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Short Communication: R(+)-XK469 inhibits hydroxylation of S-warfarin by CYP2C9

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Abstract

Introduction: XK469 is a novel topoisomerase II inhibitor structurally akin to several propionic acid derivatives, such as ibuprofen and diclofenac, which are metabolized by CYP2C9. We report eight subjects who experienced significant elevation of INR while receiving concomitant R(+)-XK469 and warfarin. The aim of the study was to investigate whether R(+)-XK469 interacts with S-warfarin by inhibition of CYP2C9.

Methods: The effect of R(+)-XK469 on S-warfarin hydroxylation was determined by the measurement of S-7-hydroxywarfarin formation in pooled human liver microsomes and cDNA expressed CYP2C9.

Results: R(+)-XK469 competitively inhibited S-warfarin hydroxylation. The K_i values of R(+)-XK469 were estimated to be $959 \pm 426 \mu\text{M}$ for human liver microsomes and $377 \pm 92 \mu\text{M}$ for CYP2C9.

Conclusion: At the recommended phase II dose of R(+)-XK469, the ratio of C_{max}/K_i is >1 . This suggests that coadministration of R(+)-XK469 and warfarin results in a clinically significant pharmacokinetic interaction due to CYP2C9 inhibition by R(+)-XK469.

Keywords

XK469; warfarin; drug interaction; CYP2C9; pharmacokinetics

Introduction

K469 (2-[4-(7-chloro-2-quinoxalinyloxy) phenoxy] propionic acid) is a synthetic quinoxaline analogue derived from the herbicide Assure (Dupont) (1). XK469 is active against a broad spectrum of malignancies particularly for solid tumors and multi-drug resistant cancers (1,2). XK469 has been suggested to work as a selective topoisomerase II β inhibitor (3). However,

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XK469 also affects other putative targets, and has been demonstrated to upregulate the expression of p53-dependent proteins (Bax, p21 and Gadd 45), downregulate cdc2-cyclinB1 kinase activity and cyclin B ubiquitination, inhibit the phosphorylation of MEK and induce apoptosis via binding to the peripheral benzodiazepine receptor (4-8).

XK469 exists as two stereoisomers (2). R(+)-XK469 is more potent than S(+)-XK469 and has been selected for further clinical trials (9). At least five R(+)-XK469 metabolites have been identified (10). Cytosolic aldehyde oxidase is the main metabolizing enzyme for XK469. In addition, glycine, taurine and glucuronide conjugation, and oxidative transformations by microsomal enzyme, are minor metabolic pathways for XK469. R(+)-XK469 is structurally similar to propionic acid derivatives such as ibuprofen and diclofenac. Although both ibuprofen and diclofenac are CYP2C9 substrates, CYP2C9 does not contribute to XK469 metabolism (11-13).

A phase I study of R(+)-XK469 in patients with advanced solid tumors showed that the recommended phase II doses are 850-1100 mg/day on days 1, 3 and 5 of a 21-day cycle and 2500 mg on day 1 of a 21-day cycle (14). Here we report a possible drug-drug interaction between warfarin and R(+)-XK469 observed in 8 subjects, from the same study, with significantly elevated prothrombin time. In addition, we evaluate CYP2C9 inhibition as a possible mechanism of R(+)-XK469-warfarin interaction.

Patients and Methods

Patients and trial design

The patient eligibility criteria and trial design has been reported previously (14). All patients provided written informed consent prior to enrollment. The starting dose of R(+)-XK469 was 15 mg per day. R(+)-XK469 was administered every 3 weeks as a 30 min infusion on days 1-5 initially. The treatment schedule was subsequently changed to infusion on days 1, 3, 5 every 3 weeks when pharmacokinetic analysis demonstrated a longer than expected half-life. In this study, all patients on concomitant therapeutic dose of warfarin were identified. Information on warfarin dose, XK469 dose, peak plasma concentration of XK469 in Day 1 of cycle 1, baseline and post-treatment INR, and concomitant medication were collected.

Chemicals and Reagents

R(+)-XK469 was supplied by the National Cancer Institute (Bethesda, MD). S-warfarin and 7-hydroxywarfarin were obtained from Genetest (Woburn, CA). Benzamide and nicotinamide adenine dinucleotide phosphate, reduced form (NADPH) were purchased from Sigma-Aldrich (St. Louis, MO). Human recombinant CYP2C9*1 (Arg₁₄₄) was purchased from Genetest (Woburn, CA). This preparation also contained cDNA-expressed human P450 reductase and human cytochrome b₅. All other reagents and HPLC solvents were of the highest quality commercially available. Human liver samples from 10 separate donors were obtained from the Liver Tissue Procurement and Distribution System (National Institutes of Health NO1-DK-9-2310, Pittsburgh, Pennsylvania). Microsomes were prepared by differential centrifugation methods (15). Total protein content in microsomes was determined by the Bradford method using bovine serum albumin as the standard (16).

