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A PHARMACOGENETIC STUDY OF ALDEHYDE OXIDASE I IN PATIENTS TREATED WITH XK469

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Abstract

XK469 (NSC 697887) is a selective topoisomerase II β inhibitor eliminated mainly via aldehyde oxidase I (AOX1). We performed a candidate gene study to investigate whether *AOX1* genetic variation contributes to interindividual variability in XK469 clearance. Forty-one *AOX1* single nucleotide polymorphisms (SNPs) and seven liver expression quantitative trait loci were genotyped in Caucasian patients with advanced refractory solid tumors (n=59) and leukemia (n=33). We found a significant decrease in clearance ($\tau = -0.32$, $P = 0.003$) in solid tumor patients with rs10931910, although it failed to replicate in the leukemia cohort ($\tau = 0.18$, $P = 0.20$). Four other *AOX1* SNPs were associated with clearance ($P = 0.01$ - 0.02) in only one of the two cohorts. Our study provides a starting point for future investigations on the functionality of *AOX1* SNPs. However, variability in XK469 clearance cannot be attributed to polymorphisms in *AOX1*.

Keywords

XK469; aldehyde oxidase I; clearance; phase I

XK469 (NSC 697887), a synthetic derivative of quinoxaline phenoxypropionic acid, is a selective topoisomerase II β inhibitor. It has been evaluated in phase I clinical trials as a single agent for treatment of refractory solid tumors and hematological malignancies [1,2], although its development was subsequently discontinued due to lack of adequate anticancer activity. XK469 exhibits linear pharmacokinetics over the range of doses and intravenous schedules tested. Drug clearance is independent of dose and highly variable among patients, with a coefficient of variation ranging from 38-46%. The determinants of this variability are unknown.

XK469 is extensively metabolized to form mainly S-oxide by aldehyde oxidase I (AOX1) [1], a major cytosolic enzyme with broad substrate specificity and high hepatic expression. The human *AOX1* gene is located at 2q32.3-2q33.1. It consists of 35 exons and spans approximately 85kb. The functional impact of *AOX1* polymorphisms on drug pharmacokinetics has not been studied.

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We report the results of the pharmacogenetic components of two clinical studies [1,2]. Our analysis is limited to Caucasian patients to eliminate the confounding factor of race. The study evaluated whether 41 *AOX1* single nucleotide polymorphisms (SNPs) and seven liver expression quantitative trait loci (eQTLs) are associated with XK469 plasma clearance in Caucasian patients with advanced refractory solid tumors (n=59) and leukemia (n=33). Patient characteristics are listed in Supplementary Tables 1 and 2. Treatment schedules, plasma collection, measurement of XK469 concentrations and estimation of drug clearance have been previously described [1,2].

We resequenced all 35 exons, exon-intron boundaries, intronic conserved noncoding sequences, and 5' (3 kb) and 3' (1 kb) flanking regions of *AOX1* in seven patients with extreme phenotypes (four high and three low clearance subjects) selected from the first 43 patients enrolled in the solid tumor trial (see Supplementary Materials and methods and Supplementary Table 3). Forty-nine SNPs were identified including a novel one (in intron 20, Chr2 position 201492254). SNP information was summarized according to dbSNP (<http://www.ncbi.nlm.nih.gov/SNP>) data, and was highly consistent with our results (Supplementary Table 4).

Out of these 49 SNPs, we selected 18 candidate tag *AOX1* SNPs using a visual genotype plot of the resequenced data created with the Genome Variation Server (<http://gvs.gs.washington.edu/GVS/>). Twenty-nine additional HapMap tag SNPs were selected using Haploview-Tagger and CEU data from phases II and III (*AOX1* ±5kb), a minor allele frequency (MAF) cutoff of 10%, and linkage disequilibrium cutoff of $r^2 \geq 0.8$. This gave us a total of 47 SNPs but as six sequencing bins overlapped with HapMap bins, there were 41 SNPs to genotype (Supplementary Table 5). SNP rs10179964 failed design, reducing the number of tag SNPs to forty. We also genotyped a nonsense mutation (rs199984835) previously reported [3]. Genotyping assays and primers are found in the Supplementary Materials and methods and Supplementary Table 6.

For selecting candidate eQTLs that may regulate *AOX1* expression, we used human liver data from a previous study [4]. Briefly, *AOX1* expression was mapped to genomic loci as eQTLs, which were identified using Bayesian regression on residual expression after adjusting for the effects of hybridization protocol, age, sex, ancestry and unmeasured confounding variables from a surrogate variable analysis [5], as previously described [4]. A Manhattan plot was generated to illustrate the eQTL mapping. The ten top eQTL signals ($\log_{10}$ Bayes factor ≥ 3) were selected. Only seven of these ten SNPs were genotyped, as three are in the same tag SNP bin (have pairwise- r^2 values ≥ 0.8) (see Supplementary Materials and methods and Supplementary Table 7).

Forty-eight SNPs (40 tag SNPs, one nonsense SNP and seven eQTLs) were genotyped. In addition to laboratory-based quality checks, each SNP was tested for deviation from Hardy-Weinberg equilibrium. The Jonkheere-Terpstra statistic was used to test for association between SNPs and clearance. Analyses were powered for additive SNP effects. The corresponding effect size was estimated using the Kendall tau coefficient of concordance (τ). Statistical analyses were carried out using the R statistical environment (<http://www.r-project.org/>) along with the extension packages *clinfun* (for the Jonkheere-Terpstra test implementation) (R package version 1.0.4; <http://CRAN.R-project.org/package=clinfun>) and *HardyWeinberg* (R package version 1.4.1; <http://CRAN.R-project.org/package=HardyWeinberg>). P-values were not adjusted for multiple testing.

