QUANTITATIVE PREDICTION OF THE INTERACTION OF MIDAZOLAM AND HISTAMINE H₂ RECEPTOR ANTAGONISTS IN RATS

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(Received July 24, 1997; accepted December 17, 1997)

This paper is available online at http://www.dmd.org

ABSTRACT:

To quantitatively evaluate drug-drug interactions involving metabolic processes in the liver, we attempted to predict the increasing ratio of the plasma concentration of midazolam (MDZ) in the presence of cimetidine (CIM) or nizatidine (NZD) in rats. Under steady-state conditions for the plasma concentration of CIM or NZD, MDZ was administered through the portal vein. The AUC of MDZ in the presence of CIM was 2.5-fold higher than that in the absence of CIM. There was no effect of NZD on the AUC of MDZ. The liver/plasma concentration ratios for CIM and NZD were 4.0 and 2.7, respectively. The estimated liver unbound concentration (CH₄/f) plasma unbound concentration (Cp,f) ratios for CIM and NZD were 1.9 and 2.4, respectively, suggesting concentric hepatic accumulation of both drugs. The oxidative metabolism of MDZ in rat liver microsomes was competitively inhibited by CIM or NZD, and the Kᵣ values of CIM and NZD were 110 and 2600 μM, respectively. Based on these data obtained in vivo and in vitro, the increasing ratios for MDZ in the presence of CIM or NZD were predicted using the equations Rₚ = 1 + Cp,f/Kᵣ and Rₕ = 1 + CH₄,f/Kᵣ. The observed increasing ratios in the presence of CIM were very close to Rₕ, compared with Rₚ. However, Cₚ,f and Cₕ,f were much less than Kᵣ and there was no difference between Rₚ and Rₕ in the presence of NZD. Consequently, Cₚ,f and Cₕ,f were greater than or equal to Kᵣ and Cₚ,f was not equal to Cₕ,f, as in the presence of CIM, and it was indicated that Cₕ,f was more suitable for quantitatively predicting the drug-drug interactions than was Cₚ,f.

In drug-drug interactions based on pharmacokinetics, inhibition of hepatic metabolism is one of the most important events and induces serious side effects. There have been many reports concerning the induction of various side effects and toxicities caused by azole anti-fungal agent-induced inhibition of drug metabolism (Baldwin et al., 1995; Neuvonen and Suhonen, 1995; Jurima-Romet et al., 1994). Furthermore, H₂ receptor antagonists (Pasanen et al., 1986; Knodell et al., 1991; Wright, 1991), new quinoline antibacterial agents (Thomsen et al., 1987; Wijnands et al., 1986; Beckmann et al., 1987), and macrolide antibiotic drugs (Warot et al., 1987; Gascon and Dayer, 1991) have also functioned as inhibitors of oxidative metabolism in the liver. Most of the enzymes related to drug-drug interactions of these drugs are CYPs. The CYP superfamily is composed of families, subfamilies, and individual isoforms (Nelson et al., 1996). It is possible to qualitatively predict drug-drug interactions if the isoform related to the metabolism of each drug is identified. Moreover, we can quantitatively predict the extent of drug interactions if the metabolic Kᵣ values of drugs are determined in liver microsomes and/or CYP-expressing cells. However, it is difficult to precisely predict the increasing ratio of the plasma concentrations of the interacting drug from this information alone. Therefore, we must solve the following problems (Sawada et al., 1996; Sawada and Iga, 1996; Sugiyama and Iwatsubo, 1996): 1) prediction of the hepatic disposition of inhibitors, 2) prediction of the concentrations of inhibitors in the hepatic vein after oral administration, and 3) prediction of drug-drug interactions in the gastrointestinal tract (Wacher et al., 1996). In this study, focusing on the first point, we attempted to predict the increasing ratio of the plasma concentrations based on in vivo and in vitro experiments in rats. To exclude the second and third points, the inhibitors were maintained at steady-state concentrations by combinations of iv administration and constant infusion, and intraportal administration of the interacting drugs was carried out. MDZ, an hypnotic drug, was used as a substrate and CIM and NZD, H₂ receptor antagonists, were used as inhibitors.

