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Photochemical Mechanism of Riboflavin-Induced Degradation of Famotidine and a Suggested Pharmaceutical Strategy for Improving Photostability

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ABSTRACT

The present study aimed to clarify the mechanism of photodegradation of famotidine with riboflavin (FMT/RF), and to develop a photochemically stabilized formulation of FMT/RF. Photochemical properties of RF were characterized by UV-VIS spectral analysis, reactive oxygen species (ROS) assay, and photostability testing. Pharmacokinetic study was conducted in rats after intravenous administration of FMT (1 mg/kg) formulation containing RF (0.01 mg/kg). The UV-VIS spectral pattern of RF partly overlapped with the sunlight spectrum, and ROS generation from photoirradiated RF was remarkable; thus, RF had high photoreactive potential. In the photostability testing, after irradiation (250 W/m^2), degradation rate for FMT in FMT/RF was ca. 11-fold higher than that in FMT alone. The addition of radical scavengers to FMT/RF led to attenuated photodegradation of FMT/RF; in particular, the addition of L-ascorbic acid (vitamin C; VC) to FMT/RF showed ca. 86% inhibition of the photodegradation of FMT/RF. The pharmacokinetic study on FMT indicated that the addition of VC (1 mg/kg) to FMT/RF had no significant impact on the pharmacokinetic behavior of FMT. These findings suggest that ROS-mediated photochemical reaction would be involved in the photodegradation pathway of FMT/RF, and the complementary use of VC might be an attractive approach to improve the photostability of FMT/RF.

Introduction

In pharmaceutical therapy, patients often receive various injectable drugs simultaneously. It is possible to reduce the invasiveness to the patient by mixing up all injectable drugs and giving them in one go via one route; however, incompatibility may occur among the applied drugs.[1,2] Incompatibility sometimes induces various undesirable reactions, including precipitation, color change, drug degradation, and yield of toxic products upon covalent binding.[3] These factors might jeopardize the safety and effectiveness of intravenous drug therapies.[4] According to the clinical case, famotidine (FMT; Fig. 1a), a histamine H_2 receptor antagonist, has been used by mixing with various drugs.[5,6] Several injectable drugs, including total parenteral nutrition and potassium chloride (KCl) preparation, contain riboflavin (RF; Fig. 1b),[5,7] and RF may lead to the photodegradation of FMT even under a daylight fluorescent lamp at room temperature.[5] The attenuation of FMT potency may cause therapeutic failure for patients with gastrointestinal diseases; however, the mechanistic aspects of the photodegradation of FMT with RF (FMT/RF) are still unclear. Thus, the clarification on the mechanism of photochemical interaction between FMT and RF may be needed to take preventive measures against the photodegradation of FMT/RF. The photochemical mechanism-based prevention can provide a desired medication for the clinical use of FMT.

RF has been commonly prescribed for the treatment of avitaminosis B_2, and it is also used as a coloring agent of KCl solution in order to avoid medical accidents, including arrhythmia and cardiac arrest.[7] Because of its high photosensitivity,[8,9] irradiated RF tends to cause photooxidation of co-existing drugs. For example, irradiated RF led to the photodegradation of various drugs, including

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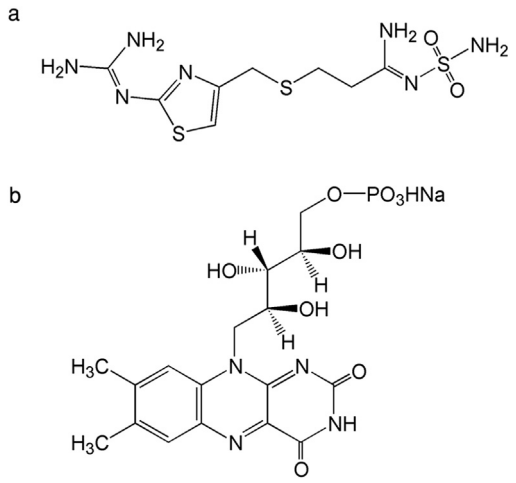

Figure 1. Chemical structures of FMT (a) and RF (b).

ceftriaxone, isoproterenol, and folate.[10-12] Reactive oxygen species (ROS)-mediated photochemical reaction may be involved in the photooxidation pathway of a drug.[13] FMT has been widely prescribed for patients with gastric ulcers, duodenal ulcers, Zollinger-Ellison syndrome, and gastroesophageal reflux disease.[14] FMT has antioxidant activity since it was shown to act as an acceptor of ROS.[15,16] From these previous observations, RF-sensitized photooxidation may be partly involved in the photodegradation pathways of FMT/RF. However, the detailed mechanisms of the photodegradation of FMT/RF have not been fully elucidated.

The present study aimed to clarify the mechanism of the photodegradation of FMT/RF in more detail. The photochemical properties of RF were characterized by UV-visible light (UV-VIS) spectral analysis and ROS assay. The degradation profile of FMT with or without RF upon irradiation was monitored, and photostability testing of FMT/RF was conducted with the addition of radical scavengers to clarify the possible involvement of a ROS-mediated mechanism in the photodegradation of FMT/RF. Furthermore, to improve the photostability of FMT/RF, a new formulation of FMT/RF was designed on the basis of the mechanisms of the photodegradation of FMT/RF. To assess the bioequivalence between FMT/RF formulations, pharmacokinetic study was carried out in rats after the intravenous administration of FMT/RF formulations.