SNP rs199984835 was monomorphic. No deviation from Hardy-Weinberg equilibrium was observed for all other polymorphisms. The median (range) XK469 clearances were 185 (61-467) and 194 (25-343) ml/h for solid tumor and leukemia patients, respectively.

Distribution of clearances was independent of patient group (two-sided $P = 0.94$, Wilcoxon rank sum test). When combining all patients, there was no evidence for an association between XK469 clearance and *AOX1* SNPs. A few significant associations were found when analyzing the solid tumor or leukemia patients separately (Supplementary Table 8). Solid tumor patients with rs10931910 showed a significant decrease in drug clearance ($\tau = -0.32$, $P = 0.003$). The median clearances conditional on genotype were 242 (AA) vs. 172 (AG) vs. 145 (GG) ml/h. These correspond to effect sizes of 41% decrease (AA vs. AG), 19% decrease (AG vs. GG) and 67% decrease (AA vs. GG) (Figure 1A). We found no evidence of an additive or multiplicative genotype-by-sex interaction (data not shown). In the leukemia cohort, there was no association with rs10931910 ($\tau = 0.18$, $P = 0.20$) and the trend in median clearance was reversed (Figure 1B). Marginal changes in clearance were observed in solid tumor patients with rs6435060 ($\tau = -0.26$, $P = 0.02$), and in leukemia patients with rs2881811 ($\tau = -0.38$, $P = 0.01$), rs2287013 ($\tau = 0.32$, $P = 0.02$) and rs4674302 ($\tau = 0.34$, $P = 0.02$). However, these findings could not be reproduced across cohorts. The eQTLs rs12674549 and rs13280706 were associated with changes in clearance in solid tumor ($\tau = -0.23$, $P = 0.03$) and leukemia ($\tau = 0.33$, $P = 0.03$) patients, respectively. All associations observed are no longer significant following adjustment for multiple comparisons (data not shown).

This comprehensive pharmacogenetic study investigated the effects of genetic variation in *AOX1* using XK469 plasma clearance as a probe for *AOX1* activity. SNP rs10931910 was associated with reduced clearance in one of the cohorts, suggesting this may be a functional variant. However, as the effect was not consistently shown in the leukemia study and the variant has no known function, its clinical significance is yet uncertain.

There are very few reports on the potential functionality of *AOX1* variants. A nonsense mutation C>G (Y126stop) identified in exon 5 (rs199984835) was predicted to encode a nonfunctional protein [3]. This SNP was found to be monomorphic in our patients and its effect on clearance could not be tested. The biological significance of the nonsynonymous SNPs rs55754655 (N1135S) and rs373177 (H1297R) is unclear. A previous study reported a modest increase in catalytic efficiency for these two variants although the effect was not observed across all substrates tested [3]. SNP rs55754655 was associated with lack of clinical response to azathioprine in inflammatory bowel disease [6], and higher dose requirements and incidence of hepatotoxicity in kidney transplant patients [7]. On the other hand, *in silico* analysis predicted that the SNPs would have no functional impact [8], and we did not observe an effect of rs55754655 on XK469 clearance. We found rs3731722 when sequencing the outliers (Supplementary Table 4) but it was not genotyped in all patients due to its low MAF (<10%). Other SNPs in the same bin had also low MAF.

The function of rs13280706 (eQTL located in *CYP7B1*) as it relates to drug clearance is unknown. There is a microRNA gene (hsa-mir-124-2) located 200 kb downstream of *CYP7B1*. Future studies could investigate the possibility that the SNP can influence the function of the microRNA and regulate *AOX1*. The other eQTL associated with clearance (rs12674549) is located in an intergenic region close to *MYOM2*. The function and regulation of this SNP and its relationship with XK469 clearance are yet unclear.

In conclusion, our study did not demonstrate a strong relationship between *AOX1* polymorphisms and XK469 clearance. Variability in enzyme activity is likely to be attributed to factors other than genetic variation in *AOX1*. Regulation of human *AOX1* activity has not been investigated extensively. The gene is downregulated by adipopectin through activation of peroxisome proliferator-activated receptor alpha [9], and decreased *AOX1* expression has been found in cancer tissues due to DNA methylation [10]. Further

studies are needed to elucidate the determinants of phenotypic variability and their impact on pharmacokinetics.

There were a few study limitations. The sample size was relatively small which may have limited our ability to detect small effects of SNPs on drug clearance. As S-oxide concentrations were not determined, we relied on XK469 clearance as the marker for AOX1 activity. We genotyped two different disease populations, and the two trials had modest differences in eligibility criteria for liver and kidney function. We did not test the influence of organ function on XK469 clearance but the median clearance was similar between cohorts. Development of XK469 has been discontinued but since AOX1 plays an important role in the metabolism of many drugs commonly prescribed such as methotrexate, 6-mercaptopurine and zaleplon [11], knowledge gained from this study could be applied to research aiming to better understand variability in AOX1 activity.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Figure 1. XK-469 clearance by *AOX1* rs10931910 genotype in (a) solid tumor and (b) leukemia patients. Plots show the median, 10th, 25th, 75th and 90th percentiles as vertical boxes with error bars. Jonkheere-Terpstra test results are shown.