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Mechanism Underlying Conflicting Drug-Drug Interaction Between Aprepitant and Voriconazole via Cytochrome P450 3A4-Mediated Metabolism

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ABSTRACT

Background Voriconazole is an antifungal drug for which therapeutic monitoring is recommended to prevent side effects. Temporary administration of the antiemetic drug fosaprepitant remarkably decreases the plasma concentration of voriconazole from the therapeutic range. The ratio of the major metabolite voriconazole *N*-oxide to voriconazole exceeded that at any other time for a patient who started chemotherapy during voriconazole therapy. We attributed this unpredictable result to cytochrome P450 3A4 induced by aprepitant that was converted from fosaprepitant in vivo.

Methods Concentrations of voriconazole and voriconazole *N*-oxide were measured using liquid chromatography-mass spectrometry/mass spectrometry in primary human hepatocytes after incubation with aprepitant. Aprepitant suppressed voriconazole *N*-oxide formation within 24 h, followed by a continuous increase. Levels of drug-metabolizing cytochrome P450 mRNA were measured using real-time PCR in primary human hepatocytes incubated with aprepitant.

Results Cytochrome P450 3A4 and 2C9 mRNA levels increased ~4- and 2-fold, respectively, over time. Cytochrome P450 3A4 induction was confirmed using reporter assays. We also assessed L-755446, a major metabolite of aprepitant that lacks a triazole ring. Both compounds dose-dependently increased reporter activity; however, induction by L-755446 was stronger than that by aprepitant.

Conclusion These results indicate that aprepitant initially inhibited voriconazole metabolism via its triazole ring and increased cytochrome P450 3A4 induction following L-755446 formation. The decrease in plasma voriconazole concentration 7 days after fosaprepitant administration was mainly attributed to cytochrome P450 3A4 induction by L-755446.

Key words aprepitant; CYP3A4; drug interaction; L-755446; voriconazole

Most anticancer drugs have severe side effects, even at optimum dosages. Supportive drugs are, therefore, integrated into regimens to prevent side effects; however, their administration is complicated. The increased prevalence of powerful chemotherapies is accompanied by increased incidence of immunosuppressant activities. Therefore, several supportive anti-inflammatory, antibiotic, and antifungal agents are often simultaneously administered with anticancer drugs.

The prodrug fosaprepitant (fos-APR) is administered by intravenous (IV) drip infusion to provide temporary support for chemotherapy. The molecular structure of active aprepitant (APR) has a triazole ring, and inhibitory effects are attributed to a coordinate bound to the active site of cytochrome P450 (CYP).[1, 2] In particular, APR is a powerful inhibitor that induces CYP3A4 activity.[3] Therefore, drug-drug interactions are closely associated with combination chemotherapy; however, the effects of APR on plasma concentrations of concomitant drugs are unknown.

Voriconazole (VRCZ) contains a triazole ring and is used to treat severe fungal invasive infections,[4–6] such as candidiasis and aspergillosis in immunocompromised patients. VRCZ binds to CYP-dependent 14α-sterol demethylase and inhibits ergosterol synthesis, which depletes ergosterol in fungal cell membranes.[7]

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Abbreviations: ALT, alanine transaminase; APR, aprepitant; AST, aspartate transamin ase; BUN, blood urea nitrogen; CAM, clarithromycin; CRP, C-reactive protein; CT, computed tomography; CYP, cytochrome P450; DMSO, dimethyl sulfoxide; D-PBS, Dulbecco's phosphate-buffered saline; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; fos-APR, fosaprepitant; IS, internal standard; IV, intravenous; LC-MS/MS, liquid chromatography-mass spectrometry/mass spectrometry; MCFG, micafungin; PHH, primary human hepatocyte; PK/PD; pharmacokinetic/pharmacodynamic; RT-PCR, real-time PCR; RIF, rifampicin; T-ALL, T-cell acute lymphoblastic leukemia; VRCZ, voriconazole; VNO, VRCZ *N*-oxide; VNO:VRCZ ratio; concentration ratio of VNO to VRCZ