Materials and Methods

Materials. CIM was obtained from SmithKline Beacham Co. (Tokyo, Japan) or was purchased from Sigma Chemical Co. (St. Louis, MO). NZD and MDZ were obtained from Zeria Pharmaceutical Co. (Tokyo, Japan) and Yamanouchi Pharmaceutical Co. (Tokyo, Japan), respectively. Tetramethyl- ammonium chloride was purchased from Nacalai Tesque (Kyoto, Japan). RAN and FAM were supplied from Glaxo Pharmaceutical Japan (Tokyo, Japan) and Yamanouchi Pharmaceutical Co. (Tokyo, Japan), respectively. All other chemicals used as reagents were of HPLC grade.

Animals. Wistar male rats (7–8 weeks of age) were purchased from Kuroda Experimental Animals Co. (Kumamoto, Japan) and Seac Yoshitomi (Fukuoka, Japan). Food and water were supplied ad libitum. The rats were maintained at a suitable temperature.
Preparation of Drug Solutions. The solution of CIM for bolus administration and constant infusion was prepared by dissolving CIM in 1 N HCl and was neutralized using 1 N NaOH. The total volume of CIM solution was adjusted to 3.5 ml by the addition of saline. The solution of MDZ used for intraportal administration was an MDZ injection solution (Dormicam Inj. Nippon Roche K. K., Tokyo, Japan) 10 mg/2 ml). The solution of MDZ used for the experiments on metabolism inhibition was the Dormicam Inj. solution diluted with purified water.

Plasma and Liver Concentration Profiles for CIM and NZD. Rats anesthetized with ether were cannulated in the femoral artery and femoral vein. To determine the infusion rates for CIM and NZD, these drugs were administered through the femoral vein at a dose of 30 mg/kg. Blood samples were collected at 5, 10, 15, 20, 25, 30, 45, 60, 120, 180, and 240 min after administration. The blood was centrifuged at 12,000 rpm for 5 min to obtain the plasma. Plasma concentrations of the drugs were measured using HPLC. Pharmacokinetic parameters were calculated using the nonlinear least-squares method (MULTI program) (Yamaoka et al., 1981). Furthermore, both bolus injection and constant infusion were used to obtain steady-state concentrations of both drugs. Under ether anesthesia, the rats were cannulated in the femoral artery and femoral vein. After the rats had recovered from anesthesia, CIM was administered through the femoral vein at a dose of 3, 5, or 9 mg/rat and was then infused into the rats at one of three constant rates (K_P = 1.4, 2.8, or 5.7 mg/hr/rat), using a syringe infusion pump (model 11; Harvard Apparatus, S. Natick, MA), for 6 hr. NZD was administered through the femoral vein at a dose of 5, 7, or 10 mg/rat and was infused at one of three constant rates (K_P = 2.5, 5.1, or 1.14 mg/hr/rat) for 6 hr. Blood samples were collected at 0, 15, 30, 60, 120, 180, and 240 min after the beginning of the infusion, and the liver was removed at the final sampling time. Blood was centrifuged at 12,000 rpm for 5 min to obtain the plasma. Plasma and liver samples were stored at −20°C, and the drug concentrations in the samples were measured using HPLC.

Effects of CIM or NZD on Plasma Concentration Profiles for MDZ. Under ether anesthesia, the rats were cannulated in the femoral artery, femoral vein, and portal vein. After the rats had recovered from anesthesia, CIM was administered through the femoral vein at a dose of 9 mg/rat and was then infused into the rats at a constant rate of 5.7 mg/hr/rat, using a syringe infusion pump (model 11; Harvard Apparatus). NZD was administered through the femoral vein at a dose of 10 mg/rat and was infused at a constant rate of 11.4 mg/hr/rat. For treatment with both drugs, MDZ was administered through the portal vein at a dose of 10 mg/kg at 100 min after the beginning of infusion. Blood samples were collected at 0, 2, 5, 10, 30, 60, 120, and 180 min after administration of MDZ. Blood was centrifuged at 12,000 rpm for 5 min to obtain the plasma. Plasma and liver samples were stored at −20°C, and the drug concentrations in the samples were measured using HPLC (Mandema et al., 1991).

