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

（リボフラビンにより誘発されるファモチジンの
光分解機構解明および光安定化手法の提案）

内田　淳
(Uchida, Atsushi)

山梨大学

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

Atsushi Uchida [1, 2, *], Satomi Onoue [2], Hiroto Ohtake [2], Yoshiki Seto [2], Tsuyoshi Teramatsu [1], Tomoko Terajima [1], Toshio Oguchi [1]

[1] Department of Pharmacy, University of Yamanashi Hospital, Chuo-city, Yamanashi 409-3898, Japan
[2] Department of Pharmacokinetics and Pharmacodynamics, School of Pharmaceutical Sciences, University of Shizuoka, Suruga-ku, Shizuoka 422-8526, Japan

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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

* *Correspondence to*: Atsushi Uchida (Telephone: +81-55-273-1111; Fax: +81-55-273-6672).
E-mail address: auchida@yamanashi.ac.jp (A. Uchida).

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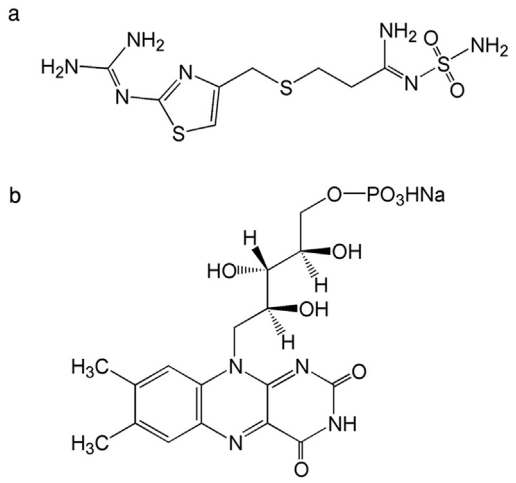

Figure 1. Chemical structures of FMT (a) and RF (b).

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 $[M+H]^+$.

Photostability Testing of FMT/RF With Radical Scavenger

FMT and RF were dissolved in 20 mM NaPB (pH 7.4). A mixture containing FMT (300 μM) and RF (2 μM) in a 5 mL clear glass vial was supplemented with radical scavengers (500 μM), including VC, Tyr, Trp, Cys, NaN_3, Na_2SO_3, His, BHT, and D-mannitol. Each sample was set in an Atlas Suntest CPS+ solar simulator and irradiated with simulated sunlight for 30 min. Each sample was diluted 100-fold, and the remaining FMT in the sample was measured by UPLC/ESI-MS analysis, as described in section *Photodegradation Profiles of FMT/RF.*

Pharmacokinetic Study

Animals

Male Sprague-Dawley rats (8-10 weeks of age; Japan SLC, Shizuoka, Japan), weighing 250-323 g, were housed 2 per cage in the laboratory with free access to food and water, and maintained on a 12-h dark/light cycle in a room with controlled temperature (24 ± 1°C) and humidity (55 ± 5%). All procedures used in the present study were conducted in accordance with the guidelines approved by the Institutional Animal Care and Ethical Committee of the University of Shizuoka and Yamanashi.

Plasma Concentration of FMT

Blood samples were obtained at a volume of 300 μL from the tail vein after the intravenous administration of FMT (1 mg/kg) containing RF (0.01 mg/kg) with or without VC (1 mg/kg) dissolved in saline at the indicated times (10, 20, 30 min, 1, 1.5, 2, 4, and 6 h). The blood samples were centrifuged at 10,000*g* for 10 min to prepare plasma samples, and the samples were kept frozen at below −20°C until they were analyzed. FMT concentrations in plasma were estimated by an internal standard method using UPLC/ESI-MS. Briefly, 250 μL of MeOH containing quinine (700 ng/mL; as internal standard) was added to 100 μL of plasma sample, followed by centrifugation at 10,000*g* for 10 min, and filtration at 0.22 μM. The concentration of FMT in the supernatant was analyzed by UPLC/ESI-MS, as described in section *Photodegradation Profiles of FMT/RF.* Analysis was also carried out using SIR for specific *m/z* 325.4 for quinine $[M+H]^+$. The pharmacokinetic parameters for FMT were calculated by two-compartmental methods using the WinNonlin® program (Ver. 4.1; Pharsight Corporation, Mountain View, CA).