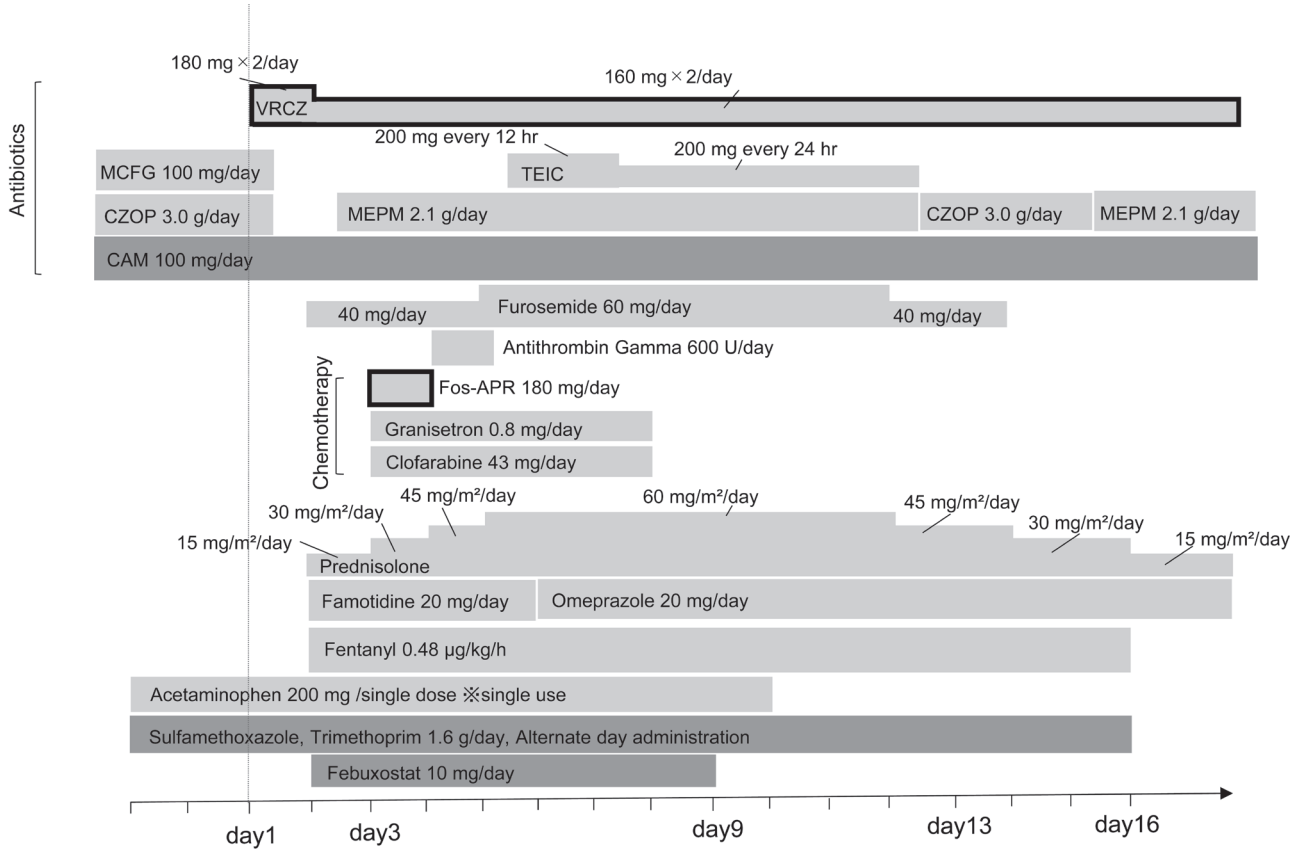

Fig. 1. Therapeutic regimen for the patient. Drugs administrated by IV or orally are shown as ( ) or ( ), respectively. VRCZ (IV) administration was started on day 1. Among the medications with possible drug-drug interactions, the combinations examined in the present study are enclosed in black boxes. CAM, clarithromycin; CZOP, cefozopran; Fos-APR, fosaprepitant; IV, intravenous; MCFG, micafungin; MEPM, meropenem; TEIC, teicoplanin; VRCZ, voriconazole.

Hepatotoxicity, visual disturbances, and phototoxicity have been identified as side effects of VRCZ since its introduction in 2002.[6, 8] The overall incidence of clinically significant hepatotoxicity (transaminitis) in a Japanese phase III study was ~13%.[9] This information is provided in the package insert provided by the manufacturer. Other side effects include fever, nausea, skin rash, vomiting, and chills.[6, 8] Therapeutic drug monitoring based on pharmacokinetic/pharmacodynamic (PK/PD) data is recommended for VRCZ to avoid these side effects.[10] The proposed trough concentration of VRCZ for effectiveness is 1–2 μg/mL. Concentrations that exceed 4–5 μg/mL are likely to have adverse effects such as liver damage.

VRCZ is predominantly metabolized by recombinant CYP2C19, CYP3A4, and CYP2C9 in the liver and small intestine in vitro.[11] The amount of CYP2C19 expressed in the human liver is low,[12, 13] and its polymorphism considerably affects the metabolic activity of VRCZ to VNO.[14–16] On the other hand, CYP3A4 is the most abundant CYP in the human liver[17–19] and has the highest specific activity in the generation of VRCZ *N*-oxide (VNO) from VRCZ.[20] Therefore, CYP3A4 is the primary enzyme involved in the metabolism of VRCZ, which inhibits CYP3A4 activity. VRCZ is frequently simultaneously administered with other drugs in clinical practice. Therefore, plasma concentrations of VRCZ and VNO must be monitored to prevent their interactions with CYP3A4.