Inhibition studies of S-warfarin hydroxylation

The incubation mixture (200 μ L) consisted of S-warfarin (0, 0.5, 1, 2.5, 5 and 10 μ M), sodium phosphate buffer at pH 7.4 (100 mM), magnesium chloride (5 mM), R(+)-XK469 (0, 50, 400, 800 μ M) and CYP2C9 enzyme (50 pmol/mL) or human liver microsomes (1mg/mL). After a 10-minute preincubation of all assay components at 37°C, NADPH (1 mM) was added to start the reaction. The reaction was terminated after 30 minutes by adding 10 μ L of ice-cold

hydrochloric acid and followed by the addition of internal standard, benzamide (25 μL of 7 $\mu\text{g/mL}$). The mixture was centrifuged at 25000 rpm for 30 minutes at 4°C and the supernatant was analyzed by HPLC. Single experiment in duplicates was performed throughout the study. Incubation conditions (enzyme content, incubation time) had been optimized with regard to the linearity and metabolic turnover.

Assay for S-7-hydroxywarfarin

One hundred μL of supernatant was used for HPLC analysis. The HPLC system consisted of a gradient pump (C-7000, Hitachi Instruments, Inc., San Jose, CA) and an autosampler (C-7200, Hitachi Instruments, Inc., San Jose, CA). A $\mu\text{Bondapak C18}$ column (3.9 $\times$ 300 mm, 10 μm ; 125Å, Waters Corp., Milford, MA) with Novapak guard column (4 μm ; 60 Å, Waters Corp., Milford, MA) was used. The mobile phase consisted of A: 100% acetonitrile and B: 5 mM heptanesulfonate in 50 mM KH_2PO_4 (pH 3.5). Elution was performed at a flow rate of 0.8 ml/min using the following gradient: 0 to 30 minutes, A:B (30:70) to (80:20) then 30.1 to 50 minutes (30:70). S-7-hydroxywarfarin was detected at 22.8 minutes by fluorescence detector (C-7400, Hitachi Instruments, Inc., San Jose, CA) at $\lambda = 320$ nm (excitation) and 415 nm and the internal standard, benzamide at 12.5 minutes by UV detector (C-7480, Hitachi Instruments, Inc., San Jose, CA) at 230 nm (emission). The limit of detection for 7-hydroxywarfarin was 0.1 nM. Inter-day (n=3) and intra-day (n=3) coefficients of variation of controls were less than 16.2% and accuracy ranged from 90.1 to 114.7%.

Analysis of enzyme kinetics and inhibition

Results were presented as mean values $\pm$ SEM. A Michaelis-Menten model (equation 1) was fitted to enzyme kinetic data by nonlinear regression analysis using GraphPad Prism software version 4.01 (GraphPad Software Inc., San Diego, CA) to derive K_m and V_{max} .

$$v = [S] \times V_{max} / ([S] + K_m) \quad (1)$$

[S] is defined as the concentration of substrate S-warfarin. The type of inhibition was evaluated using graphical analysis with Lineweaver-Burk plots. K_i value was determined using non linear regression analysis with a competitive inhibition model (equation 2):

$$v = [S] \times V_{max} / ([S] + K_m / (1 + I/K_i)) \quad (2)$$

Results

Sixteen of the 81 patients were on therapeutic doses of warfarin. Prolonged prothrombin time (International Normalized Ratio, INR > 4) was observed in 8 patients. All but 1 patient with prolonged prothrombin time were on a stable warfarin dose prior to study enrollment. One patient had hematuria and was treated with vitamin K injection. All other patients were asymptomatic with no evidence of bleeding.

The mean peak plasma concentrations of R(+) XK469 measured on day 1 of the first treatment cycle were 598 $\pm$ 80 μM and 282 $\pm$ 72 μM in subjects with and without a prolonged prothrombin time, respectively. Table 1 summarizes the different doses of R(+)XK469 administered and the peak plasma concentrations of R(+)XK469 at each dose level. Four of 8 subjects with prolonged prothrombin time had concomitant drugs that also might contribute to the prolonged prothrombin time (see Table 2).

Lineweaver-Burke plots (Figure 1) indicated that R(+)XK469 competitively inhibited 7-hydroxylation of S-warfarin in human liver microsomes and CYP2C9. Dixon plots for R(+)

XK469 inhibition of S-warfarin hydroxylation are shown in Figure 2. The K_i values of R(+)-XK469 were estimated to be $959 \pm 426 \mu\text{M}$ for human liver microsomes and $377 \pm 92 \mu\text{M}$ for CYP2C9. The K_m and V_{max} values of S-warfarin were $4.4 \pm 1.3 \mu\text{M}$ and $5445 \pm 654 \text{ pmol/min/mg protein}$ for human liver microsomes, and $3.5 \pm 0.8 \mu\text{M}$ and $51.1 \pm 4.6 \text{ pmol/min/pmol protein}$ for CYP2C9. The catalytic efficiencies (V_{max}/K_m) for human liver microsomes and CYP2C9 were $1556 \mu\text{L/min/mg protein}$ and $14.6 \mu\text{L/min/pmol protein}$.