Plasma Protein and Liver Tissue Binding Assays for CIM and NZD. The plasma protein binding of CIM and NZD was evaluated by using equilibrium dialysis. In the present study, we used 0.1 M phosphate buffer at pH 7.0 because the intracellular space is slightly acidic, compared with the pH value for blood (pH 7.4). In brief, 0.5 ml of plasma containing 30, 60, or 60 µM CIM or NZD and 0.1 M phosphate buffer (pH 7.0) containing no drugs were added to the dialysis board set on the dialysis membrane (Spectra/Por membrane; Spectrum Medical Industries, CA) and were incubated at 37°C for 6 hr. After the incubation, 0.1-ml samples were collected from both sides. Two milliliters of liver homogenate containing the drug and 0.1 M phosphate buffer (pH 7.0) containing no drugs were added to the dialysis board set on the dialysis membrane and were incubated at 37°C for 6 hr. After the incubation, a 0.5-ml sample of the homogenate and a 0.1-ml sample of the plasma were collected from the two sides. Plasma and liver homogenate concentrations of the drugs were measured using HPLC. The equations used for the calculation of f_p and f_H are as follows,

\[ f_p = C_p / (C_p + C_o) \]  
\[ f_H = C_H / (C_H + (100/n)C_o) \]

where C_p is the unbound drug concentration on the buffer side, C_o is the total drug concentration on the protein side, C_H is the bound drug concentration (C_b = C_p - C_o), and n is the percentage of homogenate. It was confirmed in preliminary studies that the protein binding was constant for 5 hr after incubation and the volumes in the buffer and protein cells were scarcely different. Moreover, tissue degradation might not have occurred, because enzymes were removed from the tissue homogenates by overnight equilibrium dialysis at 4°C before the binding study. Indeed, the sum of the unchanged drug concentrations in the buffer cell and the protein cell, as measured by HPLC, was equal to the drug concentration used in the protein binding study. Thus, it was confirmed that tissue degradation did not occur.

Measurement of the Concentrations of CIM, NZD, and MDZ. For determination of the plasma concentration of CIM, 0.1 ml of plasma, 0.1 ml of 75 µM NZD as an internal standard, 0.1 ml of 5 N NaOH, and 5 ml of CH_2Cl_2 were mixed, shaken for 10 min, and then centrifuged at 2000 rpm for 10 min. After the upper aqueous phase had been removed, 4 ml of the organic phase was transferred to another tube and completely evaporated under nitrogen gas. The residue was dissolved in 0.4 ml of the mobile phase, and 0.02 ml was injected into the HPLC system. For determination of the liver concentration of CIM, 0.5 g of liver was homogenized with saline for 1 min, on ice. Then 0.1 ml of 75 µM NZD, 0.1 ml of 0.5 N NaOH, and 5 ml of CH_2Cl_2 were mixed, shaken for 10 min, and then centrifuged at 3000 rpm for 10 min. After the upper aqueous phase had been removed, 3 ml of organic phase was transferred to another tube and completely evaporated under nitrogen gas. The residue was dissolved in 0.4 ml of the mobile phase and 0.02 ml was injected into the HPLC system. As an internal standard, 75 µM CIM was used (Shimokawa et al., 1994).

For the quantitation of MDZ, 0.1 ml of plasma, 0.1 ml of MeOH, 0.5 ml of 1 N NaOH, and 3 ml of hexane were mixed, shaken for 5 min, and then centrifuged at 3000 rpm for 5 min. After the upper aqueous phase had been removed, 2 ml of the organic phase was transferred to another tube and completely evaporated under nitrogen gas. The residue was dissolved in 0.2 ml of the mobile phase and 0.075 ml was injected into the HPLC system. A liquid chromatography pump (Shimazu LC-10AD; Shimadzu, Kyoto, Japan) and a spectrophotometric detector (Shimadzu SPD-10A; Shimadzu), with an autosampler and column heater (U-620; Sugai), were used as the HPLC system for CIM and NZD. A liquid chromatography pump (Shimazu LC-9A; Shimadzu) and a spectrophotometric detector (Shimadzu SPD-10AV; Shimadzu) were used as the HPLC system for MDZ. For the quantitation of CIM and NZD, a Senshu Pak ODS-1251 column (250 mm × 4.6 mm i.d.; Senshu Sciences, Japan) and a Senshu Pak ODS-1031 guard column (30 mm × 4.6 mm i.d.; Senshu Sciences) were used. The mobile phase was water/acetonitrile (95:5, v/v) containing 5 mM NaH_2PO_4 and 5 mM tetramethylammonium chloride. The flow rate of the mobile phase was 1.5 ml/min. CIM and NZD were detected at 228 nm. The limit of the detection for the plasma concentrations was 0.05−0.1 mg/ml under these conditions. For the quantitation of MDZ, an Inertsil ODS column (5 µm, 250 mm × 4.6 mm i.d.; GL Sciences, Japan) and a YMC-Guardpack ODS-AM guard column (5 µm, 30 mm × 4.6 mm i.d., GL Sciences) were used. The mobile phase was acetonitrile/10 mM phosphate buffer (pH 6.5) (8:2, v/v). The flow rate of the mobile phase was 1.0 ml/min. MDZ was detected at 245 nm. The limit of detection for the plasma concentrations was 50 ng/ml under these conditions.