Figure 1 shows data from an immunocompromised pediatric patient with cancer taking at least 15 drugs, including VRCZ to prevent deep mycosis and APR to prevent chemotherapy-induced nausea and vomiting, for treatment. The plasma VRCZ concentration decreased, and the concentration ratio of VNO to VRCZ (VNO:VRCZ ratio) remained consistent except at 5 days after fos-APR administration, when the plasma concentrations of both VRCZ and VNO decreased considerably, and the VNO:VRCZ ratio increased. This appeared to be caused by interactions between VRCZ and APR via CYP3A4. APR inhibits and induces CYP3A4 activity.[21, 22] We speculated that CYP3A4 activity was briefly

inhibited by APR, which was thereafter metabolized by CYP3A4 to its major metabolite L-755446 losing its triazole ring; then, L-755446 induced CYP3A4 activity. To confirm these speculations, we analyzed the inhibition and induction of CYP3A4 in primary cultured human hepatocytes and CYP3A4 reporter cells in vitro.

MATERIALS AND METHODS

Materials

The following materials were purchased from the respective suppliers: VRCZ (Tokyo Chemical Industry Co., Ltd., Tokyo, Japan), VNO (Toronto Research Chemicals Inc., Toronto, Canada), APR (LKT Laboratories Inc., St. Paul, MN), L-755446 (Acanthus Research, Mississauga, Canada), rifampicin (RIF; Sigma-Aldrich Corp., St. Louis, MO), chloroform, 2-propanol, ethanol, and dimethyl sulfoxide (DMSO; FUJIFILM Wako Pure Chemical Corporation, Osaka, Japan), and primary human hepatocytes (PHHs) and their media (Biopredic International; St. Grégoire, France). Oligonucleotides were commercially synthesized (Fasmac, Atsugi, Japan).

Patient characteristics

A 7-year-old girl (height: 118.4 cm, weight: 19.0 kg) with no medical history presented at a local hospital with intermittent diarrhea and abdominalgia. Blood tests revealed increased white blood cells and hypercholesterolemia. Computed tomography (CT) imaging revealed ascites, a swollen mesenteric membrane, lymph nodes, and suspected blood malignancy. The patient was then referred to our hospital, where she was diagnosed with T-cell acute lymphoblastic leukemia (T-ALL). The patient met the inclusion criteria and was accepted into the JALSG T-ALL11 clinical study, where she was administered nelarabine. Under treatment with clarithromycin (CAM) (100 mg/day) to prevent infection, CT imaging revealed several pulmonary nodules and recurrent leukemia. Pulmonary mycosis was suspected, and the patient was administered micafungin (MCFG) (100 mg/day) via IV drip infusion. However, this regimen was changed to VRCZ because her C-reactive protein (CRP) levels increased on day 5 and oxygen saturation decreased on day 11. Figure 1 shows the drug regimen for the patient.

Quantitation of VRCZ and VNO concentrations in patient plasma

Plasma preparation for analysis

Plasma (50 μL) was mixed with 50 μL of 0.5 μg/mL paclobutrazol in methanol (internal standard, IS) in 1.5-mL microtubes, stirred for 10 s in 200 μL of methanol, and centrifuged for 3 min at 9,600 × g and 20°C. Supernatants (1 μL) were analyzed using a Nexera X2 liquid chromatography-mass spectrometry/mass spectrometry (LC-MS/MS) system (Shimadzu, Kyoto, Japan) with a quadrupole mass spectrometer (LCMS-8040; Shimadzu).

Conditions for LC-MS/MS

VRCZ, VNO, and IS were measured as described in previous studies.[23]

Ethical statement

This human subject study was a part of a series of observational clinical studies approved by the Ethical Review Committee of Tottori University School of Medicine (approval number: 18A102) and was conducted with opt-out consent from the patients.

Cell culture and incubation with chemicals

CYP3A4 reporter (3-1-10) cells

HepG2-derived cells expressing the CYP3A4-luciferase reporter gene 3-1-10 were generated using the method outlined by Noracharttiyapot et al.[24] and cultured in Dulbecco's modified Eagle medium (FUJIFILM Wako Pure Chemical Corporation) supplemented with 10% fetal bovine serum (Gibco BRL, Rockville, MD), minimum essential medium non-essential amino acids, and antibiotic-antimycotic (both from Gibco BRL) under 5% CO_2 at 37°C.

Before 24 h of chemical treatment (APR or L-755446 in DMSO), we seeded 3-1-10 cells (3×10^4/well) in 48-well plates (BD Biosciences, Heidelberg, Germany).

Primary human hepatocytes

Primary human hepatocytes (PHHs) derived from a 45-year-old woman (lot no. HEP187301-TA05; Biopredic International) were cultured in a diffuse medium (Biopredic International, ADD222C) under a 5% CO_2 atmosphere at 37°C. Hepatocytes (4×10^5 cells) were placed in a seeding medium (Biopredic International) in Corning® BioCoat® Collagen I-coated plates (Corning Inc., Corning, NY, USA) for 24 h. Thereafter, a final concentration of 10 μM APR or RIF in DMSO was added to the culture medium at a ratio of 0.1% v/v and incubated for 24, 72, and 120 h (Fig. 2): days 1, 3, and 5, respectively (Table 1 and Fig. 4). The cells were incubated with 20 μM VRCZ for 3 h, and drug concentrations in the medium were measured. The cells were washed with Dulbecco's phosphate-buffered saline (D-PBS; Wako Pure Chemicals Industries Ltd.), suspended in passive lysis buffer (Promega, Madison,

