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ORIGINAL ARTICLE

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Metabolic profile of XK469 (2(*R*)-[4-(7-chloro-2-quinoxalinyl)oxyphenoxy]-propionic acid; NSC698215) in patients and in vitro: low potential for active or toxic metabolites or for drug–drug interactions

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Abstract As part of an ongoing phase 1 study, we studied the excretion of XK469 and its metabolism in patients and in vitro. Five primary metabolites were identified by HPLC/MS/MS. An oxidized product formed by cytosolic aldehyde oxidase was the predominant species both in urine and human hepatocytes in vitro. Conjugates of XK469 with glycine, taurine, and glucuronic acid, as well as the microsomal product, 4-oxo-XK469, were also found in urine and in vitro, but none were major contributors to the mass balance for XK469 elimination. Based upon the relative concentrations circulating in plasma, systemic exposure to parent drug was 100-fold higher than for the metabolites. Thus, both toxicity and efficacy of XK469 are most likely to be produced by the parent molecule, rather than the metabolites. Urinary recovery of parent drug was low (2% of dose in 24 h), partly because of the long half-life of XK469 (approximately 3 days). In addition, the metabolite profile in urine indicates that only 25% of the XK469-derived material was unchanged drug. Thus, urinary excretion was not a major factor in XK469 elimination. Variations in systemic exposure to XK469 will be strongly influenced by factors that alter the activity of aldehyde oxidase, including pharmacogenetics, enzyme inhibition, and enzyme induction, but no specific modifiers have been reported. The multiday half-life of XK469 hampered our ability to obtain a complete mass balance, and the possibility exists that other routes, such as biliary excretion, may also play a substantial role in XK469 disposition.

Keywords NSC698215 · Metabolites · Drug-drug interactions

Abbreviations AO: Aldehyde oxidase · XK469: 2(*R*)-[4-(7-Chloro-2-quinoxalinyl)oxyphenoxy]-propionic acid, NSC698215

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Introduction

The *R* isomer of 2-[4-(7-chloro-2-quinoxalinyl)oxyphenoxy]-propionic acid (XK469; NSC698215) was selected for phase 1 evaluation based upon its novel structure (Fig. 1) and a preclinical activity profile that appears to be different from that of other drugs that have been tested clinically [7, 8, 9, 15]. Phase 1 trials are ongoing [18, 24] under the sponsorship of the National Cancer Institute (NCI).

The primary goal of a phase 1 study is to determine the safety of a new molecule. In addition, this is the ideal time to determine excretory pathways for elimination of a drug from the body and to ascertain the profile of exposure to metabolites that could potentially contribute to efficacy and/or toxicity. Studies of metabolism in animals during preclinical testing retain their importance for an understanding of interspecies differences in activity or toxicity, but are generally weak predictors of metabolic pathways and metabolite exposures in humans. Fortunately, the widespread availability of human

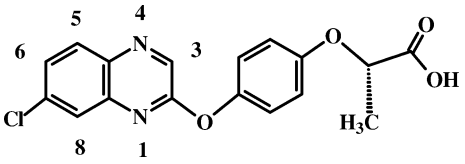

Fig. 1 Structure of (*R*)-XK469

tissue for metabolic testing in vitro, and recent improvements in our ability to interpret and extrapolate these data, have enhanced the predictivity for metabolism prior to entry of a drug into the clinic, as well as complementing ongoing clinical studies [5].

Exposure of the body to a drug is determined by the ratio of the dose and elimination. When the drug is eliminated largely unchanged in urine, dosage adjustments may be necessary based upon renal function, e.g., carboplatin [6]. At the other extreme, metabolism may be so extensive that only minor amounts of drug are excreted unchanged, and assessment of the influence of renal function on elimination is a lower priority, e.g., cytarabine [3].

For XK469, the focus on metabolism was sharpened when low urinary recovery of parent drug was found in preliminary studies [18]. In order to resolve parent compound from potential metabolites and to provide structural identification for the metabolites, an HPLC/MS/MS method was developed. Plasma and urine samples obtained from patients receiving XK469 in a phase 1 study were analyzed for parent drug and potential metabolites. XK469 was incubated in vitro with human hepatocytes and subcellular fractions of human liver to assist in the determination of specific metabolic pathways.

