Cytochrome P450 3A5 expression in the kidneys of patients with calcineurin inhibitor nephrotoxicity

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Abstract

Background. Nephrotoxicity secondary to calcineurin inhibitors is common in renal transplant recipients, occurring in 76–94% of patients. The role of drug transporters (P-glycoprotein) and drug metabolizing enzymes (cytochrome P450) as predisposing factors toward nephrotoxicity or its prevention has not been thoroughly examined.

Methods. The objective of this study was to analyse cytochrome P450 3A5 (CYP3A5) expression in kidneys of solid organ recipients by immunohistochemistry to determine if there is an association between expression of this enzyme and calcineurin inhibitor toxicity. Transplant recipients were compared with a control group.

Results. Apical tubular plasma membrane staining for CYP3A5 was present in 62% of study and 100% of control biopsies ($P = 0.0012$). Proximal and distal tubular nuclear staining intensity was similar between groups. Cytoplasmic staining in both the proximal (2.1 ± 0.9 vs 1.4 ± 0.9) and distal (2.8 ± 0.5 vs 1.8 ± 1.1) tubules was greater in the control vs study population specimens, respectively ($P = 0.0093$ and $P = 0.0005$, respectively). Regression models that controlled for use of CYP3A inhibiting and inducing medications, age, gender, race and glomerular filtration rate did not predict differences between study groups with regard to staining locations and intensity, except for the cytoplasm of the distal tubule, where intensity of staining was significantly lower in the study group ($0.9 ± 0.3; P = 0.002$).

Conclusions. This study showed decreased expression of CYP3A5 in nephrotoxic biopsies as compared with a control group. Our data suggest that the relationship between reduced presence of CYP3A5 in the kidney tubules and nephrotoxicity should be further explored to elucidate the role of this enzyme in mediating toxicity.

Keywords: cyclosporine; cytochrome P450; immunohistochemistry; nephrotoxicity; renal transplant recipients; tacrolimus

Introduction

Ciclosporin and tacrolimus are frequently prescribed calcineurin inhibitors to prevent solid organ transplant rejection episodes and are often employed as immunosuppressants for other disorders [1–3]. Nephrotoxicity is the most frequent and severe adverse event that either precludes their use or at least requires close monitoring. Nephrotoxicity secondary to calcineurin inhibitors occurs in liver (52%), heart (20–75%) and kidney (76–94%) transplant recipients [4–7]. This form of nephrotoxicity clinically presents as an elevation in serum creatinine and blood urea nitrogen, as well as disorders of electrolytes, acid-base status, renal endocrine function and urine concentrating ability. Morphologically, structural changes associated with the renal toxicity are found mainly in the tubules, afferent arterioles and glomeruli [8].

Investigators continue to explore the aetiologies for calcineurin inhibitor-related nephrotoxicity [9–11]. The examination of the role of intracellular transport proteins (drug efflux proteins) in modulating calcineurin inhibitor toxicities is appealing given that P-glycoprotein (P-gp) is known to transport both ciclosporin and tacrolimus [12]. We and others [13–15] have reported the presence of P-gp predominantly on the apical membrane of renal tubules. The role of P-gp in regulating the nephrotoxicity of calcineurin inhibitors has also been proposed, given its activity as an export pump on the apical membranes of various tissues [14,16]. It is intriguing to consider the supportive role of the cytochrome P450 drug metabolizing enzyme CYP 3A5 in ameliorating this form of drug-induced nephrotoxicity since this enzyme
can metabolize pharmacologically active agents to less active/toxic substances and also co-localizes in the genome and at anatomical locations with P-gp [17]. Additionally, some substrates (including ciclosporin and tacrolimus) are metabolized and transported by CYP3A5 and P-gp, respectively [18]. Lastly, CYP3A5 is primarily found in the anatomical location of the kidneys, where local calcineurin inhibitor metabolism may occur, whereas CYP3A4 and other CYPs are predominantly localized to the liver [19–21].