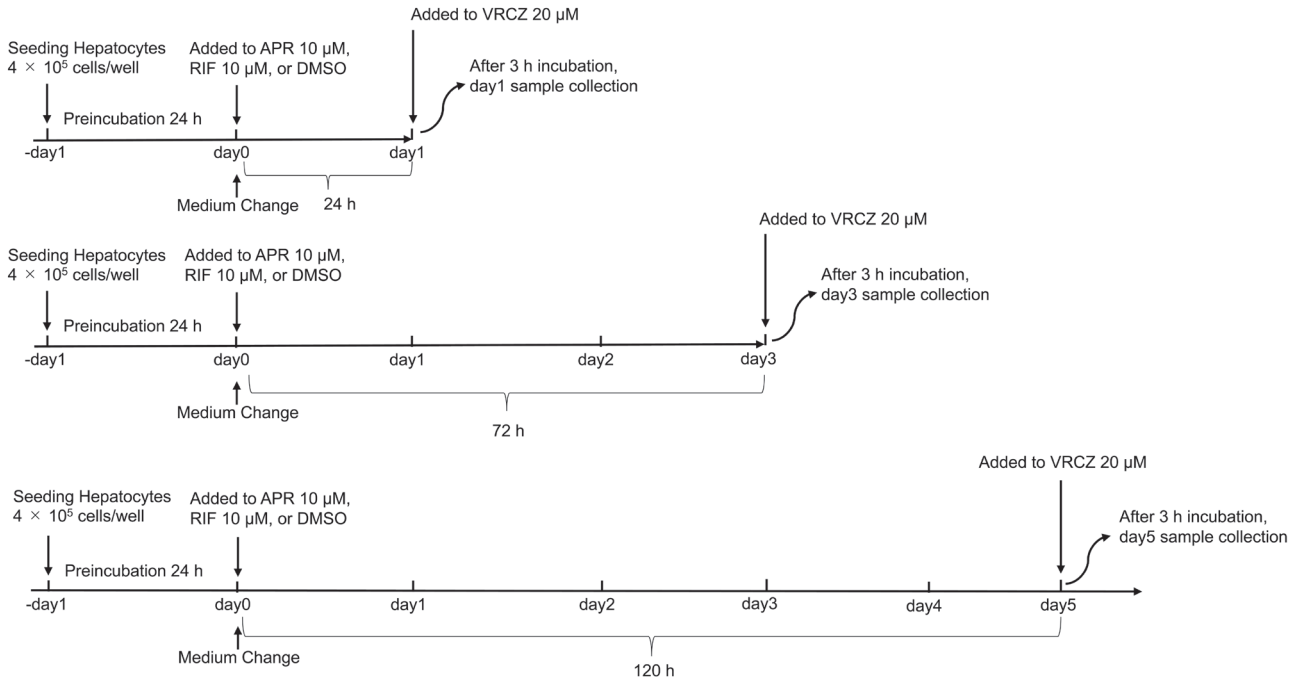

Fig. 2. Experimental schedule of drug interactions between VRCZ and APR in PHHs. APR, aprepitant; DMSO, dimethyl sulfoxide; PHHs: primary human hepatocytes; VRCZ, voriconazole.

Table 1. Effects of pretreatment with APR or RIF on VRCZ metabolism in PHHs

Day	Cont			APR			RIF		
	VRCZ	VNO	VNO: VRCZ ratio	VRCZ	VNO	VNO: VRCZ ratio	VRCZ	VNO	VNO: VRCZ ratio
	μg/mL			μg/mL			μg/mL		
1	5.16 ± 0.14	0.0673 ± 0.0073	0.0130 (1)	4.90 ± 0.48	0.00325 ± 0.00150	0.000663 (0.0510)	4.42 ± 0.08	0.0858 ± 0.0047	0.0194 (1.49)
3	4.62 ± 0.08	0.0513 ± 0.0046	0.0111 (1)	5.06 ± 0.38	0.0175 ± 0.0017	0.00346 (0.312)	4.46 ± 0.32	0.105 ± 0.013	0.0235 (2.12)
5	4.59 ± 0.13	0.0375 ± 0.0076	0.00817 (1)	4.78 ± 0.39	0.0323 ± 0.0044	0.00676 (0.827)	4.78 ± 0.39	0.0850 ± 0.0520	0.0178 (2.18)

Values present the mean ± SE of four different concentrations in medium from four different dishes. The values in brackets present the ratio to the average value of each control group (Cont; DMSO added) on days 1, 3, and 5. APR, active aprepitant; DMSO, dimethyl sulfoxide; RIF, rifampicin; PHH, primary human hepatocytes; VNO:VRCZ ratio, concentration ratio of VNO to VRCZ in culture media.

WI, USA), and centrifuged for 10 min at 2,000 × g and 4°C. We then extracted RNA from the pelleted cells for real-time polymerase chain reaction (RT-PCR).