In the context of a first-in-human study, evaluation of metabolites should be a highly focused effort designed to provide practical information that can assist further development of the molecule. Thus, our objectives were to assess: (1) relative roles of excretion and metabolism; (2) structures and quantities of metabolites that are formed; and (3) the systemic exposures to these metabolites as indicators of their potential to contribute to the activity or toxicity of XK469.

Materials and methods

Drugs and chemicals

Reference (*R*)-XK469 and [^{3}H]-(*R*,*S*)-XK469 were supplied by NCI (Bethesda, Md.). Synthetic standards of some potential metabolites (4-*N*-oxide; glycine and taurine conjugates of racemic XK469) were prepared by Dr. J.P. Horwitz (Wayne State University). All solvents were HPLC grade. All other reagents were obtained from the Sigma-Aldrich Company (St. Louis, Mo.).

The racemic mixture of [^{3}H]-(*R*,*S*)-XK469 was placed on a 250×4.6 mm Chirasil OJ column (Chiral Technologies, Exton, Pa.). At a flow rate of 0.5 ml/min, the mobile phase of 0.1% trifluoroacetic acid in a mixture of 10% ethanol and 90% methanol achieved baseline separation of *R*-XK469 and *S*-XK469. The tritiated *R*-form was collected for use in experiments in vitro.

Chromatography of biological samples

Separation of XK469 and its metabolites was performed on a HP 1050 LC (Agilent Technologies, Palo Alto, Calif.) using a Zorbax (Agilent Technologies) Extend-C18 2.1×150 mm LC column. Mobile phase, pumped at 0.27 ml/min, consisted of two parts: bottle A containing 0.15% (v/v) ammonium hydroxide and 0.065% glacial acetic acid, and bottle B containing a 1:1:1 mixture of acetonitrile, methanol, and bottle A buffer. A linear gradient program was developed for the separation of the metabolites: start at 80% A/20% B for 0–2 min, then ramp to 60% A/40% B at 18 min, ramp to 50% A/50% B at 24 min, ramp to 25% A/75% B at 28 min, and ramp to 10% A/90% B at 29 min. Detection and identification of XK469 and its metabolites were accomplished by UV monitoring at 335 nm, and by HPLC/MS/MS.

Mass spectra were acquired on a Thermo-Finnigan TSQ7000 (Finnigan; San Jose, Calif.). Ions were generated by electrospray ionization in negative ion mode using a spray voltage of 3.5 kV. Single quadrupole scanning was used to identify potential metabolites, and product ion scanning was used to identify fragmentation patterns. Total ion MS chromatograms were interrogated for masses that exhibited chlorine isotope ion clusters. Ions at these masses were then subjected to MS/MS analysis to confirm that fragmentation patterns were similar to those of the parent compound

Selected reaction monitoring was used to identify metabolites in biological samples. The reactions that were monitored were m/z 343 → 271 at 30 V, m/z 359 → 287 at 30 V, m/z 400 → 271 at 34 V, m/z 450 → 271 at 38 V, and m/z 519 → 271 at 28 V.

^{1}H-NMR spectra were obtained on a Varian (Palo Alto, Calif.) Unity 500 MHz instrument. Peak 1 metabolite was generated by multiple human liver cytosol incubations using 25 mg protein and 2–4 mg XK469 for 2–6 h. The reaction preparations were deproteinized with 2 vol 1:1 MeOH/acetonitrile, the supernatant lyophilized, and reconstituted in 1:1 MeOH/water. Peak 1 metabolite was separated and collected from parent XK469 by HPLC, using a 4.6×150 mm Hypersil BDS column (Alltech, Deerfield, Ill.) with an isocratic mobile phase of 78% aqueous (40 m *M* formic acid and 10 m *M* triethylamine) 22% acetonitrile. The collected fractions were lyophilized, rechromatographed and collected using 70% 50 m *M* formic acid 30% acetonitrile mobile phase, and lyophilized to dryness. Samples were dissolved in CD_3OD for analysis.