In our previous study, we showed that P-gp expression was reduced in transplant patients who exhibited structural signs of calcineurin inhibitor nephrotoxicity in comparison with controls, suggesting an association between P-gp and nephrotoxicity associated with these agents [14]. Since the calcineurin inhibitors are substrates for CYP3A5 metabolism in addition to being substrates for P-gp transport, we decided to analyse renal CYP3A5 expression by immunohistochemistry to further elucidate the association between nephrotoxicity secondary to calcineurin inhibitors and the drug disposition-association factor of metabolism. We hypothesized that transplant patients with structural nephrotoxicity would exhibit reduced expression of CYP3A5 in the kidney—suggesting an association between local (renal) metabolism and nephrotoxicity.

Methods

A HIPPA (Health Insurance Portability and Accountability Act) waiver to study existing pathology specimens and medical records was approved by the University Investigational Review Board (IRB). The nephropathology database was reviewed for renal biopsy specimens from solid organ transplant recipients that were coded for calcineurin inhibitor-induced toxicity (i.e. study group). Structural calcineurin inhibitor-induced nephrotoxicity was defined according to the criteria by Mihatsch et al. [8,22,23]. The nephropathological structural criteria used were previously described [14]. A control group of renal biopsy specimens from age-matched patients who lacked any morphological evidence of calcineurin inhibitor-induced toxicity was selected from the nephropathology database. This control group included biopsy specimens from patients with various renal diagnoses including acute interstitial nephritis (3), thrombotic microangiopathy (4), amyloid (1), diabetes mellitus (6), acute cellular rejection (w/o nephrotoxicity) (4), minimal change disease (5) and arterionephrosclerosis (7). All biopsies in the study and control groups showed only minor degrees of tubular damage not >20% of the biopsy cores. Charts of control and nephrotoxic patients were evaluated for demographics, clinical and laboratory data and prescribed medications that are known to induce or inhibit CYP3A. Renal laboratory data of interest including serum creatinine, estimated glomerular filtration rate from the Cockcroft-Gault formula [24], and levels of proteinuria were collected at the time of biopsy.

Immunohistochemistry

The immunohistochemistry assay was previously described [14]. Incubations were performed on 5μ thick sections cut from formalin-fixed and paraffin-embedded tissue cores. Specific to this study, slides were incubated with the primary antibody, rabbit anti-human CYP3A5 polyclonal antibody (Chemicon International, Inc.) 1/5000 in Ventana solution overnight at 4°C in a humidity chamber. Negative controls were incubated overnight in rabbit Envision+Universal Negative Control (DAKOCytomation). After rinsing with Automation buffer, the slides were incubated in secondary antibody (Envision+HRP Rabbit, DAKOCytomation, Carpinteria, CA) for 30 min at room temperature.

Evaluation of staining

The study and control biopsy specimens were reviewed in a blinded approach by the same nephropathologist (V.N.) in order to limit inter-observer variations. All biopsies showed at least five glomeruli and surrounding cortical parenchyma, insuring adequate insight into tubules. The scoring methods for assessment of CYP3A5 staining were modified from that previously reported for P-gp [16].

Proximal and distal tubular CYP3A5 expression was evaluated and scoring was then differentiated by apical membrane, cytoplasm and/or nuclei. Nuclear and apical staining was denoted as either positive or negative. Cytoplasmic staining was defined by intensity according to 0 (no staining) to 4 (maximum staining). The total percentage of tubular cells in the cortex that stained positive were classified as 1 (≤25%), 2 (>25–50%), 3 (51–75%) and 4 (>76%).