Materials and Methods

Chemicals

FMT, RF, dimethyl sulfoxide (DMSO), imidazole, nitroblue tetrazolium (NBT), *p*-nitrosodimethylaniline (RNO), Tween 20, disodium hydrogen phosphate $12H_2O$, sodium dihydrogen phosphate dehydrate, dibutylhydroxytoluene (BHT), ammonium acetate, L-tyrosine (Tyr), sodium sulfite (Na_2SO_3), and D-mannitol were purchased from Wako Pure Chemical Industries (Osaka, Japan). Sodium azide (NaN_3), L-tryptophan (Trp), L-cysteine (Cys), and L-histidine (His) were purchased from Sigma-Aldrich Japan (Tokyo, Japan). L-Ascorbic acid (vitamin C; VC) was purchased from Nacalai Tesque, Inc. (Kyoto, Japan). Methanol (MeOH) and acetonitrile (liquid chromatography grade) were purchased from Kanto Chemical (Tokyo, Japan).

UV-VIS Spectral Analysis

FMT and RF were dissolved in 20 mM sodium phosphate buffer (NaPB) (pH 7.4) at 20 μM. UV-VIS absorption spectra were recorded with a HITACHI U-2010 spectrophotometer (HITACHI, Tokyo, Japan) interfaced to a PC for data processing (software: Spectra Manager). A spectrofluorometer quartz cell with 10-mm pathlength was employed.

ROS Assay

Irradiation Conditions

Each tested sample was stored in an Atlas Suntest CPS+ solar simulator (Atlas Material Technology LLC, Chicago, IL) equipped with a xenon arc lamp (1500 W). A UV special filter was installed to adapt the spectrum of the artificial light source to natural daylight. The irradiation tests were carried out at 25°C with an irradiance of 250 W/m^2 (300-800 nm).

Determination of ROS

The ROS assay was designed to evaluate the photochemical reactivity of the tested chemicals by determining both singlet oxygen and superoxide generated from photo-irradiated chemicals.[13,17] In the present study, the ROS assay was undertaken to clarify the photoreactivities of FMT and RF. Briefly, FMT and RF were dissolved in DMSO and 20 mM NaPB (pH 7.4), respectively. Singlet oxygen was measured by spectrophotometrically monitoring the bleaching of RNO at 440 nm using imidazole as a selective acceptor of singlet oxygen. Samples, containing tested compounds (50 μM), RNO (50 μM), imidazole (50 μM), and DMSO (2%, v/v) in 20 mM NaPB (pH 7.4) with Tween 20 (0.5%, v/v), were irradiated with simulated sunlight for the indicated periods (5, 10, 20, 30, 40, and 60 min), and then measured for their absorbance at 440 nm using a SAFIRE microplate spectrophotometer (TECAN, Mannedorf, Switzerland). To determine superoxide generation, samples, containing tested compounds (50 μM), NBT (50 μM), and DMSO (2%, v/v) in 20 mM NaPB (pH 7.4) with Tween 20 (0.5%, v/v), were exposed to simulated sunlight for the indicated periods (5, 10, 20, 30, 40, and 60 min), and reductions in NBT were measured by increases in absorbance at 560 nm using a SAFIRE microplate spectrophotometer.

Photostability Testing

Photodegradation Profiles of FMT/RF

FMT and RF were dissolved in 20 mM NaPB (pH 7.4). FMT (300 μM) and FMT (300 μM) containing RF (2 μM) with or without radical scavengers (500 μM), including NaN_3, VC, and BHT, in 5 mL clear glass vials were set in the Atlas Suntest CPS+ solar simulator, and irradiated with simulated sunlight for different periods (1.5, 3, 5, 10, 20, and 30 min). Each sample was diluted 100-fold, and the remaining FMT in the sample was determined with ultra-performance liquid chromatography equipped with electrospray ionization mass spectrometry (UPLC/ESI-MS) analysis. The UPLC/ESI-MS system consisted of a Waters Acquity UPLC^{TM} system (Waters, Milford, MA), which included binary solvent manager, sample manager, column compartment, and SQD connected with Mass-Lynx software. An Acquity UPLC^{TM} BEH C_{18} column (particle size: 1.7 μM, column size: 2.1 × 50 mm^2; Waters) was used, and column temperature was maintained at 60°C. Samples were separated using a gradient mobile phase consisting of 5 mM ammonium acetate (A) and acetonitrile (B) with a flow rate of 0.25 mL/min, and the retention time of FMT was ca. 1.9 min. The gradient conditions of the mobile phase were 0-0.5 min, 5% B; 0.5-2.5 min, 5%-20% B; and 2.5-4.0 min, 20%-95% B. Analysis was carried out using selected ion recording (SIR) for specific *m/z* 338.16 for FMT $[M+H]^+$.