Evaluation of Factors Affecting Gastrointestinal Absorption of a Novel Anticoagulant FX-93 for Development of Oral Formulation

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ABSTRACT: To find out factors causing the low bioavailability of FX-93, a novel anticoagulant, its solubility, membrane permeability, and the effect of bile salt on the absorption of FX-93 were investigated. The solubility of FX-93 under physiological conditions ranged from 0.3 to 18.3 mg/mL and the dose number was calculated to be 0.02–0.27, suggesting that the intrinsic solubility of FX-93 should not be a limiting factor for oral absorption. Apparent permeability of FX-93 across Caco-2 cell monolayer suggested that its fraction of dose absorbed would range between 30% and 40% in humans. Furthermore, FX-93 was substantially absorbed from each segment of rat intestine. However, the decrease in the gastrointestinal transit rate significantly decreased maximum plasma concentration and area under the plasma concentration–time curve of FX-93 after oral dosing in dogs, suggesting that FX-93 absorption would be suppressed by some components in the small intestinal lumen. An *in situ* rat administration study indicated that bile significantly decreased the intestinal absorption of FX-93 by two-thirds, which could be attributed to the decrease in FX-93 solubility by the interaction with bile or bile acid. Nuclear magnetic resonance spectroscopy analysis suggested that FX-93 would interact with bile salt between the naphthalene ring of FX-93 and steroidal backbone of bile salt. © 2012 Wiley Periodicals, Inc. and the American Pharmacists Association J Pharm Sci 101:2134–2142, 2012

Keywords: FX-93; oral anticoagulant; bile; absorption; interaction; gastrointestinal transit; Caco-2 cells; permeability

INTRODUCTION

N-[4-[(1-acetoamidoyl-4-piperidyl) oxy]phenyl]-*N*-[(7-amidino-2-naphtyl)methyl]sulfamoyl acetic acid (FX-93, Fig. 1) is a novel anticoagulant that inhibits factor Xa, a potential target for anticoagulation therapy.[1,2] Findings from previous reports have shown that the compound or its salt forms, such as monomesilate and hydrochloride, exert a potent anticoagulant effect with high selective affinity for factor Xa and suggested that FX-93 would be a safe antithrombotic agent with low bleeding risk.[3,4] Therefore, FX-93 is expected to be useful in treating a number of thrombotic disorders such as deep vein thrombosis, disseminated intravascular coagulation, and unstable angina. As orally deliverable anticoagulants are clinically needed, the new factor Xa inhibitor is expected to be developed as an oral dosage form.[3] However, the oral bioavailability of FX-93 was estimated to be very low in rats (4%) and dogs (7%).[4] FX-93 is chemically stable in the physiological conditions because almost 100% of the compound was recovered after 1-day incubation under pH 3–7 at 40°C and after 30-min dissolution test under pH 1.2 at 37°C (unpublished data). Apparent lipophilicity of this compound is not affected by the physiological pH conditions (pH 1–7), as the ratio of unionized form to ionized form is considered to be almost the same (<0.01%) in these pH conditions based on a calculated p*K*a (>11) due to its amidino group. Furthermore, results from an *in vitro* metabolic study using liver microsome or *in vivo* rat pharmacokinetic study found little or no metabolites of FX-93, respectively.[5,6] These results suggest that

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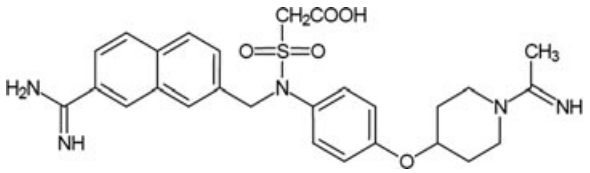

Figure 1. Chemical structure of FX-93.

the low oral bioavailability of FX-93 would be due to its poor oral absorption. To develop the oral formulation of FX-93, oral absorption of FX-93 must be improved and we, therefore, need a deeper understanding of characteristics such as solubility, membrane permeability, and other factors affecting the oral absorption, but few reports have evaluated these fundamental properties of FX-93 so far. In the present study, we tried to clarify the absorption properties of FX-93 to identify the factors responsible for the low oral absorption.

