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SHORT COMMUNICATION

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# Bioavailability and pharmacokinetics of the investigational anticancer agent XK469 (NSC 698215) in rats following oral and intravenous administration

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**Abstract** *Purpose*: To determine the oral bioavailability of R-XK469, a water-soluble investigational anticancer agent undergoing phase I clinical trials as an intravenous product. *Methods*: R-XK469 was administered to two groups of catheterized Sprague-Dawley rats via the oral and IV routes at a dose of 10 mg/kg and blood samples were collected at predetermined times. XK469 in plasma samples was quantified using a HPLC method. The pharmacokinetic parameters were computed using WinNonlin 4.0.1 software. *Results*: The pharmacokinetic parameters of XK469 following oral and IV administrations, respectively, were (mean ± SD): $C_{max}$ 138 ± 64 and 404 ± 355 μg/ml; $AUC_{0-\infty}$ 2381 ± 773 and 2854 ± 1924 μg h/ml; and elimination half-life ($T_{1/2}$) 12.9 ± 5.8 and 13.5 ± 7.8 h $T_{max}$ was 2.92 ± 1.92 h following oral dosing. Oral R-XK469 was 83% bioavailable. *Conclusion*: Together with the antitumor efficacy of oral XK469 shown in preclinical models and its schedule dependency, these results indicate the promise of developing an oral dosage form of R-XK469 for clinical development.

**Keywords** XK469 · Quinoxaline · Bioavailability · Rats

## Introduction

Racemic XK469 [sodium 2-(4-(7-chloro-2-quinoxalinyloxy)phenoxy)propionate] is a water-soluble investigational agent that exhibits broad activity against murine and human tumors in vivo, and high activity against mdr-resistant tumors [3, 4]. Based on dose-schedule studies involving a large number of trials with a wide variety of schedule variations, Polin et al. concluded that extended treatment schedules, without rest periods, produce the best efficacy in preclinical models [6]. Intravenous (IV) dosages of 40–50 mg/kg daily, or 75 mg/kg every other day, were well tolerated and could be given to reach an optimal total dose with minimal toxicity. Thus, the authors recommended a split dose regimen (daily or every other day or twice weekly) and avoidance of a 21-day schedule in clinical development.

XK469 exists as two stereoisomers that differ in their toxicity to normal myeloid progenitors in human and animal bone marrows [4]. Studies conducted by the National Cancer Institute confirmed the in vivo efficacy of the sodium salt of XK469 (NSC 656889), and also found excellent anti-tumor activity for the free acid racemate XK469 (NSC 697887), as well as for both the S(−) and R(+) free acid enantiomers (NSC 698216 and NSC 698215) administered IV on daily treatment schedules. Highly efficacious antitumor activity was observed with treatment regimens of 53 mg/kg per dose and 80 mg/kg per dose given IV daily for 4 days on two successive weeks in the Colon adenocarcinoma 38 model.

Pharmacology and dose range-finding toxicology studies were performed with both the R(+) and S(−) enantiomers. S-XK469 is rapidly converted in vivo to R-XK469 in rodent and non-rodent animal models [1, 8, 9], and hence the decision was made to advance the R(+) enantiomer into clinical development. The R(+) enantiomer is currently in phase I clinical trials at our institution and the University of Chicago [5, 7].

Polin et al. have reported that oral racemic XK469 is as active as IV XK469 [6]. However, it requires 35% more dose to produce the same efficacy by the oral route. Given the need to use a frequent administration schedule for XK469 and based on comparable efficacy by the IV and oral routes, it is imperative to elucidate the pharmacokinetics and oral bioavailability of XK469 for it to be developed as an oral medication. The pharmacokinetics of XK469 have been studied in several

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species including mice, rats, and dogs following IV administration [1, 8, 9]. We report on studies of the pharmacokinetics of XK469 following both oral and IV administration in dual cannulated rats to determine its oral bioavailability.

## Materials and methods

*Materials* R-XK469 (NSC 698215) was obtained from the Pharmaceutical Management Branch of the US National Cancer Institute (Bethesda, Md.). Diclofenac sodium was purchased from the United States Pharmacopeial Convention (Washington, DC), and AR grade glacial acetic acid, HPLC grade chloroform and acetonitrile, and HCl were purchased from VWR Scientific (Chicago, Ill.). Saline for injection USP and heparin sodium USP were purchased from a local pharmacy. Sodium bicarbonate was purchased from Sigma Chemicals (St Louis, Mo.).

*XK469 injection/oral solution* XK469 was dissolved in 0.1 *M* sodium bicarbonate. The pH of the solution was adjusted to 7. The solution was sterilized by filtration and stored at room temperature. The compound is stable in solution for several months (personal communication, Dr. V. Rao, NCI).

