX2-79 (**4b**) (SO_2NH derivative of ZXX2-77 (**4a**); 4-amino-4′-chlorobenzenesulfonanilide), which shows less potent COX inhibitory activities (COX-1 $IC_{50} = 12$ μM, COX-2 $IC_{50} = 150$ μM) than those of ZXX2-77 (**4a**) *in vitro*, was found to be more absorbable ($C_{max} = 16$ μM at a dose of 30 mg/kg in rats) than ZXX2-77 (**4a**). Furthermore, ZXX2-79 (**4b**) not only showed a potent analgesic effect in a formalin test but also caused little gastric damage. These findings indicate that demethylated sulfonamide compounds are more easily absorbed than are *N*-methylated sulfonamide compounds and suggest that COX-1-selective inhibitors will be useful as analgesics that do not cause gastric damage.
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Keywords: cyclooxygenase; COX; COX-1; COX-1-selective inhibitor; sulfonamide; analgesic; absorption; gastric damage; writhing test; formalin test

INTRODUCTION

Cyclooxygenases (COXs), which are known as target enzymes of nonsteroidal anti-inflammatory drugs (NSAIDs), play key roles in prostaglandin synthesis from arachidonic acid. COXs have been reported to have three different subtypes, COX-1, COX-2, and COX-3.[1–3] While COX-2 is thought to be inflammatory stimuli-inducible,[4–6] COX-1 is expressed constitutively and is thought to be related to mucosal protection by production of prostaglandin.[7] Thus, COX-1 inhibition is thought to be the main reason for gastric damage caused by NSAIDs.[8,9] COX-3 has been identified as an isoform of COX-1 and as a target of acetaminophen (paracetamol).[3]

COX-2-selective inhibitors have attracted attention worldwide as new alternative anti-inflammatory drugs with less gastric damage than that caused by past NSAIDs. Currently, COX-2-selective inhibitors are used clinically for treatment of rheumatism[10] and have been reported to

Correspondence to: Hiroki Kakuta (Telephone: +81-86-251-7977; Fax: +81-86-251-7926;
E-mail: kakuta@pharm.okayama-u.ac.jp)

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show antitumor activity based on their antiangiogenic activity.[11,12] In contrast, development of COX-1-selective inhibitors has proceeded slowly because of the bad image of COX-1 inhibition as the main reason for NSAID-induced ulcer formation.

There are only a few currently available COX-1 inhibitors, including SC-560 (**1**),[13] mofezolac (**2**),[14–16] and FR122047 (**3**)[17,18] (Fig. 1). The latter two COX-1 inhibitors were developed before the discovery of COX-2. Interestingly, these COX-1 inhibitors have been reported to cause little gastric damage.[16,18] Wallace et al.[19] also reported that COX-1 inhibition alone causes little gastric ulcer formation and that gastric damage caused by COX inhibition requires both COX-1 and COX-2 inhibition. COX-1 inhibitors tend to show an analgesic effect rather than anti-inflammatory activity; for example, mofezolac (**2**) is used as a pain killer (Disopain tablets) in Japan.[16] On the other hand, COX-2 inhibitors have been reported to act as anti-inflammatory agents rather than pain relievers.[17,20,21] Thus, COX-1-selective inhibitors should attract more attention as analgesics that do not cause gastric damage.

The current limited availability of COX-1-selective inhibitors prompted us to examine the usefulness of COX-1-selective inhibitors. We have reported that a benzensulfoanilide-type COX-1 inhibitor, ZXX2-77 (**4a**) (Fig. 1), has an analgesic effect without causing gastric damage.[22] ZXX2-77 (**4a**) shows potent COX-1 inhibitory activity *in vitro* (Tab. 1), but the analgesic effect *in vivo* was weak after oral administration. We hypothesized that the reason for the weak analgesic effect is the low rate of absorption, and we evaluated the relationships of *in vivo* activity with the *in vitro* activity and drug absorption in this study. The results demonstrated that the weak *in vivo* activity of ZXX2-77 (**4a**) when orally administered was based on the low rate of absorption, and ZXX2-79 (**4b**) (Fig. 1), which shows less potent COX-1 inhibitory activity than that of ZXX2-77 (**4a**), was found to be absorbed effectively to elicit a potent analgesic effect without causing gastric damage. In this article, the blood concentration profile and analgesic activity of and the gastric damage caused by each compound are described.

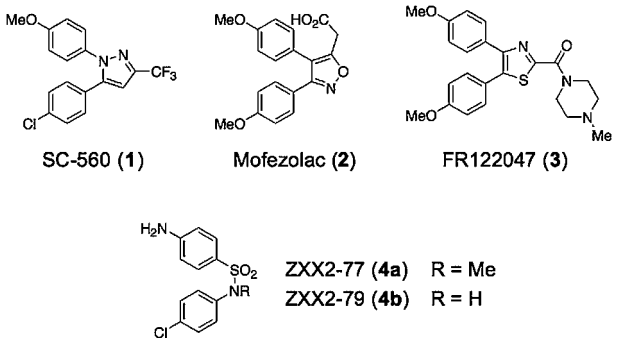

Figure 1. Chemical structures of known and our COX-1 inhibitors.

MATERIALS AND METHODS

Compounds

ZXX2-77 (**4a**) and ZXX2-79 (**4b**) were synthesized according to our previous report.[22] SC-560 (**1**), FR122047 (**3**) were purchased from Cayman. Mofezolac (**2**) was extracted from Disopain® tablets purchased from Mitsubishi Tanabe Pharma, Osaka, Japan. Aspirin and indomethacin were purchased from Sigma, St. Louis, MO.

COX Inhibitory Activity

IC_{50} values against each COX were assessed with a colorimetric COX (ovine) inhibitory screening assay kit (Cayman Chemical, Ann Arbor, MI; catalog No. 760111) according to the supplier's protocol. Each experiment was performed at least twice and their mean value was calculated.

Animals

Male ICR mice and male SD rats, weighing 15–30 and 170–200 g, respectively, were acquired from Charles River Co. Ltd., Kanagawa, Japan. Only water was provided *ad libitum* during a period of 12 h before experimentation. The study was conducted according to internationally accepted principles of laboratory animal use.

Samples for Blood Concentration Measurement

Groups of rats ($n = 5$ in each) were treated with solutions of ZXX2-77 (**4a**) or ZXX2-79 (**4b**) at a dose of 30 mg/kg (1% ethanol and 0.5% CMC in distilled water) at a volume of 5 mL/kg of body weight by oral administration. At the indicated times, 0.25 mL of blood was taken by heart puncture under diethyl ether anesthesia. Each blood sample was centrifuged at 4400*g* for 5 min at 4°C. To 100 μL of the resulting plasma were added 100 μL of ice-cold 5 mM ammonium acetate