Patient samples

Plasma and urine samples were obtained from patients enrolled in a phase 1 clinical study approved by the

Wayne State University Institutional Review Board and the Research in Human Subjects Committee at the FDA. Patients received intravenous doses of XK469 administered daily for 5 days by brief infusion. Blood samples were drawn throughout day 1 and day 4 and collected into heparinized tubes. Plasma was separated by centrifugation, and aliquots were frozen at −20°C until analysis. Total urine output was collected over 24 h on day 1 and day 4. Aliquots were frozen at −80°C until analysis.

Sample preparation

Urine samples (0.5 ml) were loaded onto a preconditioned 1-ml LC18 Supelclean SPE column (Supelco, Bellefonte, Pa.), washed with 0.5 ml deionized water, and eluted with 0.75 ml methanol. The methanol was removed under vacuum, and the sample reconstituted in 0.1 ml 10% methanol in water, of which 10 µl was injected into the HPLC.

Samples from incubations in vitro were prepared in similar fashion except that 1.0 ml of the incubation medium was used for processing. Extraction efficiencies were determined by comparing peak areas for both UV and radioactive signals from non-extracted samples to those for extracted samples, and were found to be >95% for peak 1 and XK469.

Plasma samples were processed by solvent deproteinization. To 0.3 ml plasma was added 0.3 ml methanol and 0.3 ml acetonitrile. The samples were vigorously vortexed and cooled on ice for 10 min. The samples were then centrifuged at 4000 *g* for 5 min and the supernatant was recovered and lyophilized. The sample was then reconstituted in 60 µl 10% methanol, and 10 µl was used for analysis.

Studies in vitro

Human livers that were medically unsuitable for organ transplantation were obtained under the auspices of the Washington Regional Transplantation Consortium (Washington, DC). Subcellular human liver fractions (microsomes and S100 cytosol) were prepared as described previously [13]. All incubations were conducted in 0.1 *M* potassium phosphate buffer, pH 7.4, containing 2 m *M* Na-EDTA, 0.1 m *M* $MgCl_2$, and 100 µ*M* XK469. Both cytosolic and microsomal incubations had a final protein concentration of 0.8–1.0 mg/ml. NADPH, 1 m *M*, was added to the incubations when the experiment dictated. Samples were incubated at 37°C for 30–60 min, and then immediately processed as above. Inhibitor stock solutions were prepared in ethanol, and added to incubations at levels such that the final ethanol level never exceeded 1% (v/v).

Cryopreserved human hepatocytes and Williams' medium were obtained from In Vitro Technologies (Baltimore, Md.). After thawing and equilibration, as specified by the supplier, the cells in Williams' medium were exposed to 100 µ*M* XK469 for 4 h at 37°C. At the end of the incubation, hepatocytes were removed by centrifugation at 1000 *g* for 5 min, and the medium immediately processed as the subcellular fractions above.

Quantitation

Stock solutions of XK469, 0.3–300 µ*M*, were prepared and injected into the HPLC. Peak areas at 335 nm were used to construct a response curve from which the amounts of XK469 were estimated in sample preparations. Metabolite concentrations were estimated by comparing peak areas at 335 nm of the metabolites to the peak area of XK469. UV areas for peak 1 were scaled by 0.67 because the ratio of UV absorption to ^3H counts for samples in vitro was 1.5-fold higher for peak 1 than for XK469. Insufficient UV areas were obtained to determine scale factors for the other metabolites found in vitro; thus, it was assumed that their UV extinction coefficients were similar to those of the parent compound.

Preparation and analysis of methyl esters

To further characterize the cytosolic metabolite (peak 1), several incubations of XK469 in cytosolic fractions were conducted, and the supernatants were pooled. The metabolite was separated and collected by HPLC using a 4.6×100 mm Hypersil ODS column. Mobile phase flowed at 1.0 ml/min and consisted of 18% acetonitrile, 0.4% glacial acetic acid, and 0.1% ammonium hydroxide. The collected fractions were pooled and lyophilized.

