Investigation of the Safety of Topical Metronidazole from a Pharmacokinetic Perspective

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Metronidazole (MTZ) ointment has been used widely as a hospital preparation against cancerous malodor. Although cancerous tissue with ulcer-like symptoms is likely to have a higher capacity to absorb drugs than normal skin, the extent to which MTZ is absorbed when a topical preparation is applied to cancerous tissue remains uncertain. Furthermore, few studies have investigated the drug interactions involving MTZ despite its long use in clinical practice. In the present study, plasma concentration of MTZ was measured in a breast cancer patient using MTZ ointment for cancerous malodor and basic research was also conducted with the objective of investigating the safety of topical MTZ from a pharmacokinetic perspective. 4.75 µg/mL (2.78 µM) of MTZ was detected in the patient’s plasma, which was close to the plasma concentration after oral dosage of MTZ. In a metabolic inhibition study using human liver microsomes, cytochrome P450 (CYP) 2C9-mediated hydroxylation of S-warfarin was almost unaffected by MTZ at the corresponding concentrations. In addition, 3-d repeated oral administration of MTZ (200 mg/kg/d) to rats did not show any significant effects on the hepatic mRNA levels of various CYP isozymes and CYP2C protein levels. These results suggest that the reported interaction of oral MTZ and S-warfarin was not due to CYP2C9 inhibition and that drug interactions via inhibition of CYP2C9 is unlikely to occur when MTZ ointment is applied to ulcerous skin. This information should be valuable for assessing the safety of MTZ ointment used for mitigating cancerous malodor.

Key words metronidazole; cytochrome P450; drug interaction; cancerous malodor

Invasion and metastasis of malignant tumors to the skin (continuous, hematogenous, lymphogenous metastasis to the skin), complicated by the infection of ulcerated skin cancer tissue, results in the emission of an intense malodor. 1–3 Malodor causes discomfort to the patient, of course, but also to people in the vicinity of the patient, such as family members and healthcare workers, and can make it difficult to maintain human relationships, damage the self-esteem of the patient, and cause a decrease in quality of life. Volatile sulfides and volatile fatty acids produced by anaerobic bacteria (such as Bacteroides fragilis) that have infected sites of ulceration are suggested to contribute to malodor. 1–5 The application of topical metronidazole (MTZ) preparations has been reported to be effective against cancerous malodor, 2,3,5 but because topical MTZ preparations are not sold in Japan, many hospitals prepare these agents in-house. A survey of the literature conducted by the Japanese Society of Hospital Pharmacists7 in 2010 reported that topical MTZ preparations were prepared at standards between 0.75% and 1%, with an average one-time dose of 70 g. The plasma MTZ concentration of 32 ng/mL has been reported when 1 g of 1% MTZ gel was applied to the face once daily for 7 d. 9 Cancerous tissue with ulcer-like symptoms is likely to have a higher capacity to absorb drugs than normal skin, due to the fact that the protective mechanisms have been compromised. 9,10 However, the extent to which MTZ is absorbed when a topical preparation is applied to cancerous tissue with ulcer-like symptoms remains unclear.

Clinically, patients with cerebral hemorrhage and high prothrombin time values have been reported with concomitant use of MTZ and warfarin (WAR). 11 In addition, while an increase in blood concentrations of the S-enantiomer of WAR (S-WAR) reportedly occurs with concomitant use of MTZ and S-WAR, concomitant use of MTZ and the R-enantiomer of WAR (R-WAR) has no effect on R-WAR blood concentrations. 12 A similar interaction has also been observed in a rat in vivo study 13 and the involvement of inhibition of S-WAR metabolism by MTZ has been suggested, 13,14 but the mechanism of this interaction has yet to be clarified in detail. WAR, provided as a racemic mixture of the S-WAR and R-WAR enantiomers, undergoes stereoselective and regioselective hydroxylation by cytochrome P450 (CYP) in the liver 15 with little contribution of renal clearance (urinary excretion <2%). While S-WAR is predominantly metabolized by CYP2C9 into S-7-hydroxy WAR and S-6-hydroxy WAR, R-WAR is metabolized into R-6-, 8- and 10-hydroxy WAR by CYP1A2 and CYP3A4. CYP2C8, CYP2C18 and CYP2C19 are also involved in the metabolism of R-WAR. 17–20 Therefore, it is possible that the above interaction was caused by inhibition of CYP2C9-mediated 7- and 6-hydroxylation of S-WAR by MTZ.