*Animal studies* Two groups (seven animals in each) of male Sprague-Dawley rats of minimum size 250 g cannulated in the femoral and/or jugular veins were purchased from Taconic (Germantown, N.Y.). The animals were acclimatized for at least 1 week before initiation of the experimental procedures. After an overnight fast (water was provided ad libitum), the first group of animals (jugular vein cannulated) were treated with 10 mg/kg oral R-XK469 solution by gavage and the animals were fasted up to 2 h following drug administration. The second group (femoral and jugular vein cannulated) received 10 mg/kg R-XK469 bolus injection through the femoral vein followed by a saline flush. Blood samples (0.3 $cm^3$ each) were drawn from the jugular vein catheter into a heparinized syringe at 0 (pretreatment), 0.25, 0.5, 1, 1.5, 2, 4, 8, 24, 30, and 48 h after drug administration to determine the pharmacokinetics of XK469. After each blood draw, fluid was replaced by flushing the catheter with 0.6 $cm^3$ of 10 IU of heparin/ml in saline. The catheter injection port was swabbed with alcohol before and after each use, and plugged when not in use. The samples were centrifuged, and the plasma was separated and stored at −20°C until assayed. The Animal Investigation Committee of Wayne State University approved the study protocol.

*HPLC analysis of XK469 in plasma* A rapid, sensitive, simple, and reliable reverse-phase HPLC method developed in our laboratory was used to analyze the XK469 content in rat plasma samples in this study. The details of the method in brief are as follows. To 0.2 ml plasma in a screw-capped glass tube was added 20 µl internal standard solution (100 µg/ml diclofenac sodium in methanol). Following mixing, 0.5 ml 0.5 *N* HCl was added and mixed. The mixture was extracted with 4 ml chloroform by shaking the tubes for 30 min on a table-top rotary shaker. After centrifugation at 4000 rpm for 10 min, the organic phase was separated and evaporated under a stream of nitrogen at 40°C. The dried residue was reconstituted in 0.2 ml mobile phase and 100 µl was injected onto the column.

*HPLC conditions* A Waters Nova-Pak C-18 column (300×3.9 mm, 4 µm particle size) fitted with a guard column (Waters Nova-Pak C-18, 4 µm; Waters Corporation, USA) was used. The mobile phase consisted of a mixture of acetonitrile/0.5% acetic acid in water (45:55) at a flow rate of 1 ml/min. The eluent was monitored at 243 nm to detect the analytes. The concentration of analytes in the plasma was determined by back calculation from a linear regression equation developed from the ratio of external reference standards to the internal standard in pooled untreated rat plasma that was prepared for analysis as described above and analyzed with every set of samples. A linear relationship in the XK469 concentration range of 1–100 µg/ml was observed with a mean interday deviation of less than 6%.

*Pharmacokinetic analysis* Pharmacokinetic parameters of XK469 were computed from the plasma concentration-time data using each rat as an independent subject employing non-compartmental methods using WinNonlin 4.0.1 (Pharsight). The $AUC_{0-48}$ for each animal in the two groups was calculated using the linear trapezoidal rule. The bioavailability fraction (F) of oral R-XK469 administered as an aqueous solution was calculated using the equation:

$$F = AUC_{0-\infty}\ \text{oral}/AUC_{0-\infty}\ \text{IV},$$

(Since the oral and IV doses given were the same)

Systemic clearance (CI) and apparent volume of distribution for oral treatment were computed based on F = 0.834 of XK469 observed in this study. The mean pharmacokinetic parameters of XK469 following oral and IV treatments were compared using a paired *t*-test at $P = 0.05$.

## Results

The time course of the mean plasma levels of XK469 following oral and IV administration at 10 mg/kg are shown in Fig. 1 and the corresponding pharmacokinetic parameters are summarized in Table 1. The bioavailability of XK469 following oral administration in rats was 83%. The time to reach peak plasma levels following oral administration ($T_{max}$) ranged from 1.5 to 4 h