In Vitro Metabolism of MDZ. The concentrations of MDZ for in vitro metabolism experiments were 1, 2, 5, 10, 20, and 50 µM. After 0.32 ml of a reaction mixture containing 0.04 ml of microsomes and an NADP-generating system (0.02 ml of 100 mM glucose-6-phosphate, 0.02 ml of 20 mM NADP, 0.02 ml of 20 units/ml glucose-6-phosphate dehydrogenase, 0.004 ml of 10 mM EDTA, 0.016 ml of 125 mM MgCl_2, and 0.2 ml of 0.2 M Na_2HPO_4/KH_2PO_4 buffer, with 0.4 mg/ml microsomal protein) had been preincubated for 2 min, the reaction was started by addition of 0.04 ml of a CIM (0.3 M) or NZD, RAN, or FAM (1, 2, or 5 mM, respectively) solution and 0.04 ml of a MDZ solution. After incubation at 37°C for 5 min, the reactions were stopped by addition of 0.4 ml of acetonitrile and were centrifuged at 3000 rpm for 2 min. Then 0.4 ml of supernatant was removed to another tube, and the plasma concentration of MDZ was determined. The K_m and V_max values for MDZ and the K values for H_2 receptor blockers were calculated by using Lineaweaver-Burk plots. For the in vitro kinetic study, we confirmed that there was a good
linear relationship between incubation time and metabolite concentration until 5 min after incubation; thereafter, it showed nonlinearity.

**Analysis of Data.** As shown in Results, the \( K_M \) values for MDZ in the presence and absence of CIM and in the presence and absence of NZD were 30.3, 8.1, 9.6, and 5.9 \( \mu \text{M} \), respectively. The average \( C_{p,f} \) values for MDZ were 0.9, 0.3, 0.1, and 0.1 \( \mu \text{M} \), respectively. Thus, the increasing ratio of the plasma concentration of the inhibited drug (MDZ) can be estimated using the following equation (Sawada et al., 1996; Sawada and Iga, 1996; Sugiyama and Iwatsubo, 1996):

\[
R_H = \frac{C_p}{C_p} = \frac{\text{AUC}_f}{\text{AUC}_5} = 1 + \frac{C_{p,f}}{K_i} \tag{3}
\]

where \( C_p \) and AUC represent the blood concentration and the AUC in the absence of inhibitors (CIM or NZD), respectively. \( C_{p,f} \) and AUC\(_f\) represent values measured in the presence of inhibitors. \( C_{H,f} \) and \( K_i \) refer to \( H_2 \) blockers and \( H_2 \) blocker inhibition of MDZ metabolism, respectively. It is difficult to directly measure \( C_{H,f} \), so that value was calculated using the total liver concentration (\( C_H \)) and \( f_H \), as follows:

\[
C_{H,f} = C_H \times f_H \tag{4}
\]

As another analytical method, the increasing ratio of the plasma concentration can be estimated using \( C_{p,f} \) instead of \( C_{H,f} \), according to the following equation.

\[
R_p = \frac{C_p}{C_p} = \frac{\text{AUC}_f}{\text{AUC}_5} = 1 + \frac{C_{p,f}}{K_i} \tag{5}
\]

where \( C_p \) represents the total plasma concentration.

**Statistical Analysis.** The statistical analysis was performed by using the Student \( t \) test, and \( p = 0.05 \) was taken as the minimum level of significance. Data were expressed as mean \( \pm \) SD.