Although MTZ has long been used in clinical practice, few studies have investigated the drug interactions involving MTZ, with some controversial findings. 21–24 In the present study, both clinical and basic researches were conducted with the objective of investigating the safety of topical MTZ preparation from a pharmacokinetic perspective. As a case study, a breast cancer patient with cancerous malodor using MTZ ointment was surveyed with regard to deodorizing effects and adverse events, and MTZ concentrations in the plasma were monitored. Also, with the objective of elucidating the effects of MTZ on the expression and activity of various
Materials and Methods

**Patient** Background: Female; age, 55 years; height, 159 cm; weight, 49 kg; and body surface area, 1.48 m².

Diagnosis: Invasive ductal breast cancer, T4bN3M0 Stage IIIC.

History of Present Illness: The patient became aware of a mass in the right breast in January 2011, but left it untreated. Peripapillary edema and induration gradually expanded, to the point that discharge appeared, and the patient consulted a local physician in March. The patient was referred to this hospital on suspicion of breast cancer. Based on extensive examinations, cancer of both left and right breasts was diagnosed.

Prior History: Nothing of note.

Physical Findings: Right side: Clinical breast examination revealed a 10×10 cm enlargement of the breast overall, and a 4.3×4.3 cm cutaneous erosion centered on the nipple. Several of the size of the forefinger were also palpable among the axillary lymph nodes.

Histopathological Findings at Diagnosis: Invasive ductal carcinoma, solid-tubular>scirrhous carcinoma, nuclear grade 3 (A3+B3), with intraductal component, papillary, intermediate grade, estrogen receptor (+), progesterone receptor (−), human epidermal growth factor receptor type 2 (3+).

Course of Treatment: Preoperative chemotherapy [FEC100 (epirubicin 100 mg/m², cyclophosphamide 500 mg/m², fluorouracil 500 mg/m²)] was started in April 2011. At an outpatient examination on day 8 of chemotherapy, pus was observed from the papillary cutaneous erosion site, and the patient complained of malodor. Administration of an MTZ ointment prepared at the hospital began. The patient was instructed to apply MTZ ointment to gauze (where it contacts the skin), which was used to absorb seepage from the cutaneous erosion site, so that the affected area was covered. The gauze to which the MTZ ointment was applied was to be replaced at appropriate times, such as when seepage absorbed in the gauze became a concern or when bathing. The patient was given a questionnaire and was asked to record the following information: number of times and dose of MTZ ointment used each day; pathological condition such as odor and seepage; and presence or absence as well as changes (improved, unchanged, worse) in the conditions of symptoms of adverse drug reactions, such as pain or itching. With an emphasis on minimizing the burden on the patient, blood samples were collected using a Terumo vacuum blood collection tube containing a serum separating agent. After centrifugation, the plasma was stored frozen at −30°C until drug concentrations were assayed. This study was approved by the ethics review boards at Musashino University and Saiseiki Yokohamashi Nanbu Hospital.