Luciferase assays

The concentrations of APR and L-755446 added to the culture medium were determined within the range where cytotoxicity was not observed based on the preliminary test results, depending on the human blood concentrations in the interview form. The 3-1-10 cells were incubated with APR (1, 2.5, 5, or 7.5 μM) or L-755446 (0.1, 0.25, 0.5, 0.75, 1, or 3 μM) for 48 h, washed with D-PBS, suspended in passive lysis buffer (Promega), and centrifuged for 10 min at 2,000 × g and 4°C. Whether proteins of interest in cell extracts regulated genes at the level of transcription was assessed using Luciferase Assay Systems and a GloMax® 96 Microplate Luminometer (Promega) according to the manufacturer's instructions. Data are presented as ratios of luminescence emitted by cell extracts to control normalized to protein concentrations determined using Coomassie Brilliant Blue dye.

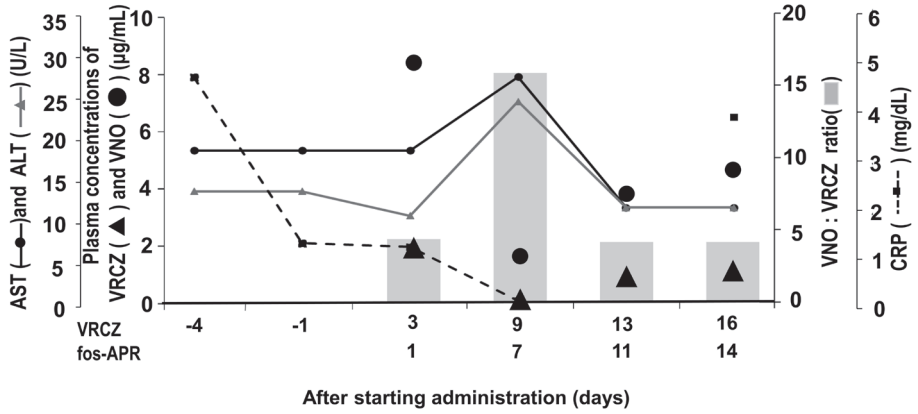

Fig. 3. Plasma VRCZ and VNO concentrations and VNO:VRCZ ratios. Administration of VRCZ or fos-APR was started on day 1. Details of the medical therapy is shown in Fig. 1. ALT, alanine transaminase; AST, aspartate transamin ase; CRP, C-reactive protein; Fos-APR, fosaprepitant; VRCZ, voriconazole; VNO, voriconazole *N*-oxide; VNO:VRCZ ratio, plasma concentration ratio of VNO to VRCZ.

RNA purification and RT-PCR

Total RNA was extracted according to the Sepasol RNA I super G protocol (Nacalai Tesque, Kyoto, Japan). First-strand cDNA was synthesized from 2 μg of total RNA in a 10 μL reaction mixture using Moloney murine virus reverse transcriptase (Promega), oligo (dT) primer, 5× buffer, and DEPC water (both from Nacalai Tesque). Messenger RNA levels of liver-specific genes, CYPs, and glyceraldehyde-3-phosphate dehydrogenase (GAPDH) were measured by RT-PCR using cDNA with First Start Essential DNA Green Master (Roche, Basel, Switzerland) and the following specific forward and reverse (5′ → 3′) primers:

CYP1A2: GTTCCTGCAGAAAACAGTCCA and CTGTGCTTGAACAGGGCAC;

CYP2B6: CCCTTTTGGGAAACCTTCTG and GTCCCAGGTGTACCGTGAAG;

CYP2C9: ATTTTGGCCTGAAACCCATA and TCTTTCAGCCAGTGGGAAAAT;

CYP2C19: ATTTTGGCCTGGAACGCATG and TCTTTCAGCCAGTGGGAAATG;

CYP2D6: GATGAGCTGCTAACTGAGCACA and GATGTAGGATCATGAGCAGGAG;

CYP3A4: CCAAGAAGCTTTTAAGATTTGATTTT and TCTTCGAGGCGACTTTCTTTCAT CCT.

Values above the threshold PCR cycle number (Ct), where the increase in signals is associated with exponential growth in PCR products, were detected using Light Cycler® 96 (Roche). Relative mRNA values in all samples were normalized to levels of GAPDH expression.

Quantitation of VRCZ and VNO concentrations in media

Medium (50 μL) recovered at 48 h after incubation with drugs was mixed with paclobutrazol (0.5 μg/mL) in 50 μL methanol (IS). The mixture was stirred in methanol (200 μL) for 10 s and centrifuged for 3 min at 9,600 × g and 20°C. Thereafter, 1 μL of supernatant (200 μL) was analyzed according to the method outlined by Yamamoto et al.[23]

Statistics

Data are presented as means ± standard error, and statistical significance was determined using Student *t*-test. Statistical significance was set at $P < 0.05$.

RESULTS

Plasma VRCZ and VNO concentrations

The patient was administered VRCZ (180 mg: 9.5 mg/kg twice on day 1 and 160 mg: 8.4 mg/kg twice daily after day 2). Plasma VRCZ concentrations were 1.9, 0.1, 0.9, and 1.1 μg/mL on days 3, 9, 13, and 16 after administration, respectively. Plasma concentrations of VNO were 8.4, 1.6, 3.8, and 4.6 μg/mL on days 3, 9, 13, and 16 after VRCZ administration, respectively (Fig. 3). The VNO:VRCZ ratios were 4.4, 16.0, 4.2, and 4.2 on days 3, 9, 13, and 16 after VRCZ administration, respectively.