Discussion

Altered plasma protein binding and inhibition of warfarin metabolism can lead to prolonged prothrombin time. XK469 is highly protein bound (96-98.5%) and could potentially displace warfarin from protein binding sites (17). In this study, we evaluated the inhibitory effect of R(+)-XK469 on S-warfarin, the more potent enantiomer metabolized by CYP2C9, as a potential mechanism for drug-drug interaction. Our study showed that R(+)-XK469 competitively inhibited CYP2C9. Although R(+)-XK469 is a relatively weak inhibitor of CYP2C9 compared with fluconazole ($K_i = 8 \mu\text{M}$), the peak plasma concentrations of R(+)-XK469 achievable in patients exceeds the estimated K_i value for CYP2C9 (18).

The current R(+)-XK469 dose recommended for a phase II study is 850-1100 mg administered every other day for a total of three doses in a three-week regimen and 2500 mg on day 1 of a 21-day cycle. Based on the equation $[AUC]_i/[AUC] = (1 + [I]/K_i)$ for competitive inhibition and the reported C_{max} at 1100 mg and 2500 mg of R(+)-XK469, the ratio of C_{max}/K_i would be greater than 1. This suggests that XK469 is likely to cause clinically relevant CYP2C9 inhibition. Cancer patients are at a higher risk of developing thrombotic complications and are often anticoagulated with warfarin. Physicians should closely monitor their patients' INR and be watchful for signs and symptoms of bleeding, when R(+)-XK469 is coadministered with warfarin. Consideration should be given to withholding or reducing the warfarin doses scheduled in conjunction with R(+)-XK469.

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Figure 1. Lineweaver-Burk plots showing competitive inhibition of S-warfarin hydroxylation by R(+) XK469 in (A) human liver microsomes and (B) CYP2C9. The R(+)XK469 concentrations used were 0 (■), 50 (▲), 400 (▼), 800 (◆) μM.

Figure 2. Dixon plots for R(+)XK469 and (A) human liver microsomes, and (B) CYP2C9. S-warfarin concentrations tested were 0.5 ($\blacksquare$), 1 ($\blacktriangle$), 2.5 ($\blacktriangledown$), 5 ($\blacklozenge$) and 10 ($\bullet$) μ M.

Table 1

Dose levels

Dose levels (mg/day)	No of patients treated	No of patients on warfarin	Mean Cmax (μM)
Schedule A: Given on Day 1-5 every 21 days			
15	2	1	3.5
30	2	0	11.9
60	2	0	25.5
120	1	0	41.5
240	4	2	80.9
Schedule B: Given on Day 1, 3, 5 every 21 days			
360	3	0	168.2
540	6	2	207.4
675	5	1	250.0
850	7	1	304.9
1100	20	3	424.4
1400	1	1	502.1
Schedule C: Given on Day 1 every 21 days			
1600	5	1	498.9
2000	3	1	718.2
2500	14	4	852.5
3200	5	0	847.9

Table 2
A summary of patients with elevated INR with concomitant use of R(+XK469).

Patients	Reason for anti-coagulation	Warfarin dose (mg)	Days after XK469	INR Pre	INR Post	R(+XK469) dose (mg)	R(+XK469) Cmax (µM)	Concurrent medication
I	DVT	5	24	1.3	8.5	540	202	Dexamethasone, enoxaparin, fentanyl patch, fluconazole*, hydromorphone hydrochloride, levofloxacin, lorazepam, paracetamol, sertraline.
II	DVT	2.5	-	2.2	8.2	1100	531	Gabapentin, hydrocodone bitartrate, methylprenisolone, ondansetron.
III	DVT	2.5	3	2.6	6.7	1100	572	Atenolol, diphenoxylate hydrochloride, furosemide, propranolol, ramipril.
IV	Prophylaxis	8	4	1	10.0	1400	502	Amlodipine, docusate sodium, fentanyl patch, ferrous sulphate, gaifloxacina, hydrochlorothiazide, morphine, ondansetron, sertraline, valsartan.
V	AF	2.5	14	3.3	8.5	2000	591	Amiodarone*, atorvastatin, carvedilol, digoxin, enalapril, glibenclamide, fluconazole*, furosemide, metformin, potassium
VI	AF	4	28	2.5	4.4	2500	918	Budesonide, escitalopram, fexofenadine, fluticasone propionate, lorazepam, montelukast sodium, rabeprazole*
VII	PE	2.5	35	-	5	2500	617	Citalopram, dextropropoxyphene*, glipizide, lansoprazole*, morphine, paracetamol
VIII	AF	3	36	1.5	4.7	2500	882	Diltiazem, digoxin, glibenclamide, metoprolol, sertraline, tamsulosi, urecholine, vitamin B12,

AF: atrial fibrillation

DVT: deep vein thrombosis

PE: Pulmonary embolism

* reported drug-drug interaction