**Materials** MTZ tablet (Flagyl® 250mg) was purchased from SHIONOGI Co., Ltd. (Osaka, Japan). Hydrophilic ointment was purchased from Maruishi Pharmaceutical Co., Ltd. (Osaka, Japan). MTZ and 7-hydroxycoumarin were purchased from Wako Pure Chemical Industries, Ltd. (Osaka, Japan). Sulfaphenazole and rabbit anti-rat glyceraldehyde 3-phosphate dehydrogenase (GAPDH) antibody (Lot. 060M4775) were purchased from Sigma-Aldrich Japan Corp. (Tokyo, Japan). Rabbit anti-rat CYP2C11 antibody (Lot. 110608) and Goat anti-rat immunoglobulin G (IgG)-horse-radish peroxidase (HRP) antibody were purchased from Nason Corp. (Kanagawa, Japan). S-WAR, S-7-hydroxy WAR and S-6-hydroxy WAR were purchased from Cayman Chemical Company (Ann Arbor, MI, U.S.A.). Immobilon-P (PVDF membrane) was purchased from Millipore (Billerca, MA, U.S.A.). High Capacity cDNA Reverse Transcription Kit (Lot. 0909069) and RNase Inhibitor were purchased from Applied Biosystems (Carlsbad, CA, U.S.A.). THUNDERBIRD SYBR® qPCR Mix was purchased from TOYOBO Co., Ltd. (Osaka, Japan). Various primers were purchased from Invitrogen (Tokyo, Japan). HLM 50-Donor Pool (Lot No. 88114) and NADPH regeneration systems were purchased from BD Gentest (Tokyo, Japan). With regard to other reagents, commercially available products of the highest grade were purchased.

Preparation of MTZ Ointment MTZ ointment was prepared at Saiseikai Yokohamashi Nanbu Hospital as follows: 20 MTZ tablets, containing 5g MTZ in total, were well milled, sieved, and mixed with 3mL propylene glycol, after which 485g hydrophilic ointment was gradually added and mixed to give 500g of 1% MTZ ointment. The prepared ointment was stored in 100mL containers at room temperature. The homogeneity of the prepared ointment was confirmed by measuring MTZ content in 30mg each of 3 samples taken from one container (data not shown).

Determination of Plasma Concentration of MTZ To 100μL of plasma was added 200μL of acetonitrile (containing 5μg/mL tinidazole as internal standard), vortexed for 5 min, and centrifuged (15000×g, 25°C, 8 min) for deproteinization. Two hundred and fifty microliter of supernatant was collected and dried with nitrogen gas, after which the residue was dissolved in mobile phase, and MTZ was assayed by HPLC-UV.

HPLC The HPLC apparatus consisted of the LC-20AB prominence LIQUID CHROMATOGRAPH (Shimadzu, Kyoto, Japan), SPD-20AV prominence UV/VIS DETECTOR (Shimadzu), SCL-10A VP SYSTEM CONTROLLER (Shimadzu), DGU-20A3 prominence DEGASSER (Shimadzu), SIL-10A AUTO INJECTOR (Shimadzu), CTO-10AS VP COLUMN OVEN (Shimadzu) and measured data were recorded and analyzed using the analysis software Chromato-PRO (Run Time Instruments, Kanagawa, Japan). Inertsil C18 ODS-3 was used as a column (mean particle size: 5μm, 4.6×150mm, GL Sciences Inc., Tokyo, Japan). As a mobile phase, acetonitrile:10mM potassium phosphate buffer (pH 7.4) (3:7) was used. Flow rate was 1.5mL/min, column temperature was 40°C and detection wavelength was 318nm. The detection limit for MTZ was 0.2μg/mL, and the retention time for MTZ and tinidazole was 1.73 min and 2.44 min, respectively.

Effects of MTZ Treatment on the Expression of Metabolic Enzymes in the Liver
Animals Ten-week-old male Sprague-Dawley (SD) rats were purchased from Tokyo Laboratory Animals Science Co., Ltd. (Wakayama, Japan). Rats were housed in a controlled environment at a temperature of 23.1 ± 1°C, humidity of 51.2 ± 3% and under a 12-h light–dark cycle (light: 08:00 to 20:00). Rats were provided food and water ad libitum. Rats were given oral administration of 50 mg/kg MTZ (low dosage group), 200 mg MTZ (high dosage group) or vehicle (corn oil: control group) once a day for 3 d. At 1 h after last administration, laparotomy was performed under ether anesthesia, blood was collected from the abdominal vena cava in a heparinized syringe, and the liver was resected. Blood samples were centrifuged to obtain plasma and stored at −80°C until MTZ concentration was assayed. Excised livers were washed with ice cold saline, frozen in liquid nitrogen and stored at −80°C. All animal experiments were approved and carried out following the Guideline for Animal Experimentation of Musashino University.