**Results**

**Plasma and Liver Concentration Profiles for CIM and NZD.** Plasma concentration profiles for CIM (fig. 1A) and NZD (fig. 1B) at three infusion rates are shown in fig. 1. The plasma concentrations reached steady state 2 hr after injection of the two drugs at any infusion rate, and the plasma concentrations at steady state were almost proportional to the infusion rates for both drugs. Fig. 2 shows the plasma concentration dependence of the \( K_{p,H} \) values for CIM (fig. 2A) and NZD (fig. 2B). The \( K_{p,H} \) values were within the range of 2–5 for CIM and 2–4 for NZD, regardless of the plasma concentration of the drugs.

**Effects of CIM and NZD on Plasma Concentrations of MDZ.** Fig. 3 shows the plasma concentration-time profile for MDZ after administration through the portal vein, in the presence or absence of
CIM (fig. 3A) or NZD (fig. 3B). Table 1 summarizes the kinetic parameters for the metabolism of MDZ in vivo.

The half-life for the β-phase of the plasma concentration-time profile and the AUC for MDZ in the presence of CIM were 1.6 ± 0.6 hr and 61.9 ± 3.7 µg/hr/liter, respectively; values in the absence of CIM were 1.1 ± 0.4 hr and 25.1 ± 6.0 µg/hr/liter, respectively. The AUC in the presence of CIM was significantly (2.5-fold) greater than that in the absence of CIM (p < 0.05). The half-life and the AUC were only 1.2-fold and 1.0-fold higher, respectively, in the presence of NZD than in the absence of NZD.

**f_p and f_H Values for CIM and NZD.** We measured the f_H values for CIM (36 µM) and NZD (36 µM) using liver homogenates. The f_H values for CIM and NZD were obtained by extrapolation of data from 5, 10, 20, and 25% homogenates to 100% homogenates, according to eq. 2, and examined the effect of the dilution ratio of the homogenate on the unbound fraction. The f_p values obtained for CIM and NZD were within the ranges of 0.4–0.5 and 0.7–0.8, respectively, suggesting weak binding of both drugs. Thus, a linear relationship between f_H and concentration was observed. Furthermore, the f_p values for CIM and NZD were 0.80 and 0.96, respectively, regardless of the drug concentration (data not shown).

**C_H/C_p Ratios.** Table 2 shows the liver concentration, plasma concentration, K_H/f_p, f_H and f_p for CIM and NZD, determined from the experiments described above, and the C_H/C_p ratios calculated using those values. The C_H/C_p ratios for CIM and NZD were 1.9 and 2.4, respectively, and were greater than 1.

**Inhibitory Effects of Histamine H2 Receptor Antagonists on the Metabolism of MDZ.** Fig. 4 shows the inhibitory effects of CIM, NZD, FAM, and RAN on the metabolism of MDZ by rat liver microsomes, as analyzed using Lineweaver-Burk plots. In the presence or absence of CIM, NZD, FAM, or RAN, the metabolism of MDZ showed only one pattern; metabolism showed competitive inhibition by all of the histamine H2 receptor antagonists. The calculated K_M and V_max values were 12.75 ± 11.79 µM and 3.15 ± 1.0 nmol/min/mg protein, respectively. The K_j values for CIM, NZD, FAM, and RAN were 108, 2562, 9380, and 6814 µM, respectively.

**Comparison Between Predicted and Observed Values for the Increasing Ratio of the Plasma Concentration of MDZ.** Table 3 shows the predicted increasing ratio of the plasma concentration of MDZ (R_p) in the presence of CIM or NZD, calculated according to eqs. 3 and 5, and the observed R value (AUC/AUC). The increasing ratio (R_p) calculated from C_p using eq. 5 was underestimated, compared with the observed increasing ratio (R). However, the increasing ratio (R_p) calculated from C_H using eq. 3 was very close to the observed value.