Statistical Analysis

For statistical comparisons, one-way ANOVA with pairwise comparison by Fisher least significant difference procedure was used. A *p* value of less than 0.05 was considered significant for all analyses.

Results and Discussion

Photochemical Properties of FMT and RF

A photosensitizing drug can be excited by sunlight, composed of VIS (400-700 nm), UVA (320-400 nm), and part of UVB (290-320 nm).[18] The photo-excited drug may elicit photochemical reactions.[19] The UV-VIS absorption spectral measurement of such drugs has been commonly used as an immediate and simple method for predicting their photoreactivity.[20,21] The UV-VIS spectral patterns of FMT and RF were recorded in 20 mM NaPB (pH 7.4) (Fig. 2). RF showed strong absorption in the UVA-VIS range, and the lowest energy bands of RF had maxima at approximately 370 and 440 nm. The UV-VIS spectral patterns of RF overlapped with the sunlight spectrum. In contrast, the absorption of FMT in the UVA-VIS range was negligible. In theory, RF can thus absorb photon energy and be excited upon exposure to sunlight, whereas FMT does not have photoreactive potential.

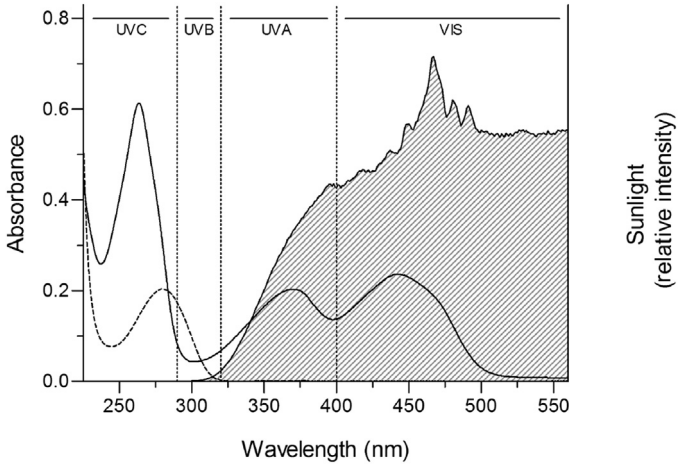

Figure 2. Average intensity of sunlight at the earth's surface and UV-VIS absorption spectra of RF and FMT in 20 mM NaPB (pH 7.4). RF, solid line; and FMT, broken line. Average intensity of sunlight (shaded) was taken from a previous report.[18]

The primary events in any photosensitization process can be the absorption of photon energy and subsequent generation of ROS: superoxide through electron or hydrogen transfer-mediated free radical generation (type I photochemical reaction), and singlet oxygen through energy transfer from an excited triplet photosensitizer to oxygen or biomolecules (type II photochemical reaction).[22] The ROS assay was designed for the photoreactivity assessment of pharmaceutical substances on the basis of ROS generation from photoirradiated compounds.[13] The ROS assay was carried out on both FMT and RF at a concentration of 50 μM (Fig. 3). For comparison, the ROS assay was also conducted for quinine, a typical phototoxic drug, as a positive control, and sulisobenzone, a sunscreen agent with no phototoxic potential, as a negative control, at the same concentration (50 μM). FMT and RF kept in the dark did not show any ROS generation (data not shown). The kinetics of ROS generation from irradiated RF indicated that superoxide was rapidly generated compared with singlet oxygen, suggesting that the exposure of RF under simulated sunlight (250 W/m^2) leads to the generation of ROS, mainly through a type I photochemical reaction in the early stage after irradiation. The exposure of RF to simulated sunlight for 1 h resulted in the generation of both singlet oxygen ($\Delta A_{440nm} \times 10^3$: 379.2 ± 8.7) and superoxide ($\Delta A_{560\ nm} \times 10^3$: 479.7 ± 16.1). In contrast, FMT exhibited negligible ROS generation [singlet oxygen ($\Delta A_{440\ nm} \times 10^3$): 5.7 ± 2.0; and superoxide ($\Delta A_{560\ nm} \times 10^3$): 7.2 ± 1.6] from FMT upon light exposure for 1 h. As observed in a previous study, sulisobenzone had no ability to yield ROS upon exposure to simulated sunlight. In contrast, the exposure of quinine to simulated sunlight for 1 h led to the generation of ROS [singlet oxygen ($\Delta A_{440\ nm} \times 10^3$): 196.6 ± 8.7; and superoxide ($aA_{560\ nm} \times 10^3$): 232.9 ± 5.5], and the ROS-generating ability of RF was higher than that of quinine at a concentration of 50 μM. According to the results from UV-VIS spectral analysis and ROS assay, RF had high photoreactive potential through type I and II photochemical reactions, possibly leading to the photodegradation of FMT/RF.