Determination of MTZ Concentration in the Rat Plasma The concentration of MTZ in the rat plasma was measured as described above for that in the patient’s plasma.

Real-Time Reverse Transcription-Polymerase Chain Reaction (RT-PCR) RNA was extracted from about 20 mg of frozen liver using the illustra RNAspin Mini RNA Isolation Kit according to the protocol for the above kit. The resulting solution was diluted 50-fold using Tris ethylenediaminetetraacetic acid (EDTA) buffer (10 mM Tris–HCl, 1 mM EDTA, pH 8.0, TE buffer), and purity was confirmed and RNA concentration (µg/ml) was calculated by measuring absorbance at 260 and 280 nm using a spectrophotometer (Pharmaspec UV-1700, Shimadzu). A high-capacity cDNA synthesis kit was used to synthesize cDNA from 1 µg of RNA according to the protocol for the above kit. TE buffer was used to dilute the cDNA 10-fold to prepare cDNA TE buffer solution. The expression of each gene was detected by preparing primers listed in Table 1 and performing real-time PCR.

To each well of a MicroAmp® Fast 96-Well Reaction Plate (Applied Biosystems), 5 µL of THUNDERBIRD SYBR® qPCR Mix, 0.5 µL of forward primer for target gene (300 nM), 0.5 µL of reverse primer (300 nM), 4 µL of cDNA TE buffer solution and 0.02 µL of 50×ROX reference dye were added. Denaturation temperature was set at 95°C for 3 s, annealing temperature at 56°C for 5 s and elongation temperature at 72°C for 30 s. The fluorescence intensity of the amplification process was monitored using the Applied Biosystems 7500 Fast Real-Time PCR System (Applied Biosystems). As target genes, major CYP isozymes (CYP1A1, CYP1A2, CYP1B1, CYP2C6, CYP2C11, CYP2D1, CYP2E1, CYP3A1 and CYP3A2) were selected. The fold change in mRNA levels of CYP isozymes upon MTZ treatment was determined by normalizing the gene expression levels by those of β-actin (2^ΔΔCt method). The average value in control rats was set at 100%.

Western Blotting About 300 mg of frozen liver was homogenized using homogenize buffer (0.1 M Tris–HCl, 1 M KCl, 1 mM EDTA, pH 7.4). The resulting suspension was centrifuged for 20 min at 9000×g, and the supernatant was centrifuged for 60 min at 105000×g. The precipitate (microsomal protein) was resuspended using Tris–Glycerol–EDTA buffer (10 mM Tris–HCl, 20% glycerol, 0.1 M KCl, 1 mM EDTA, pH 7.4). All procedures were carried out at 4°C. Protein concentrations were measured by the Lowry method using bovine serum albumin as a standard. Electrophoresis was performed by the Laemmli’s method. One microgram of microsomal protein was diluted 2-fold using the loading buffer (84 mM Tris, 20% glycerol, 0.004% bromophenol blue, 4.6% sodium dodecyl sulfate (SDS) and 10% 2-mercaptoethanol, pH 6.3), boiled for 5 min and applied to 7.5% polyacrylamide gel. After electrophoresis, the isolated proteins were transferred to a polyvinylidene difluoride (PVDF) membrane using trans-blot SD Cell (Bio-Rad Laboratories, Inc., Hercules, CA, U.S.A.). After blocking for 60 min using 2% skim milk, the resulting membrane was reacted for 60 min at room temperature with primary antibody (rabbit anti-rat CYP2C11 antibody and rabbit anti-rat GAPDH antibody were diluted 10000-fold and 2500-fold, respectively). After washing the membrane using TBS-TWEEN (20 mM Tris–HCl, 137 mM NaCl and 0.1% Tween 20, pH 7.6), the resulting membrane was reacted for 60 min at room temperature with secondary antibody (anti-rat IgG-HRP antibody was diluted 10000-fold). After washing the membrane, the membrane was reacted with the ECLprime detection reagent and visualized with LAS-3000 (FUJIFILM, Tokyo, Japan), a luminoimage analyzer. Band density, in the linear response range, for CYP2C and GAPDH were analyzed, with the average values in control rats set at 100%.