Side effects in the patient administered VRCZ

We determined the side effects of high VRCZ concentrations from interviews with the patient or laboratory data (Fig. 3). Neither visual disturbance nor phototoxicity was observed. Aspartate transaminase (AST) and alanine transaminase (ALT) values were transiently increased on day 9 after VRCZ administration and

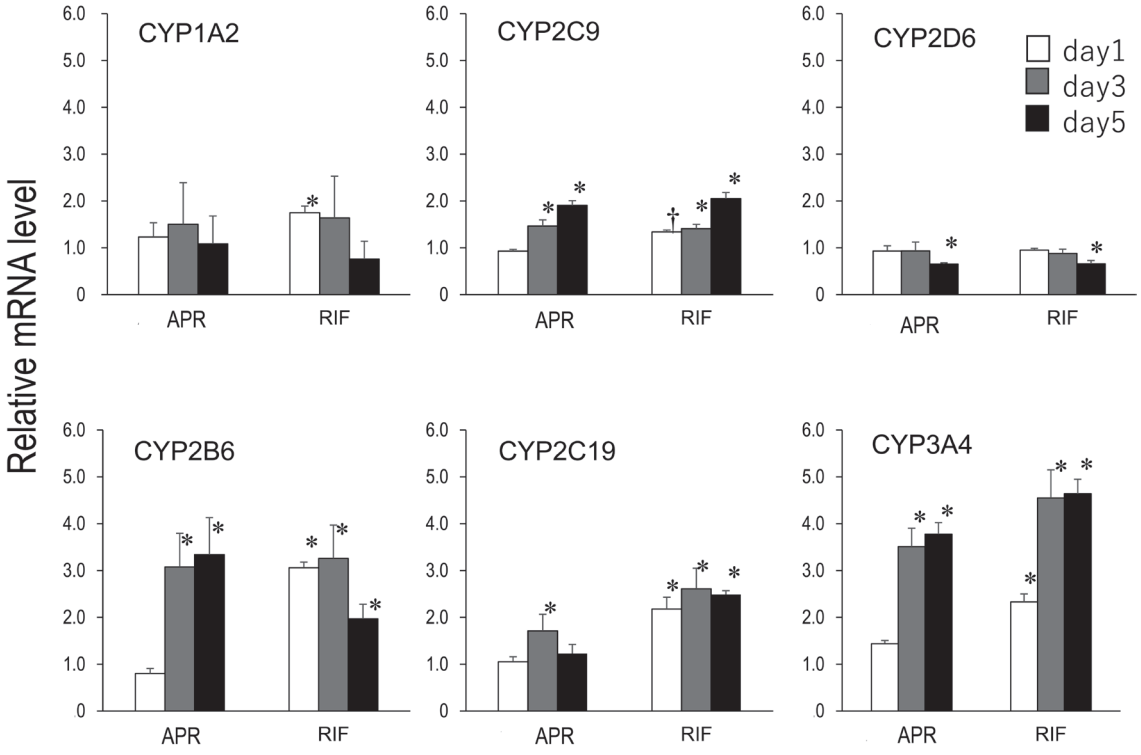

Fig. 4. Effects of APR on CYP mRNA expression in PHHs. Bar: relative ratio to the control (DMSO added) group on days 1, 3, or 5 after treatment. Data are presented as means ± standard error ($n = 4$). RIF is a positive control inducer of CYP3A4 mRNA. *$P < 0.05$ (*vs.* corresponding controls on each day). APR, aprepitant; CYP, cytochrome P450; DMSO, dimethyl sulfoxide; PHH, primary human hepatocytes; RIF, rifampicin.

almost recovered to normal levels before administration on day 13. The profile of γ-GTP was similar (data not shown). Serum creatinine and blood urea nitrogen values (indicating renal function status) did not change significantly during this period. Oxygen was administered at the same time as VRCZ; oxygen saturation improved 13 days after administration. The CRP value was decreased to < 0.1 mg/dL on day 9. No correlation was observed between the behavior of CRP values and the metabolic capacity of VRCZ (VNO:VRCZ ratios) in the patient. Among the drugs used shortly before and after the beginning of VRCZ administration (Fig. 1) and in addition to fos-APR, those metabolized via the same CYP 3A4 as voriconazole include MCFG,[25] CAM,[26] prednisolone,[27] fentanyl,[28] granisetron,[29] and omeprazole (also metabolized via CYP2C19).[30] CAM has an inhibitory effect on CYP3A4 activity,[31] while prednisolone induces CYP3A4 activity.[32–34] Owing to the consistency in continued administration of the dosage of CAM, the VNO:VRCZ ratio in the patient's plasma remained high. Conversely, owing to the increasing dosage of prednisolone, a moderate reduction in the VNO:VRCZ ratio in the patient's plasma was expected. The interactions between concomitant drugs and VRCZ *via* CYP3A4 might explain the transiently low concentration of VRCZ on day 9. Therefore, we focused on fos-APR owing to its ability to inhibit and induce CYP3A4 activity.