**Discussion**

There has been little attempt to precisely predict the increasing ratios of blood concentrations of interacting drugs. For complete prediction, the following problems must be solved (Sawada et al., 1996; Sawada and Iga, 1996; Sugiyama and Iwatsubo, 1996): 1) prediction of the disposition of inhibitors in the liver, 2) prediction of the concentrations of inhibitors in the hepatic vein after oral administration, and 3) prediction of drug-drug interactions in the gastrointestinal tract.
intestinal tract. The first point is especially important because many drugs are concentratively taken up by liver cells (Yamazaki et al., 1996). To evaluate this point, von Moltke et al. (1996) estimated $K_{p,H}$ values using a mixture of plasma and liver homogenate in an in vitro study, and they attempted to predict the increasing ratios of the blood concentrations of the interacting drugs. However, they did not take into consideration the concentrative hepatic uptake of drugs through active transport. Nakamura et al. (1994) reported the concentrative hepatic uptake of H$_2$ receptor antagonists such as CIM and NZD, using isolated rat hepatocytes. From those findings, it has been concluded that, when histamine H$_2$ receptor antagonists are used as inhibitors of drug metabolism, $C_{H,f}$ but not $C_{p,f}$ is an important determinant (Sawada et al., 1996; Sawada and Iga, 1996; Sugiyama and Iwatsubo, 1996).

As shown in table 2, the predicted $C_{H,f}/C_{p,f}$ values for CIM and NZD were approximately 1.9 and 2.4, respectively, suggesting the existence of concentrative hepatic uptake of both drugs. In contrast, Nakamura et al. (1994) reported that the intracellular/medium concentration ratios for CIM and NZD were 7–10 and 1–3, respectively, as determined using isolated hepatocytes. Those values were higher than our results. One of the reasons for this discrepancy could be that the concentrative hepatic uptake was saturated because of the high concentrations of CIM and NZD in our experiments. Furthermore, heterogeneous distribution of inhibitors in the liver can be hypothesized. In this study, the $C_{p,f}$ for the cytosol may be underestimated if the inhibitors are not distributed to certain organelles in liver cells. However, we could not avoid this problem, because liver homogenates were used to determine the nonspecific binding of drugs to liver.
tissue in this study. This problem may be resolved in future studies by performing in vitro uptake experiments using ATP-depleted hepatocytes without an energy source for active transport of drugs (Sugiyama and Iwatsubo, 1996). As shown in table 3, the increasing ratio of the plasma concentration of MDZ estimated from the $C_{H,f}$ data was very close to the observed value, suggesting the suitability of the hypothesis in this analysis. It may possible to predict the extent of drug-drug interactions after iv administration to humans based on the pharmacokinetic parameters ($K_{m}, K_c$, and $V_{max}$) for inhibitors obtained using human liver microsomes and/or human CYP-expressing systems, the $f_{H,i}$ and $K_{p,H}$ values obtained using experimental animals such as rats, and the time profiles of the total plasma concentration and the unbound concentrations of inhibitors.

Many clinical cases and clinical tests of the interaction of MDZ and H$_2$ receptor antagonists (CIM, RAN, and FAM) have been reported (Ellwood et al., 1983; Dundee et al., 1984; Fee et al., 1987; Ochs et al., 1986). To predict this interaction, the second and third points mentioned above are important because both drugs are almost always orally administered in clinical settings. Recently, it was reported that MDZ was mainly metabolized by CYP3A4 in the intestine (Wacher et al., 1996). However, at the present time, the extent of the inhibitory effects of CIM and NZD on metabolism in the intestine is unclear, and further studies are necessary. Moreover, it should be considered that drug concentrations in the portal vein are higher than those in the systemic circulation after oral administration of inhibitors (Hoffman et al., 1995; Fujieda et al., 1996; Paine et al., 1996). Therefore, we must take into consideration the point mentioned above, to estimate $C_{H,f}$ and $C_{H,i}$ as the concentrations of drugs near metabolic enzymes. The extent of the inhibitory effect is underestimated if the concentrations of drugs in the systemic circulation are used as the concentrations of inhibitors. Therefore, it is necessary to estimate the drug concentrations in the portal vein, considering the absorption ratio of the drugs, and to combine those values with the blood concentrations of the drugs in the systemic circulation to predict the concentrations of inhibitors near metabolic enzymes.

In conclusion, the increasing ratio of the blood concentration of MDZ in the presence of H$_2$ blockers could be quantitatively estimated from the $C_{H,f}$ values for the inhibitors and the $K_c$ values obtained using liver microsomes. Moreover, when the inhibitors were actively transported into the liver, the inhibition ratio was underestimated using the $C_{H,f}$ values for the inhibitors. To predict the increasing ratio of blood concentrations in the case of oral administration of both an interacting drug and an inhibitor, in future studies, the development of models considering metabolism and absorption in the gastrointestinal tract would be necessary.

References