Effects of MTZ on the S-WAR Metabolism by HLM After preincubation for 5 min at 37°C, 50 µL of S-WAR solution (final concentration: 0–10 µM for S-WAR, 0.2% for DMSO), 44 µL of HLM suspension (final concentration: 1.0 mg/mL) and 6 µL of nicotinamide adenine dinucleotide phosphate (NADPH) regeneration system solution were placed in a tube and incubated for 30 min at 37°C. The reaction

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was stopped by mixing with 200 µL of ice-cold acetonitrile (containing 60 µM 7-hydroxycoumarin as internal standard). Centrifugation was then performed at 12000×g for 15 min at 4°C, and 5 µL of the supernatant was injected to LC-MS/MS to quantify 7-hydroxy WAR and 6-hydroxy WAR. The initial linear rate conditions have been confirmed with regards to microsomal protein concentration (up to 1.0 mg/mL) and incubation time (up to 30 min). The relationship between S-WAR concentration and respective metabolic rate was fitted to the Michaelis–Menten equation using the nonlinear least-squares regression program MULTI [30] in order to estimate kinetic parameters (maximum velocity: \( V_{\text{max}} \) and Michaelis constant: \( K_m \)).

For the inhibition study, the final S-WAR concentration was set at 5 µM and the incubation in the above procedure was carried out in the presence and absence of MTZ (final concentration: 10–6000 µM) or sulfaphenazole (final concentration: 10 µM) as a positive control.

**LC-MS/MS** The LC-MS/MS apparatus consisted of the Accela LC System (Thermo Fisher Scientific Inc., Waltham, MA, U.S.A.) and LTQ Orbitrap XL with eTd (Thermo Fisher Scientific Inc.), and measured data were recorded and analyzed using the analysis software Xcalibur (Thermo Fisher Scientific Inc.). Inertsil C18 ODS-3 was used as a column (mean particle size: 5 µm, 4.6×150 mm, GL Sciences Inc.). The mobile phase consisted of distilled water (0.1% acetic acid) (A) and methanol (0.1% acetic acid) (B) and a linear gradient elution profile was used as follows: 50% B for 0–1 min, 50–90% B for 1–11 min, 90% B for 11–18 min, 90–50% B for 18–18.01 min, and finally 7 min equilibration before the injection of the next sample. Flow rate was 400 µL/min and column temperature was 40°C. The mass spectrometer was operated in positive electrospray ionization (ESI) mode, and the ion transitions monitored were \( m/z \) 309 to \( m/z \) 163 for both 6-hydroxy WAR and 7-hydroxy WAR and \( m/z \) 163 to \( m/z \) 107 for 7-hydroxycoumarin. The detection limit for both 6-hydroxy WAR and 7-hydroxy WAR was 1.6 nM, and the retention time for 6-hydroxy WAR, 7-hydroxy WAR and 7-hydroxycoumarin were 13.4 min, 13.9 min and 8.6 min, respectively.

**Statistical Analysis** Values were expressed as means±standard deviation (S.D.) unless otherwise stated. The significance of differences was assessed by Student’s t-test and the differences were considered to be significant when p values were calculated to be below 0.05.

**RESULTS**

**Patient** Number of times and doses of MTZ ointment used per day: MTZ ointment was used continuously for 56d. The ointment was used 1–3 times/d, for averages of 1.7 times/d during week 1, 2.0 times/d during week 2, 1.4 times/d during weeks 3–6, and 1.0 times/d during weeks 7 and 8, with usage decreasing over time. The average dose inferred from patient interviews was 4.5 g/d during week 1, 1.5 g/d for week 2 and 1.0 g/d for weeks 3–8.

Improvement of subjective symptoms: Seepage and subjective symptoms of odor were not observed after 2d of MTZ ointment use. Although subsequent seepage was observed on days 13, 14, 20, 34, and 35, the subjective symptom of odor was not observed.