Methylation of XK469, synthetic 4-N-oxo-XK469, and purified peak 1 was accomplished by dissolving the compounds in 250 µl DMSO alkalinized with 0.05 *M* K_2CO_3, adding 4 mg trideuterated methyl iodide and reacting for 16 h at 50°C. The resulting trideuterated methyl esters were analyzed by HPLC/MS. The mobile phase consisted of 50% buffer A/50% acetonitrile ramped to 20% buffer A/80% acetonitrile in 12 min. A flow rate of 0.3 ml/min was used with a 2.1×50 mm phenyl–hexyl column (Phenomenex, Torrance, Calif.). Ionization of compounds was accomplished with atmospheric pressure chemical ionization, 3 kV corona voltage, and the MS was operated in negative ion mode.

Results

Discovery of metabolites

UV chromatograms of urine were screened for peaks with absorbance at 335 nm and mass spectral analysis (Fig. 2). The primary fragment of the molecular ion of XK469 (*m*/*z* 343) was found at mass *m*/*z* 271. We

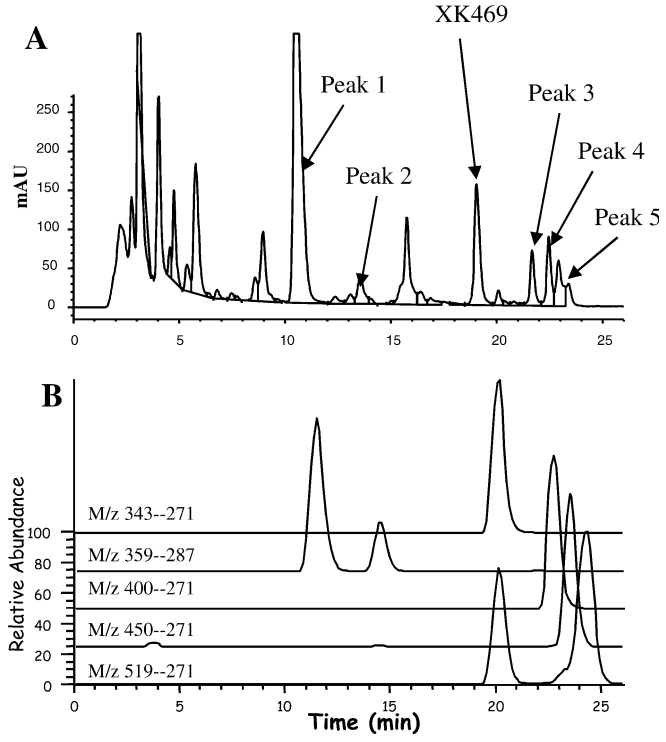

Fig. 2 Analysis of the 72–96 h urine collection from a patient receiving 260 mg/m^2 XK469. **A** UV (335 nm) HPLC tracing identifying the parent drug and metabolite peaks. **B** Selected reaction monitoring confirming the identification of the metabolite peaks. The *m*/*z* tracings are shifted 0.9 min for retention time, compared with the UV signal. The intensity of the *m*/*z* signal from the mass spectrometer is not directly comparable for each species

interpreted this to be the neutral loss of the propionic acid moiety (72 amu), leaving the 4-(7-chloro-2-quinoxalinyl)oxy-phenol. The loss of the phenol ring results in an ion found at *m*/*z* 163.

Two metabolites, labeled as peak 1 and peak 2 in Fig. 2, were found to contain molecular ions of *m*/*z* 359, 16 amu higher than the parent drug, and were assumed to be oxidative compounds. Fragmentation of the *m*/*z* 359 ion in these two potential metabolite peaks yielded an ion at *m*/*z* 287, consistent with a loss of propionic acid, similar to the parent drug. Peak 2 was found to coelute and fragment the same as the synthetically prepared 4-N-oxide-XK469.

Three other metabolites (peaks 3, 4, and 5) were found with molecular ions of *m*/*z* 400, 450, and 519, respectively. Fragmentation of these ions in each case was found to yield a primary ion at *m*/*z* 271. We interpreted these results as modifications to the propionic acid moiety, most likely due to conjugation on the free acid. Peaks 3 and 4 were identified as the glycine and taurine conjugates of XK469 through comparison to synthetic standards of retention times and fragmentation patterns.

Peak 5, with a mass ion 176 amu higher than the parent drug, was considered to be the product of glucuronidation. Pretreatment of the urine sample prior to extraction with beta-glucuronidase was found to eliminate the *m*/*z* 519-containing peak that is labeled peak 5. Selected reaction monitoring MS/MS of *m*/*z* 519 → 271 produced additional small peaks that eluted before peak 5. These signals may be due to rearrangements within the glucuronide or adduct formation with parent XK469, but were too small for UV detection or further analyses.