MATERIALS AND METHODS

Materials

FX-93 monomesilate and FX-93 monohydrochloride were synthesized by the Chemical Technology Laboratories, Astellas Pharma Inc. (formerly Yamanouchi Pharmaceutical Company, Ltd., Ibaraki, Japan). Caco-2 cells were obtained from Dainippon Sumitomo Pharma Company Ltd. (formerly Dainippon Pharmaceuticals Company, Osaka, Japan). Dulbecco's modified Eagle's medium (DMEM), nonessential amino acids (NEAAs), fetal bovine serum (FBS), L-glutamate, trypsin (0.25%)–ethylenediaminetetraacetic acid (EDTA) (1 mM), and antibiotic–antimycotic mixture for cell culture were purchased from Life Technologies, Inc. (Rockville, Maryland). Propranolol (PPL) and sodium taurocholate were obtained from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Nadolol (NDL) and fluorescein isothiocyanate (FITC)–dextran 4000 (FD-4) were purchased from Sigma Chemical Company (St. Louis, Missouri). Propantheline bromide and sulfasalazine were purchased from Sigma–Aldrich (Tokyo, Japan). Extract gall powder (bile) was obtained from Katayama Chemical, Inc. (Osaka, Japan). All other reagents were of analytical grade.

Animals

Male Fisher rats aged 8 weeks weighing 130–150 g (Japan SLC, Inc., Hamamatsu, Japan) and male Wistar rats aged 8 weeks weighing 180–200 g (Japan SLC, Inc.) were used for an *in situ* regional absorption study and intestinal administration study, respectively, to evaluate effect of bile on absorption of FX-93. All rats, maintained at 20°C–26°C and 40%–70% of humidity, were allowed free access to standard laboratory chow (CLEA Japan, Inc., Tokyo) and water, but they were fasted for at least 20 h before operation. Male beagle dogs weighing 11.6–14.0 kg (NARC Corporation, Chiba, Japan), maintained at 20°C–26°C and 40%–70% of humidity, were used for an oral administration study. The same dogs were used repeatedly in all experiments after a washout period of at least 7 days between trials. Dogs were fasted for at least 20 h before dosing and were fed approximately 250 g of standard laboratory canine diet (Oriental Yeast Company, Ltd., Tokyo, Japan) just after the study. Water was withheld from 30 min before dosing until 2 h after dosing. All experimental procedures using rats and dogs were approved by the Institutional Animal Care and Use Committee of Astellas Pharma Inc. in accordance with the Principles of Laboratory Animal Care (NIH publication #85-23).

Solubility of FX-93

FX-93 was dissolved in 0.1 mol/L HCl (pH 1) or Britton–Robinson buffer ranged from pH 3 to 11. Saturated solutions of FX-93 monomesilate and FX-93 monohydrochloride in each buffer solution at 25°C were prepared, and the solubility of FX-93 in the supernatant after filtration was measured by high-performance liquid chromatography (HPLC) assay.

Transport Study Using Caco-2 Cells

Caco-2 cells were cultured as described previously.[7] Briefly, Caco-2 cells were grown in DMEM with 4.5 g/L D-glucose supplemented with 10% (v/v) FBS, 1% (v/v) L-glutamate, 1% (v/v) NEAA, and 1% (v/v) antibiotic–antimycotic mixture in a CO_2 incubator (CPD-170W; Hirasawa Works, Tokyo, Japan) at 37°C, 5% CO_2, and 98% relative humidity. Culture medium was changed every 48 h. For preparation of 21-day cultured Caco-2 cell monolayers, Caco-2 cells from passages 58–78 were harvested with trypsin–EDTA solution and then seeded onto HTS Multiwell™ inserts (pore size: 1 μm, area: 0.31 cm^2, BD, Franclin Lakes, New Jersey) at 1×10^5 cells/insert and cultured for 21 days. Culture medium was changed every day for 3 days after seeding and every 48 h thereafter. Hank's balanced salts solution (HBSS) supplemented with 20 mM glucose adjusted at pH 6.0 with 20 mM 2-(N-morpholino)ethanesulfonic acid (MES) (0.5 mL) or pH7.4 with 20 mM 4-(2-hydroxyethyl)-1-piperazineethane sulfonic acid (HEPES) (1.5 mL) was placed in the apical side or basal side, respectively. Transport study was initiated by adding FX-93 monomesilate (100 μg/mL) or model compounds with different *in vivo* absorbability (PPL, high; NDL, intermediate; FD-4, low: 300 μM) to the apical chamber. Samples (100 μL) were drawn from the basal side at 15 min and 1 h and an equal volume of fresh HBSS was immediately added to the basal side after each sampling. All the experiments were