Photostability of FMT/RF

For further photochemical characterization, photostability testing was carried out for FMT (300 μM) with or without RF (2 μM)

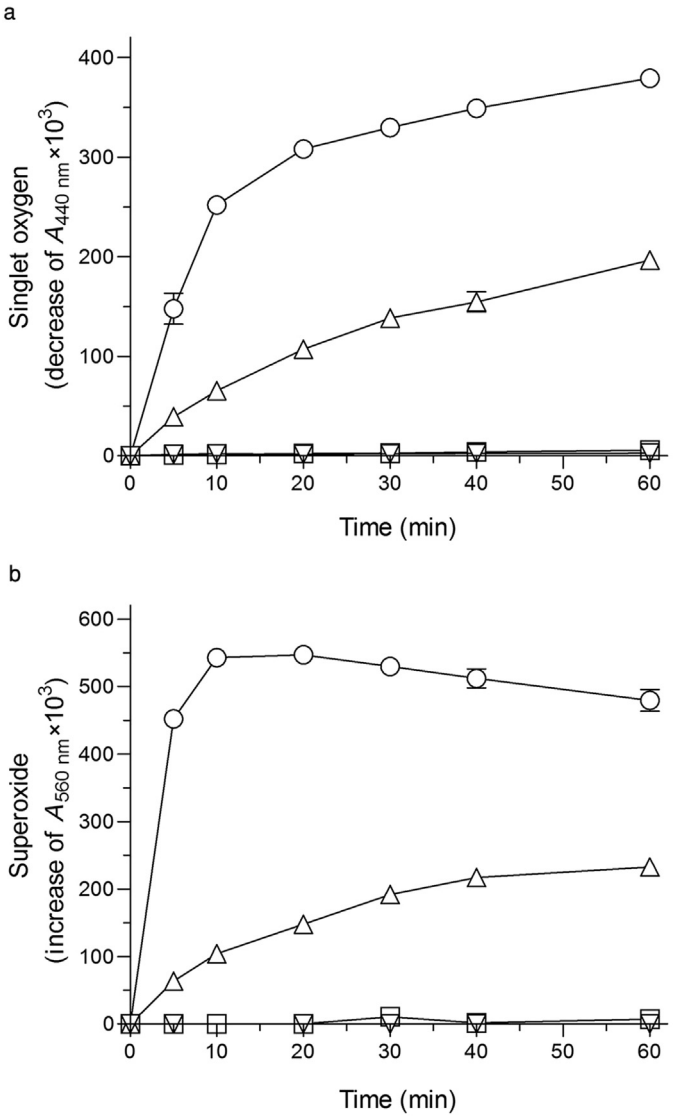

Figure 3. Generation of ROS from photoirradiated RF and FMT. Time course of singlet oxygen (a) and superoxide (b) generation from RF (50 μM) and FMT (50 μM) in 20mM NaPB (pH 7.4) exposed to simulated sunlight (250 W/m^2) for the indicated periods. ○, RF; □, FMT; Δ, quinine (50 μM, positive control); and ∇, sulisobenzone (50 μM, negative control). Data represent the mean ± SD of three experiments.

in 20 mM NaPB (pH 7.4) (Fig. 4). FMT and FMT/RF were exposed to the simulated sunlight (250 W/m^2) at 25°C for the indicated periods, and then subjected to UPLC/ESI-MS analysis. No samples kept in the dark showed any degradation for at least 30 min (data not shown). The exposure of FMT to the simulated sunlight for 30 min resulted in slight degradation of FMT potency (ca. 8%); however, the presence of RF accelerated the photodegradation of FMT, as evidenced by a ca. 84% reduction of FMT potency in FMT/RF. These findings might be consistent with the previous observation that FMT served as a ROS acceptor.[16]

In an attempt to evaluate the possible role of ROS in the photodegradation of FMT/RF, a series of experiments were performed in which several radical scavengers (500 μM) were included in FMT/RF solution during irradiation. The radical scavengers used were as follows: NaN_3, a singlet oxygen scavenger; BHT, a hydroxyl radical scavenger; and VC, a superoxide scavenger.[23] The addition of all radical scavengers led to a protective effect against the photodegradation of FMT/RF; in particular, VC showed ca. 86% attenuation