Effects of APR on VRCZ metabolism in PHHs

We seeded PHHs and incubated them for 24 h with 10 μM APR or RIF, followed by incubation with 20 μM VRCZ on days 1, 3, and 5. Table 1 shows that the amounts of generated VNO time-dependently decreased from 0.0673 to 0.0375 μg/mL in the control (DMSO) group. RIF is a typical CYP3A4 inducer that increased the VNO concentration by 0.0858 or 0.105 μg/mL; however, the level did not change significantly for 5 days. Although incubation with APR resulted in low levels of VNO formation on day 1, levels substantially and time-dependently increased from 0.00325 to 0.0323 μg/mL. The VNO:VRCZ ratios decreased in the controls and were 1.49-, 2.12-, and 2.18-fold higher in the RIF and 0.0510-, 0.312-, and 0.827-fold higher in the APR group on days 1, 3, and 5, respectively, compared with each time point in the control group.

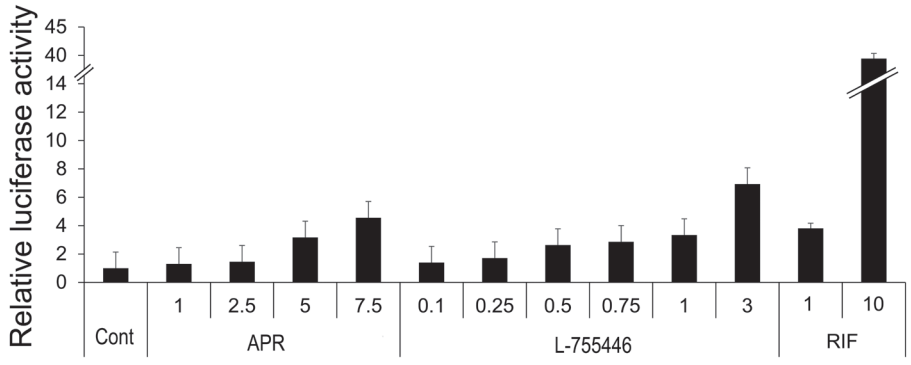

Fig. 5. Effects of APR or L-755446 on CYP3A4 gene reporter assays. Controls (Cont) contained DMSO. The X-axis shows the final concentrations of chemicals (μM). The Y-axis shows luciferase activity relative to that of Cont. Data are presented as means ± standard error (n = 6). APR, aprepitant; CYP3A4, cytochrome P450 3A4; DMSO, dimethyl sulfoxide; RIF, rifampicin.

Effects of APR on the expression of CYP mRNA in PHHs

We semi-quantified CYP mRNA expression in PHHs incubated with APR. The mRNA expression of CYP2B6, CYP2C9, and CYP 3A4 increased time-dependently, whereas that of CYP1A2, CYP2D6, and CYP2C19 mRNA did not change significantly (Fig. 4).

CYP3A4 gene reporter assays

We used CYP3A4 gene reporter assays to confirm whether APR induces CYP3A4 activity. Figure 5 shows that although APR and L-755446 both dose-dependently induced reporter activity of the CYP3A4 gene at the transcriptional level, the induction by L-755446 exceeded that by APR.

DISCUSSION

Anticancer drugs are associated with severe adverse side effects, which can be prevented by simultaneously administering several supportive therapy drugs to patients. Such combination therapies involve drug-drug interactions, most of which occur through CYP activity. We assessed a pediatric patient with cancer who was administered at least 15 types of drugs, including VRCZ, which is associated with several serious side effects. Therefore, we monitored the drugs based on VRCZ PK/PD data.

We disregarded the metabolism of VRCZ and fos-APR in the small intestine because these drugs were administered via IV infusion. We assumed that the dosage of VRCZ does not change, the condition of the patient is stable, and doses of concomitant medications are consistent. The VNO:VRCZ ratio should essentially be constant during medication; however, it can increase when the plasma VRCZ concentration decreases as shown herein. We thought that this decrease attributed to an increase in hepatic CYP3A4 activity. Drug-drug interactions with APR have been systematically reviewed.[35] Our results are in line with those of a previous study,[3] where APR increased hepatic CYP3A4 and CYP2C9 activities. Meanwhile, few inhibition studies have been investigated on APR or fos-APR.[36, 37] Marbury et al. found that fos-APR increased the area under the plasma concentration-time curve from 0 h to infinity by ~1.8-fold on day 1 but did not affect midazolam pharmacokinetics on day 4.[37] Based on these data, fos-APR is likely to inhibit VRCZ pharmacokinetics within 24 h of administration. Therefore, fos-APR-inhibited VRCZ metabolism might not have been the evident at the monitored time points. Conversely, CAM inhibits CYP3A4 activity but does not seem to be involved in changed VNO:VRCZ ratios. CAM was administered throughout the treatment period.