**Plasma Concentration of MTZ in the Patient** 4.75 µg/mL (27.8 µM) of MTZ was detected in plasma taken from the patient on day 8. The plasma concentration of MTZ was below the detection limit on days 15, 16, and 57.

**Effects of MTZ Treatment on the Expression of Metabolic Enzymes in the Rat Liver**

**Plasma Concentration of MTZ in Rats** The plasma concentration of MTZ was 13.6±3.5 µg/mL (79.3±20.7 µM) and 59.9±15.6 µg/mL (350±91 µM) in the rats (n=5 each) given oral administration of 50 mg/kg and 200 mg/kg MTZ once a day for 3d, respectively.

**mRNA Expression of CYP Isozymes in the Liver** Figure 1 shows the mRNA expression of each CYP isozyme in
livers excised from rats given oral administration of MTZ or vehicle once a day for 3d, and Western blotting was performed as described in Materials and Methods. The fold change in protein expression of CYP2C upon MTZ treatment was determined by normalizing the band densities by those of GAPDH, and the average value in control rats was set at 100% (Mean±S.D., n=5 (control and 50 mg/kg MTZ) or 6 (200 mg/kg MTZ)).

**Protein Expression of CYP2C in the Liver**  
Figure 2 shows the protein expression of CYP2C in livers excised from rats given oral administration of 50 mg/kg MTZ (A; gray column), 200 mg/kg MTZ (B; closed column) or vehicle (A and B; open column) once a day on 3d, and Western blotting was performed as described in Materials and Methods. The fold change in protein expression of CYP2C upon MTZ treatment was determined by normalizing the band densities by those of GAPDH, and the average value in control rats was set at 100% (Mean±S.D., n=5 (control and 50 mg/kg MTZ) or 6 (200 mg/kg MTZ)).

**Effects of MTZ on the S-WAR Metabolism by HLM**  
Figure 3 shows the metabolic activities of S-WAR 6-hydroxylation and 7-hydroxylation by HLM. The Km and Vmax values were estimated to be 6.4±1.6 µM and 2.9±0.4 pmol/min/mg protein, respectively, for 6-hydroxylation, and 4.8±1.1 µM and 11.3±1.2 pmol/min/mg protein, respectively, for 7-hydroxylation (expressed as fitted value ±S.D.). The CLint values for 6-hydroxylation and 7-hydroxylation were calculated as 0.45 µL/min/mg protein and 2.4 µL/min/mg protein, respectively.

Figure 4 shows the metabolic activities for 6-hydroxylation and 7-hydroxylation of S-WAR (5 µM) by HLM in the absence or presence of inhibitors. Both activities were strongly inhibited by 10 µM sulfaphenazole. On the other hand, MTZ showed weak inhibitory effects against both hydroxylation pathways of S-WAR, with only 30% inhibition observed at the highest concentration of 6000 µM.
DISCUSSION

In the present study, plasma concentration of MTZ was measured in a breast cancer patient using MTZ ointment for cancerous malodor and basic research was also conducted with the objective of investigating the safety of topical MTZ from a pharmacokinetic perspective.

The patient cooperating in the study used an average of 4.5 g/d of MTZ ointment up until day 7 after the start of use, and 4.75 µg/mL (27.8 µM) of MTZ was detected in a plasma sample on day 8. Plasma concentration of 32 ng/mL has been reported when 1 g of 1% MTZ gel was applied to the face once daily. Assuming a linear drug disposition, if 4.5 g/d is applied, the plasma concentration would be calculated as 144 ng/mL, but the plasma concentration observed in this patient was 30-fold higher. This higher plasma concentration was presumably due to the fact that skin barrier function of the stratum corneum does not function as well in ulcerous skin as in normal skin, and absorption via blood vessels near the epidermis was promoted by the invasion and metastasis of the cancer. Assuming a similar extent of absorption as observed in this study, plasma MTZ concentration after application of 70 g (reported as the average daily dose) of MTZ ointment was calculated as 74 µg/mL, which is about 4 times higher than the maximum plasma concentration (about 20 µg/mL) occurring with repeated oral administration of 1 MTZ tablet (250 mg) twice daily for 7 d. Furthermore, MTZ ointment was used continuously for only 56 d on the patient presented in this study because the skin ulcer site showed improvement from the antitumor effects of chemotherapy. However, when used for the purpose of eliminating or mitigating cancerous malodor occurring with the invasion and metastasis of malignant tumors to the skin, MTZ ointment is used for a longer period of time, and the amount used tends to increase over time, which could result in even higher concentration of MTZ in plasma.