Statistical analysis

Descriptive statistics were defined by means ± SDs, medians and ranges and percentages. Differences in demographics, clinical parameters and immunohistochemistry measures between the study and control groups (including gender, race, diagnosis of diabetes mellitus, presence of CYP3A inhibitors or inducers, CYP3A5 presence in proximal and distal tubules and nuclear and apical staining presence) were compared using Fisher’s Exact test for categorical measures. Wilcoxon rank sum tests were used for evaluation of differences in continuous measures between the groups including age, weight, serum creatinine, glomerular filtration rate, proteinuria at biopsy, cytoplasmic staining intensity and total percentage of the renal cortical cells with positive CYP3A5 staining.

Logistic regression modelling for two-level variables and linear regression modelling for continuous measures were used to evaluate differences in measures between groups while controlling for the confounders—gender, race, age, glomerular filtration rate and presence of prescribed CYP3A inducing and inhibiting drugs. Results from univariate statistical tests were used as a guide in selecting variables for multivariable statistical modelling. Variables that had either a statistically significant impact on the measure of interest or a confounding influence on the association of the group (study/control) vs outcome were retained in multivariable models. Since there is no statistical test for confounding, measures that altered the primary univariate association by ≥20% was used as a guideline for keeping variables in the models.

The sample size for this study was determined a priori and was based on the ability to detect statistical differences in the
Results

Twenty-nine nephrotoxic and 30 control biopsy specimens were analysed (Table 1) [14]. The percentage of patients who were prescribed potential CYP3A inducers was higher in the study (79%) vs control (24%) renal biopsy groups (P < 0.0001). These drugs included hydrocortisone, prednisone, carbamazepine and vitamin D. There were no significant differences between the transplant (21%) and control (17%) groups in the percentage of patients who were prescribed CYP3A inhibitors (P = 0.99). The prescribed drugs listed in the potential inhibitor grouping included diltiazem, fluconazole, trazodone, sertraline, fluoxetine and paroxetine.

Figure 1 shows the location and percentage of biopsies with positive CYP3A5 staining for the study and control subjects. Proximal tubular nuclear staining was not statistically different between groups; present in 62% of study vs 77% of control biopsies (P = 0.35). The apical membrane of the proximal tubules stained positive for CYP3A5 in only 62% of study biopsies as compared with 100% of controls (P = 0.0012). Distal tubular nuclear staining was present in 97% of study and control biopsies (P = 0.99). The apical membrane of distal tubules stained positive for CYP3A5 in only 58% of study vs 83% of control subjects (P = 0.0470). Cytoplasmic staining for CYP3A5 occurred in both the proximal (mean score: 2.1 ± 0.9 vs 1.4 ± 0.9) and distal (mean score: 2.8 ± 0.5 vs 1.8 ± 1.1) tubules significantly more frequently in the control specimens vs study biopsies (P = 0.0093 and P = 0.0005, respectively) (Figure 2). The percentage of renal cortical cells that stained positive for CYP3A5 was 98% in the control vs 82% in the study group (P = 0.0581) (Figure 3). Photomicrographs showing representative CYP3A5 staining in the kidney biopsies of study and control subjects is provided in Figure 4.

Regression models that controlled for concomitantly prescribed CYP3A inhibitors and inducers as well as age, gender, race and glomerular filtration rate did not predict statistically significant differences between the patient groups with regard to staining patterns. Multivariable modelling was not possible in assessment of staining differences at the level of the apical membrane of the proximal tubule (100% of the controls had this staining). The difference in the distal tubule apical membrane staining, however, was not found to be different with multivariable modelling: The difference in intensity of staining in the cytoplasm of the distal tubules between groups remained statistically significantly lower (0.9 ± 0.3; P = 0.002, mean difference between the groups) in the nephrotoxic group even when controlling for other factors, while a non-significant difference in intensity of staining in the cytoplasm of proximal tubules was found with the multivariable modelling approach.