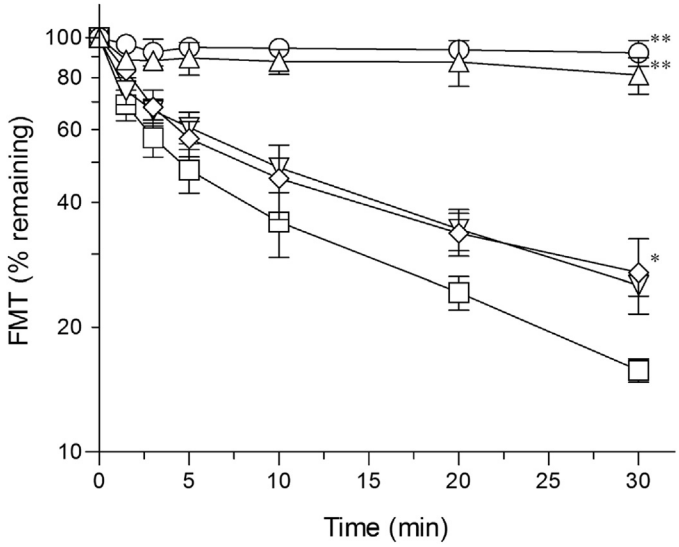

Figure 4. Photodegradation profile of FMT (300 μM) with or without RF (2 μM) and several radical scavengers in 20 mM NaPB (pH 7.4) irradiated with simulated sunlight (250 W/m^2). ○, FMT; □, FMT/RF; ∇, FMT/RF with NaN_3 (500 μM); Δ, FMT/RF with VC (500 μM); and ◊, FMT/RF with BHT (500 μM). Data represent the mean ± SD of three experiments. *, $p < 0.05$; and **, $p < 0.001$ with respect to FMT/RF at the same time point.

of photodegradation (Fig. 4). A clear linear relationship was obtained according to the following equation: $\ln A = \ln A_0 - kt$ (apparent first-order kinetics), where A is the remaining peak area of FMT, k is the slope (degradation rate constant), and t is the time (min). The initial degradation rate constant ($k_{initial}$) for FMT/RF with VC was ca. 7-fold lower than that for FMT/RF alone (Table 1). These results were in agreement with the data from the ROS assay, suggesting that ROS from irradiated RF caused the photodegradation of FMT/RF, and a type I photochemical reaction might be mainly involved in the photodegradation pathway of FMT/RF.

Attenuating Effect of Radical Scavengers on the Photodegradation of FMT/RF

In general, the protection of photolabile drugs from light by using a film coating has been widely applied to avoid the photodegradation of drug under irradiation.[24] Light protection may also be a viable approach to improve the photostability of FMT/RF, although light protection using shading film might delay the identification of physicochemical incompatibility, including color change and precipitation. Thus, to improve the photostability of FMT/RF, differential approaches may be needed on the basis of the mechanisms of photodegradation of FMT/RF.

To develop a photochemically stabilized formulation of FMT, with the exposure of FMT/RF to various radical scavengers under

Table 1
Initial Degradation Rate Constants of Photodegradation for FMT With or Without RF and Radical Scavengers

Variable	$k_{initial}$ (min^{-1})
FMT (300 μM)	0.0176 ± 0.0170
FMT (300 μM)/RF (2 μM)	0.144 ± 0.0381
FMT/RF with NaN_3 (500 μM)	0.0959 ± 0.0322
VC (500 μM)	0.0203 ± 0.0223
BHT (500 μM)	0.112 ± 0.0206

$k_{initial}$, initial degradation rate constant (from $t = 0$ to 5 min after irradiation). Data represent the mean ± 95% confidence interval of three experiments.

simulated sunlight (250 W/m^2) for 30 min, the remaining FMT of each sample was determined by UPLC/ESI-MS analysis (Fig. 5). The radical scavengers used were as follows: (i) superoxide scavengers: VC, Tyr,[25] Trp,[25] and Cys[26]; (ii) singlet oxygen scavengers: Na_2SO_3,[27] NaN_3, and His; and (iii) hydroxyl radical scavengers: BHT and D-mannitol.[28] The addition of all test radical scavengers to FMT/RF tended to attenuate the photodegradation of FMT/RF. In particular, VC, Tyr, and Trp strongly inhibited the photodegradation of FMT/RF, and the remaining levels of FMT in FMT/RF with VC, Tyr, and Trp were found to be ca. 81%, 91%, and 88%, respectively. Thus, the addition of superoxide scavenger to FMT/RF could provide a protective effect against the photodegradation of FMT/RF.