With the objective of clarifying the possibility of drug interactions caused by MTZ that has been absorbed into the blood, we investigated whether MTZ has any effects on the expression or activity of various CYP isozymes in the liver. Rats were given oral administration of MTZ once a day for 3 d and the average plasma MTZ concentration at 1 h after the last dose was measured as 13.6 µg/mL and 59.9 µg/mL (for the dose of 50, 200 mg/kg, respectively) which are comparable with that in the present patient (4.75 µg/mL) or reported maximum concentration after repeated oral administration of MTZ in humans (about 20 µg/mL). The mRNA levels of various CYP isozymes and CYP2C protein levels, analyzed by realtime RT-PCR and Western blotting, respectively, showed no significant difference between control group and both MTZ-treated groups (Figs. 1, 2). Although CYP2C9, the primary enzyme that metabolizes S-WAR in humans, is not present in rats, rat CYP2C6 reportedly corresponds to CYP2C9 in humans based on the following findings: 1) With regard to metabolic activity, human CYP2C9 and rat CYP2C6 both catalyze S-WAR 7-hydroxylation, diclofenac 4-hydroxylation and phenytoin 7-hydroxylation, and are selectively inhibited by sulphinphazole. 2) With regard to expression, both human CYP2C9 and rat CYP2C6 are known to be induced by phenobarbital. The nuclear receptors pregnane X receptor (PXR), constitutive androstane receptor (CAR), and glucocorticoid receptor (GR) play an important role in regulating the expression of human CYP2C9. The mechanism by which the expression of rat CYP2C6 is regulated remains unclear, but it has been suggested that steroid receptors are involved, and induction of CYP2C6 by phenobarbital is reportedly inhibited by RU486, a glucocorticoid-progesterone antagonist. Based on these similarities in the regulation mechanism of CYP2C expression in humans and rats, the results of this study suggesting that the interaction between MTZ and S-WAR in rats is not due to changes in CYP2C expression may also be clinically applicable.

The CLint values for S-WAR 6-hydroxylation and 7-hydroxylation obtained in the present HLM study were within the range of reported values (0.014–12 µL/min/mg protein and 0.26–33 µL/min/mg protein, respectively) which were also estimated by in vitro studies using HLM. In an S-WAR metabolic inhibition study using HLM, S-WAR metabolism was only inhibited about 30% when MTZ was added at a high concentration (6000 µM). As discussed above, with repeated oral administration of MTZ in clinical use, the maximum plasma concentration is reportedly about 120 µM. Considering the reported plasma protein binding of about 10%, the maximum unbound concentration of MTZ is calculated to be about 110 µM. This suggests that the clinical interaction between S-WAR and MTZ is not based on the inhibition of CYP2C9 activity by MTZ. Furthermore, the present findings also demonstrate that metabolic activity of CYP2C9 is unlikely to be inhibited even if relatively large amount of MTZ ointment is applied to ulcerous skin.

The findings in this study suggest that the clinical drug–drug interactions between MTZ and S-WAR is unlikely because MTZ did not inhibit S-WAR metabolism by HLM and did not change the levels of major CYP isozymes in the rat liver, both at clinically relevant concentrations. In the future, in addition to investigating the effects and adverse drug reactions of MTZ in clinical use, we would also like to provide information on the efficacy and safety of MTZ ointment with the objective of mitigating cancerous malodor by conducting investigations of the effects of MTZ on CYP expression and activity using other systems such as human hepatocytes.

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