The above results indicated that the effects of APR interaction with CYP3A4 shifted from inhibition to induction after the administration. Flavine monooxygenase 3 and CYP2C19 were reported to be considerably involved in the conversion of VRCZ to VNO in pediatric liver.[38] In general, CYP expression level is very low at newborn, and increase at adult level within a few years after birth. Especially, 1) the expression level of CYP3A4 reaches the adult level at ≥ 4 years of age,[39] 2) the expression profile of hepatic nuclear factors, required for CYP3A4 expression and induction, is significantly correlated with CYP3A4 expression in pediatric liver,[40, 41] and 3) drug-drug interactions via CYP3A4 induction among concomitant medications have been reported in children as well as adults.[42, 43] We deemed that the in vitro model used in this study is possible to evaluate the ability of CYP3A4 induction by

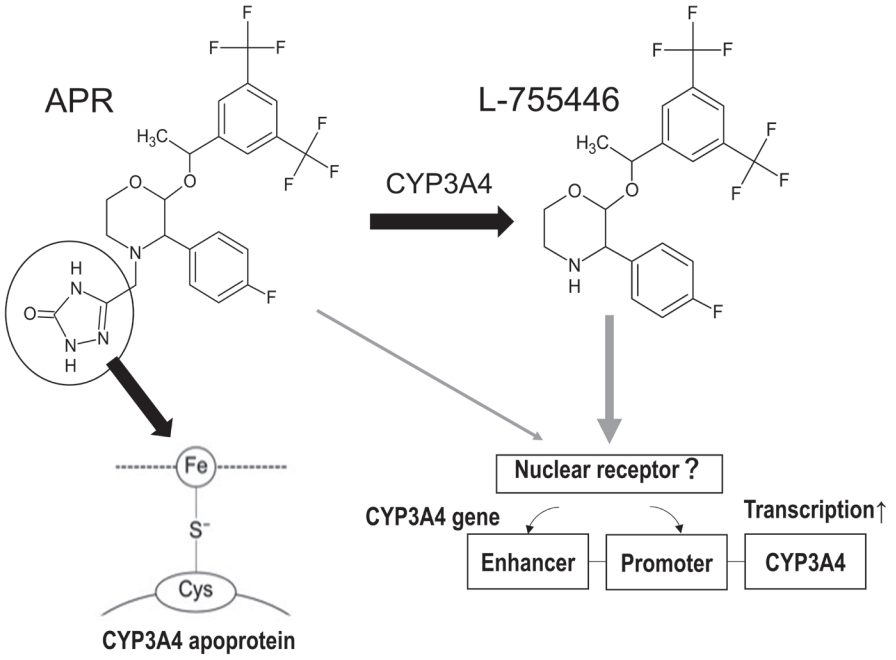

Fig. 6. Putative aspects of CYP3A4-mediated drug-drug interactions of APR. APR, aprepitant; CYP3A4, cytochrome P450 3A4.

drugs in the liver of a 7-year-old child.

The VNO value in the culture medium of PHH incubated with APR was < 10% of that in the control group on day 1 (Table 1). On days 3 and 5, the values time-dependently reached those of the controls. In addition, PHHs incubated with APR induced CYP3A4 and CYP2C9 mRNAs and involved VRCZ metabolism (Fig. 4). In contrast, RIF also increased the amount of VNO, although the levels did not significantly differ among the RIF groups. These results suggested that APR immediately inhibited the metabolizing activity of VRCZ and then increased VRCZ metabolism mediated by CYP3A4. Our experimental findings showed that APR both inhibits and induces CYP3A4 activity, which is consistent with the results reported by Marbury et al.[37] However, it is comprehensively difficult to explain the extreme drop in plasma VRCZ concentration solely based on APR effects on day 7; APR is mainly metabolized to L-755446 by CYP3A4 and is immediately decreased in the plasma. L-755446 loses a triazole ring during metabolism (Fig. 6) and does not inhibit CYP3A4 activity. We then confirmed whether L-755446 induced CYP3A4 activity using CYP3A4 gene reporter assays. We found that not only APR but also L-755446 induced CYP3A4 activity, and the induction level was three times more potent in L-755446 than in APR (Fig. 5); this indicates that APR induces CYP3A4 is induced even better by L-755446 depending on APR metabolism. Changes in plasma VRCZ concentrations and metabolite ratios were attributed to CYP3A4 induction by L-755446. In analysis using QSAR, it has been reported that APR shows affinity for pregnane X receptor (predicted to bind, probability = 0.89, similarity score = 0.78).[44, 45] However, the details of the mechanism with which APR or L-755446 promotes CYP3A4 gene transcriptional activity are unknown (Fig. 6).

The interview form of APR states that drug-drug interactions occur through the CYP3A4 shift from inhibition to induction.[46] However, the molecular mechanism underlying this shift is unclear. This study clarified most of the involved mechanisms. Elucidation of the mechanisms underlying drug-drug interactions is essential to understanding their pharmacological outcomes and preventing related side effects in clinical practice.

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The authors declare no conflict of interest.

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