Discussion

Both ciclosporin and tacrolimus are substrates for the CYP3A subfamily of drug metabolizing enzymes. Ciclosporin is known to be metabolized by CYP3A to at least 25 metabolites. Of these, one of the nine

Table 1. Patient demographics and clinical laboratory results

<table>
<thead>
<tr>
<th></th>
<th>Nephrotoxic (n = 29)</th>
<th>Control (n = 30)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>44 ± 13</td>
<td>40 ± 20</td>
<td>0.6511</td>
</tr>
<tr>
<td>Weight (kg)</td>
<td>78 ± 22</td>
<td>77 ± 27</td>
<td>0.7312</td>
</tr>
<tr>
<td>Gender (% male)</td>
<td>63</td>
<td>56</td>
<td>0.8439</td>
</tr>
<tr>
<td>Race (% Caucasian)</td>
<td>62</td>
<td>59</td>
<td>0.9674</td>
</tr>
<tr>
<td>Diabetes mellitus</td>
<td>31</td>
<td>28</td>
<td>0.9127</td>
</tr>
<tr>
<td>CYP3A inhibitors (%)</td>
<td>21</td>
<td>17</td>
<td>0.99</td>
</tr>
<tr>
<td>Serum creatinine (mg/dl) at biopsy</td>
<td>3.5 ± 2.5</td>
<td>2.2 ± 1.5</td>
<td>0.0001</td>
</tr>
<tr>
<td>Glomerular filtration rate (ml/min) at biopsy</td>
<td>40 ± 21</td>
<td>69 ± 50</td>
<td>0.0527</td>
</tr>
<tr>
<td>Proteinurin (mg/dl) at biopsy</td>
<td>2056 ± 2527</td>
<td>3481 ± 7285</td>
<td>0.1827</td>
</tr>
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Table modified with permission from reference [24].
Fig. 2. Tubular cytoplasmic staining. Cytoplasmic staining was defined by intensity according to 0 (no staining) to 4 (maximum staining). (A) Represents cytoplasmic staining in the proximal tubule in the control and nephrotoxic transplant biopsies. (*P = 0.0093). Error bars represent the minimum and/or maximum values. Control group: minimum = 0, 25th percentile = 1.0, median = 2.2, 75th percentile = 3.0, maximum = 3.0. Nephrotoxicity group: minimum = 0, 25th percentile = 1.0, median = 1.0, 75th percentile = 2.0, maximum = 3.0. (B) Represents cytoplasmic staining in the distal tubule in the control and nephrotoxic transplant biopsies. (*P = 0.0005). Error bars represent the minimum and/or maximum values. Control group: minimum = 2.0, 25th percentile = 2.5, median = 3.0, 75th percentile = 3.0, maximum = 4.0. Nephrotoxicity group: minimum = 0, 25th percentile = 1.0, median = 2.0, 75th percentile = 3.0, maximum = 4.0. *Denotes the median values on the graph.

Fig. 3. Percentage of Cortical Cells Staining Positive for CYP3A5. The cortical cells staining positive for CYP3A5 was greater in control (98%) vs nephrotoxic transplant biopsies (82%). (*P = 0.0581). The total percentage of renal cortical cells that stained positive were classified on a scale of 1 to 4: 1 (<25% of cells stained), 2 (25–50% of cells stained), 3 (51–75% of cells stained) and 4 (>75% cells stained). Error bars represent the minimum and/or maximum values. Control group: minimum = 3.0, 25th percentile = 4.0, median = 4.0, 75th percentile = 4.0, maximum = 4.0. Nephrotoxic group: minimum = 1.0, 25th percentile = 2.5, median = 4.0, 75th percentile = 4.0, maximum = 4.0.

readily identified metabolites also has some activity, but the nephrotoxicity potential has not been reported [25–27]. Ten metabolites of tacrolimus have been identified, with two of these exhibiting some activity [28]. It was thus feasible to explore the association of reduced CYP3A5 and nephrotoxicity in this pilot study since lower expression of CYP3A5 could contribute to more active/toxic levels of pharmacological agents in the kidney tissues.