However, FMT/RF with Tyr or Trp induced physicochemical incompatibility as indicated by a color change to a yellow-brown color. Namely, the oxidative reaction of Tyr and Trp caused the generation of yellow-colored products.[29] Furthermore, the accumulation of aromatic amino acids, including Tyr and Trp, in systemic circulation may increase the risk of hepatic encephalopathy in patients with liver failure.[30] VC exerts several functions, including the detoxification of exogenous compounds and cytochrome P-450 activity.[31] VC is also responsible for activating collagen proline hydroxylase in the conversion of proline to collagen hydroxyproline.[32] It plays a major role as an antioxidant and scavenger of ROS generated upon exposure to sunlight.[33] In addition, VC led to reductions in the incidence of most malignancies in humans.[31] In a clinical context, the maximum intravenous dose of VC was 2.8 g/day in humans[34]; thus, VC has high tolerability potential. From these attractive features, VC has been widely used as an ingredient of anti-aging cosmetic products and anti-cancer therapy drugs.[31,33] VC was also shown to have a photostabilizing effect on photosensitive drugs such as loratadine and pizotifen.[35] On the other hand, VC can also act as a pro-oxidant under a specific condition.[36] When Fe^{3+} is present, VC can convert Fe^{3+} into Fe^{2+}, which subsequently reacts with oxygen or hydrogen peroxide resulting in generation of ROS.[36] Herein, the pro-oxidative action of VC may affect VC-assisted photostabilization of FMT/RF. In the present study, Fe^{3+} was not added to test samples; thus, VC might play a role as an antioxidant. VC could be theoretically suitable for inhibiting the ROS-mediated photodegradation of FMT/RF in the absence of Fe^{3+}.

Pharmacokinetic Behavior of FMT

On the basis of the results from photostability testing, FMT/RF with VC (FMT/RF/VC) might be a viable photochemically stabilized FMT formulation. However, the addition of VC to FMT/RF may affect the pharmacokinetic behavior of FMT.

To prove bioequivalence between FMT/RF formulations with and without VC (1 mg/kg), pharmacokinetic study was carried out in rats after the intravenous administration of FMT (1 mg/kg) containing RF (0.01 mg/kg) with or without VC (1 mg/kg). Plasma concentration-time curves of FMT were obtained by UPLC/ESI-MS analysis after intravenous administrations of FMT/RF and FMT/RF/VC in rats (Fig. 6). The pharmacokinetic profile of FMT in the FMT/RF/VC group was likely to be similar to that in the FMT/RF group. The relevant pharmacokinetic parameters for intravenously administered FMT, including area under concentration versus time curve (AUC_{0-6}), distribution rate constant (k_a), and elimination rate constant (k_β), are listed in Table 2. The AUC_{0-6}, k_a, and k_β values in the FMT/RF/VC group were calculated to be 1.67 ± 0.22 μg·h/mL, 2.52 h^{-1}, and 4.67 h^{-1}, respectively. On the other hand, the AUC_{0-6}, k_α, and k_β values in the FMT/RF group were calculated to be 2.87 ± 0.44 μg·h/mL, 4.61 h^{-1}, and 4.76 h^{-1}, respectively. There were no significant differences in the pharmacokinetic parameters of FMT between the FMT/RF/VC and FMT/RF groups. Thus, the addition of VC to FMT/RF had no effects on the pharmacokinetic behavior of FMT in rats.

In general, drug-drug or drug-excipient interaction through direct and indirect pathways may affect the disposition and pharmacokinetic behavior of a drug, thereby affecting its safety and/or efficacy.[37,38] The administration of VC caused a rapid and pronounced decrease in the rate of excretion of sulfated metabolites of some drugs, including salicylamide and acetaminophen, in healthy adult volunteers.[39-41] In addition, VC has antioxidant activity, thereby affecting the radiosensitivity of radiosensitizing drugs such as metronidazole.[42] Thus, careful characterization of the physicochemical and pharmacokinetic properties is essential for the development of photochemically stabilized photolabile drug formulations by the addition of VC.