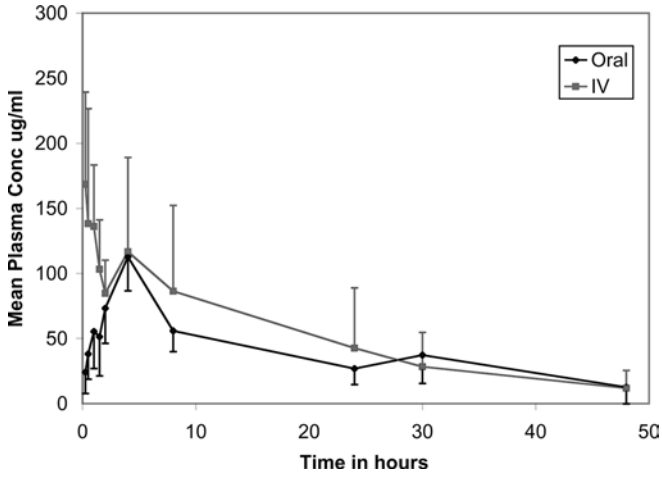


**Fig. 1** Mean plasma concentrations of XK469 following oral and IV administration at 10 mg/kg in SD rats

**Table 1** Pharmacokinetic parameters of XK469 following oral and IV administration at 10 mg/kg in SD rats

| Parameter | Oral | | IV | |
|---|---|---|---|---|
| | Mean | SD | Mean | SD |
| $C_{max}$ (μg/ml) | 138* | 64.0 | 404* | 354 |
| $T_{max}$ (h) | 2.92 | 1.20 | | |
| $T_{1/2}$ (h) | 12.9 | 5.8 | 13.5 | 7.8 |
| $AUC_{0-t}$ (μg h/ml) | 2071 | 533 | 2505 | 1720 |
| $AUC_{0-\infty}$ (μg h/ml) | 2381 | 773 | 2854 | 1924 |
| CI (ml/h/kg) | 3.94[a] | 1.58 | 5.17 | 3.49 |
| Vz (ml/kg) | 66.7[a] | 22.8 | 88.8 | 47.4 |
| MRT (h) | 21.1 | 8.6 | 18.7 | 10.7 |

[a]Corrected for $F = 0.83$ observed in this study
*Significantly different, $p \leq 0.05$

with a mean value of 2.9 h. Except peak plasma levels, none of the other pharmacokinetic parameters of XK469 were significantly different ($P > 0.05$) following oral and IV treatments.

## Discussion

In a previous study using a chiral HPLC method we found that S-XK469 is rapidly converted to R-XK469 following IV administration to BDF1 mice, while only about 5% of R-XK469 is converted to S-XK469 [8]. Collaborative NCI/Wayne State University/Ohio State University studies have documented that enantiospecific (S–R) chiral conversion of XK469 also occurs in vivo in rats, dogs and cynomolgus monkeys [1, 8, 9]. No S-isomer was detected in plasma/urine of any of 15 patients following IV administration of R-XK469 in the ongoing phase I clinical trial at Wayne State University [5]. Further, Zheng et al. found no trace of the S-isomer in circulation or in urine or feces following administration of the R-isomer up to 72 h in Fischer-344 rats [9]. Based on these observations and reports, chiral analysis of the rat plasma samples was unnecessary and R-XK469 levels were quantified by reverse-phase HPLC in the present study.

Chan et al. reported $T_{max}$ (3 h) and oral bioavailability values following oral administration of R-XK469 to Fischer-344 rats similar to the values reported here [2]. They found a similarly high variability in $AUC_{0-\infty}$ for XK469 in rats following IV administration [2, 9]. The reason for this variability is not experimental design, because the pharmacokinetic study design included dense blood sampling of each individual rat. It cannot be the analytical method, because the assay method is highly reproducible with a mean interday deviation $< 6\%$. It cannot be in-life methodology, because the pharmacokinetic study of IV-administered drug used dual catheterized rats, wherein drug was administered via the femoral vein while blood samples were drawn from the jugular vein. We suspect that biliary rhythms, coupled with high bioavailability from the gastrointestinal tract, play a significant role in the disposition of XK469 and contribute to the observed high variability in AUC. In the present study in rats, we found additional peaks in plasma concentration-time profiles (see Fig. 1, 5 h after IV administration). Chan et al. [2] and Zheng et al. [9] reported biliary excretion of drug, which could contribute to recirculation of drug. Regardless of the reason, a high degree of variability in AUC values in the rat is a reproducible finding that appears to be due to an in vivo aspect of its disposition.

The antitumor efficacy of XK469 has been evaluated following both IV and oral administration [3, 4, 6]. The percentage T/C and log tumor cell kill values for doses in the range 32–69 mg/kg administered IV (daily days 1–5 and 10–15) and orally (daily days 1–15) are similar in the mammary adenocarcinoma 16/C tumor model [6]. The high oral bioavailability of XK469 explains its antitumor effectiveness in preclinical models following both IV and oral treatments [4, 6].

Given the schedule dependency and efficacy observed in preclinical models, XK469 may be well-suited to be an oral chemotherapeutic agent. Our results along with those of Chan et al. [2] and Zheng et al. [9] indicate that oral XK469 therapy is a viable clinical alternative to IV dosing and support the development of a more practical and convenient treatment regimen. Uniformly high bioavailability will minimize problems from intersubject variations in absorption and achieve nearly constant exposure, which in animals is associated with maximal efficacy. However, before evaluating the efficacy of extended oral XK469 dosing in the clinic, its oral bioavailability in humans has to be confirmed as large interspecies variations in the pharmacokinetics of XK469 have been reported [2, 8, 9]. The terminal elimination half-lives of R-XK469 are 8, 12–24, 13, and $55.5 \pm 40$ h in mice, rats, dogs and humans, respectively. Further, the gastrointestinal tract toxicity observed in mice treated at higher doses was not observed in any of the 15 patients who received 9–346 mg/m$^2$ daily for 5 days despite evidence of enterohepatic recycling and

possibly high local concentrations of XK469 in the intestine [5]. The data from the current study support exploration of oral dosing of XK469 in a phase I clinical trial to assess safety, pharmacokinetics, and oral bioavailability.

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