No evidence of di-oxygenated or conjugated mono-oxygenated species was found in urine samples.

Generation of metabolites from human liver in vitro

Incubation of 100 μ*M* XK469 with human hepatocytes produced all five of the metabolites that were found in urine from patients. Peak 1 was the predominant species produced. For the three individual liver donors, 1.7, 3.4, and 6.7% of parent drug was converted to peak 1 in 4 h. Peak 1 was found to be about 20-fold more abundant than peak 2. The conjugated species were detected at levels $<0.06\%$ of the parent at 4 h. Other peaks were also observed at a similar level or lower, but were not pursued further due to the low impact on the mass balance.

Human liver microsomes treated with XK469 produced peak 2 only when NADPH was present in the incubation. No discernible amount of peak 1 was observed after microsomal incubations. In contrast, peak 1 was generated when XK469 was incubated with the cytosolic fraction of liver homogenate, without the requirement for added cofactors. The range of activity for the formation of peak 1 found in five liver donor preparations was 1–12 nmol/mg/30 min. Peak 1 formation in human liver cytosol was found to be inhibited 50% by 20 μ*M* isovanillin, 70% by 2 μ*M* menadione, 75% by 10 μ*M* chlorpromazine, and 50% by 1 μ*M* raloxifene, but only 25% by 50 μ*M* allopurinol. This pattern of enzyme activity and inhibition is consistent with what has been reported to be aldehyde oxidase (AO) activity [12, 13, 17].

Methylation of collected peak 1 resulted in the addition of two methyl groups, while parent drug and synthetic 4-N-oxo-XK469 added a single methyl group. These findings suggest that peak 1 has two active protons, and the oxygen addition in peak 1 is most likely to have the potential for a phenolic proton.

^{1}H-NMR analysis of XK469 revealed a sharp peak at 8.677 ppm which was assigned to the proton on the 3 position of the quinoxaline ring. The ^{1}H-NMR spectrum of a sample of human liver cytosol-generated peak 1 did not contain a peak at 8.677 ppm, indicating that the oxidation had occurred at the 3 position.

Metabolic profile in urine

The relative contribution of the metabolites to urinary excretion of XK469 is presented in Table 1. Parent compound comprised 25% of the total XK469-related

Table 1 Recovery of urinary metabolites over 24 h following the first dose of XK469. An oxidized metabolite, peak 1, was the dominant species. The maximum expected recovery in urine was 20% (see Discussion)

Patient	Dose (mg/m^2)	Fraction of total XK469 species found						Total recovery (% dose)
		Peak 1	Peak 2	Peak 3	Peak 4	Peak 5	XK469	
5	25	0.74	0.02	0.02	0.10	0.02	0.11	10.2
6	35	0.21	0.11	0.04	0.13	0.03	0.48	4.4
8	68	0.48	0.02	0.05	0.18	0.04	0.23	5.2
9	95	0.49	0.05	0.00	0.08	0.02	0.36	11.2
11	186	0.47	0.05	0.02	0.20	0.04	0.22	11.5
12	260	0.64	0.02	0.03	0.06	0.04	0.21	5.9
13	260	0.47	0.02	0.05	0.11	0.04	0.30	5.1
14	260	0.76	0.00	0.01	0.07	0.01	0.15	6.3
15	260	0.64	0.02	0.02	0.10	0.05	0.17	4.3
Average		0.54	0.03	0.03	0.11	0.03	0.25	7.1
Std. dev.		0.17	0.03	0.02	0.05	0.01	0.12	3.0

compounds. The oxidized metabolite produced by AO, peak 1, accounted for $54 \pm 17\%$ of total urinary excretion in the nine patients examined and was the most prominent species in eight of nine patients. Peak 4 (taurine conjugate) was the next most prevalent metabolite at 11%. The products of microsomal oxidation and the products of XK469 conjugation with glycine or glucuronic acid (peaks 2, 3 and 5, respectively) did not contribute substantially to the urinary mass balance of XK469. When the amounts of the five metabolites and parent drug were summed, $7.1 \pm 3.0\%$ of the administered dose was recovered in urine during the first 24 h.