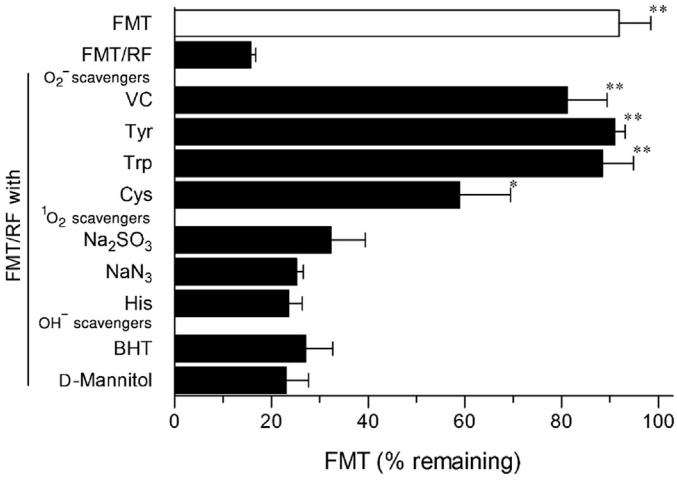

Figure 5. Photodegradation of FMT (300 μM) with RF (2 μM) and its attenuation by several scavengers (500 μM). Data represent the mean ± SD of three experiments. *, $p < 0.05$; and **, $p < 0.01$ with respect to FMT/RF.

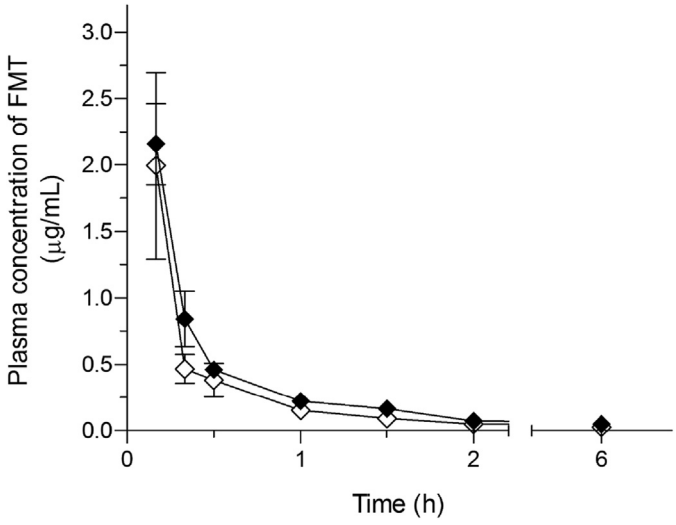

Figure 6. Plasma concentration–time profiles of FMT after intravenous administration of FMT (1 mg/kg) containing RF (0.01 mg/kg) with/without VC (1 mg/kg) in rats. ◊, FMT/RF; and ◆, FMT/RF/VC. Data represent the mean ± SE of four determinations.

Table 2
Pharmacokinetic Parameters of FMT After Intravenous Administration of FMT (1 mg/kg)/RF (0.01 mg/kg) With or Without VC (1 mg/kg)

Variable	k_α (h^{-1})	k_β (h^{-1})	$AUC_{0\text{-}6}$ (μg-h/mL)
FMT/RF	4.61 (2.36-6.86)	4.76 (1.31-8.21)	2.87 ± 0.44
FMT/RF/VC	2.52 (1.61-3.43)	4.67 (3.25-6.09)	1.67 ± 0.22

k_α, distribution rate constant; k_β, elimination rate constant; and $AUC_{0\text{-}6}$, area under the curve of plasma concentration versus time from $t = 0$ to 6 h after administration. $AUC_{0\text{-}6}$ represents the mean ± SE, and k_a and k_β represent the mean (95% confidence interval) of four determinations.

Conclusions

In the present study, the mechanism of the photodegradation of FMT/RF was partly deduced. According to UV-VIS spectral analysis, ROS assay, and photostability testing, RF had high photoreactive potential, and ROS generation from irradiated RF was involved in the photodegradation pathway of FMT/RF. The addition of VC to FMT/RF could improve the photostability of FMT/RF, and it had a negligible effect on the pharmacokinetic behavior of FMT. From these findings, the complementary use of VC would be a viable approach to improve the photostability of photolabile drugs, and it might provide reliable and consistent medication for the clinical use of photolabile drugs.

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