While it is known that CYP3A4 is predominantly localized to the liver and intestines [29], immunohistochemistry analyses have shown little to no staining for this enzyme in the kidneys [20]. CYP3A5, on the contrary, is predominantly localized to the kidney [20]. Studies evaluating the presence of CYP3A5 in normal kidneys and human renal cell cancer immunohistochemically suggest a predominant intracytoplasmic tubular localization of the enzyme [30]. We extend previous observations by carefully correlating morphological signs of calcineurin inhibitor toxicity with immunohistochemical staining patterns for CYP3A5.

In agreement with previous reports, our results showed that both the proximal and distal tubules stained for CYP3A5 [30]. We found staining signals in the nuclei, apical tubular plasma membranes and the tubular epithelial cytoplasm. Nuclear CYP3A5 expression was similar between study and control biopsy specimens. On the contrary, nephrotoxic specimens showed significantly less CYP3A5 expression in the apical membrane of both the proximal and distal tubules than the controls, underscoring the potential role of CYP3A5 in the development of calcineurin inhibitor nephrotoxicity. This observation is further consolidated by clinical data since nephrotoxic patients (vs controls) more frequently consumed CYP3A4-inducing medications, which should have theoretically resulted in over-expression than reduction of CYP3A5.

Our finding regarding the presence of reduced CYP3A5 in the study population requires inquiry regarding the potential role of disease or demography in local tissue enzyme expression patterns. Although our control population represented a diversity of kidney diseases, it is adequately chosen given the retrospective nature of our study. Cases of glomerulonephritis are often accompanied by ‘secondary’ tubulo-interstitial injury and inflammation. Thus, the differences reported in our study cannot simply be explained by significant differences in the degree of tubulo-interstitial injury found in the study groups.

The variables of age, race and CYP3A5 genotype can also be hypothesized to be associated with
CYP3A5 expression. Hepatic CYP450 enzyme expression and activity has been reported to decline with age and renal function, and our nephrotoxic patients had lower levels of kidney functions [31–35]. To further investigate the effects of demographic factors and measured level of renal function, we employed multivariable regression models in our study to evaluate the effects of age, race, gender and glomerular filtration rate on biopsy staining locations and intensities. Although controlling for these factors influenced the association between staining patterns and patient group, none was an independent predictor of differences in staining intensities or locations. We did not include genotyping for wild-type and variants of CYP3A5 in our study and hence are unable to associate genotype with nephrotoxicity risks.

Conclusions

Our study is the first to report an association between reductions in tubular CYP3A5 expression in a nephrotoxic calcineurin inhibitor-treated transplant patient population as compared with a control population. This reduction was notably observed in patients treated with a significantly greater number of medications known to induce CYP3A expression. Our preliminary data suggest that further studies should be conducted to determine whether reduced CYP3A5 expression in the kidney might be one factor promoting the development of structural nephrotoxicity secondary to calcineurin inhibitor therapy. Additional study is required to analyse the effects of renal diagnoses, glomerular filtration rate and pharmacogenetics on CYP3A5 expression and/or function in the kidney and other metabolizing organs.

Acknowledgements. This study was supported by a research grant from the American College of Clinical Pharmacy (ACCP) Research Institute. In addition, this study was presented in part at the 2003 Annual Meeting of the American College of Clinical Pharmacy, Atlanta, GA and at the 37th Annual Meeting of the American Society of Nephrology, St Louis, MO.

Conflict of interest statement. None declared.

References


Fig. 4. Immunohistochemical incubations to detect P450 3A5. (A) Control group: Strong staining (brown) is found in tubules (apical epithelial surfaces, nuclei and cytoplasm). (B) Study group with structural calcineurin inhibitor toxicity: Only minimal staining is noted in rare tubular epithelial cell nuclei. (20× magnification).


Received for publication: 14.7.06
Accepted in revised form: 16.2.07