Metabolic profile in plasma

Metabolites were detectable in plasma samples obtained from patients treated with XK469. A typical profile is presented in Fig. 3. However, in contrast to the relatively high abundance in urine, the levels for each of the metabolites circulating in plasma were estimated to be $<1\%$ of the parent drug concentration in the three patients examined.

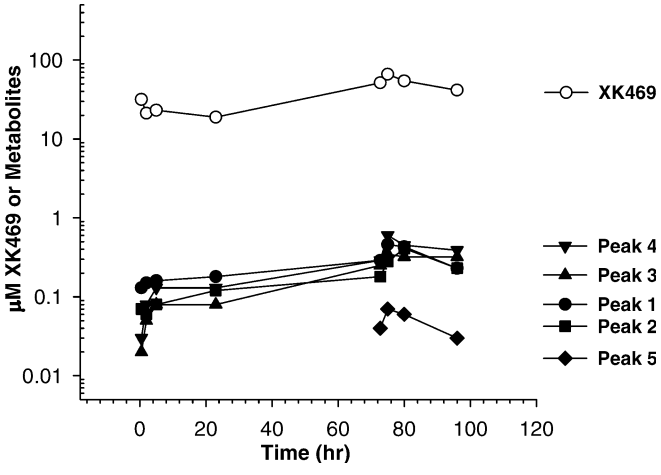

Fig. 3 Plasma concentrations of XK469 and its metabolites in a patient who received a dose of 68 mg/m^2 per day

Discussion

In this study, we sought to identify the metabolic pathways and metabolites for XK469 and to assess the utility of this information. The metabolic pathways provide specific focal points for addressing the potential of drug–drug interactions or pharmacogenetic differences within a population. Knowledge of the structure and quantity of the metabolites helps to determine the potential for biological activity, and can guide the amount of effort to be expended for further evaluation of beneficial or toxic properties.

A complete accounting of all elimination pathways, i.e., a mass balance, is a useful starting point to determine the relative roles of excretion and metabolism. The preliminary report for 24-h urinary recovery of parent drug was 1–4% [18], and we found similar values (Table 1). In addition to a low absolute recovery, unchanged XK469 constituted only 25% of the total XK469-derived species found excreted in urine. Therefore, metabolism was assumed to provide the major portion of XK469 elimination, and was closely examined in vitro and in vivo.

Although microsomes contain the most common enzymes for biotransformation of drugs, the results from this study illustrate the necessity of examining non-microsomal metabolism to obtain the complete metabolic profile. As summarized in Table 2, the profile of metabolism via microsomes would be quite misleading in terms of the determinants of whole-body exposure. In

Table 2 Pattern of metabolite generation in various preparations in vitro

	Microsomes		Cytosol	Hepatocytes
	Without cofactors	With NADPH	Without cofactors	
Peak 1	–	–	+ + + +	+ + + +
Peak 2	–	+ +	–	+
Peak 3	–	–	–	+
Peak 4	–	–	–	+ +
Peak 5	–	–	–	+

the standard approach using microsomes, peak 2 would have been identified incorrectly as the dominant species. Incubation of XK469 with the cytosolic fraction of human liver did not generate peak 2, but did produce a large amount of peak 1.

Hepatocytes, which contain the complete enzymatic machinery of the liver, including cofactors, generated both peak 1 and peak 2, as expected. In addition, three conjugated metabolites were produced: peaks 3, 4, and 5. Some additional small peaks were also observed, but were not pursued due to their low contributions to the mass balance.

Each of these conjugate species might be produced by microsomes or cytosol with appropriate conditions and cofactors, but only if they are suspected in advance. In particular, glycine and taurine conjugates (peaks 3 and 4) are so rare in humans that it is unlikely that a screening program would focus upon these pathways.

Due to the relatively large amount of peak 1 that was found in patient urine, and to elucidate possible drug-drug interaction and pharmacogenetic issues, it was a high priority to determine the specific enzyme system that was responsible for its formation. Based upon its *m*/*z* value, localization within the cytosol, lack of required cofactors, and pattern of inhibition by various compounds in vitro [12–14, 17, 19], AO appears to be the enzyme responsible for the formation of the major metabolite, peak 1.

The overall metabolic profile in vitro was generally consistent with the observed pattern for urinary metabolites. Recovery of XK469-derived species in urine indicates that AO was most likely to be the principal metabolic pathway for XK469 in vivo. Thus, variations in exposure to XK469 will be strongly influenced by variations in activity of AO, including the impact of pharmacogenetics, enzyme inhibition, and enzyme induction. Detailed information is unavailable about the factors that affect AO activity.

Several compounds have been reported to have inhibitory effects on AO in vitro, including raloxifene [17], chlorpromazine [11], cimetidine [20], hydralazine [10], and methadone [21]. However, no reports on clinical modulation of this enzyme have been found [2]. Polymorphisms have not been described for this enzyme; nonetheless, wide variability in functional expression has been documented [1, 4, 16, 22, 23].

The conjugation of XK469 with the amino acids glycine and taurine, while somewhat novel or unusual in human metabolic reports, does not contribute substantially to the total mass balance for XK469. On the other hand, the *N*-oxide, peak 2, and the glucuronide, peak 5, are more common structures for metabolites. Nonetheless, neither was a major contributor to the mass balance.

Regardless of the formation pathway, the metabolites can potentially contribute to the biological activity of the parent drug. However, in this study, the systemic exposures for each of the identified metabolites were only approximately 1% of the values for parent XK469. Thus, the impact of metabolites on the toxicity or efficacy of XK469 appears to be minimal, assuming that the metabolites are not at least 100-fold more potent than XK469. Three metabolites have been tested. The glycine and taurine conjugates, as well as the 4-N-oxo-XK469, have been found to be less potent than the parent compound in cellular cytotoxicity assays in vitro (J. Horwitz; personal communication). Therefore, the formation of each of these metabolites appears to be a detoxifying reaction.

For a drug that has a long half-life, a complete mass balance is often impractical due to the difficulties of comprehensive urine collections over three to five half-lives. For XK469, initial reports have indicated that the plasma half-life of XK469 is at least 2 days, and often 3 days or longer [7, 18], which would suggest the unrealistic requirement for continual urinary collections over a 2-week period.

As noted in Table 1, the total urinary recovery for parent XK469 and its five metabolites in the first 24 h was only 7% of the day-1 dose. If the excretion kinetics were first-order with a half-life of 3 days, and if urine were the sole route of elimination, then the maximum expected recovery would have been 20% of the dose. With only one-third of the maximal value recovered in urine in the form of XK469 and these five metabolites, other possible elimination routes should be considered. Of course, excretion can be more complex, and it is possible that the early phases are dominated by distribution processes, which divert the drug from excretion and into tissue stores. Also, neither was biliary excretion examined, nor were fecal samples obtained. If additional metabolites were formed but not discovered, use of a tracer dose of radiolabeled drug in vivo might be helpful. However, no additional metabolites of quantitative significance were found when radiolabeled XK469 was incubated with hepatocytes in vitro.

Urine is the most convenient route of elimination to monitor, but other routes should not be overlooked. Collection and analysis of fecal material over several weeks is even more difficult logistically than prolonged urine collections. Further, unless radiolabeled drug is administered to patients, the analytical methods are even more challenging due to the complex matrix.

In this work, the metabolic studies in vitro and in vivo were conducted simultaneously. In most contemporary drug development programs, emphasis on studies in vitro has increased, and information regarding metabolic pathways and metabolites is routinely available prior to entry into the clinical stage [4, 16]. Nonetheless, the current study is an example of the need for reliable preclinical systems for prediction of metabolic pathways and also initial clinical monitoring to confirm the relevance of the data in vitro. If these analyses are conducted during phase 1 studies, then the potential influences on safety and activity of organ dysfunction and comedication use can be assessed before phase 2 studies begin.

In conclusion, we have established in this first report that, compared with its known metabolites, parent XK469 is the only species of biological interest in humans. Despite the difficulties studying a drug with a long half-life, we have shown that metabolism is an important determinant for XK469 disposition, especially the AO pathway. Further research is required before our understanding of XK469 disposition in humans is complete.

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