CYP3A4 Polymorphisms and Variation in Cyclosporine Metabolism

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A. Study Purpose and Rationale

Cyclosporine is an important mainstay of anti-rejection therapy but it has a narrow therapeutic index (therapeutic window between 100- 150 ng/mL) and highly individualized pharmacokinetics. In chronic overdose it contributes to chronic allograft nephropathy and graft loss. Currently cyclosporine trough drug levels are used to monitor and adjust the dosing regimen. The CYP3A family of enzymes metabolizes many drugs and provides 30% of the cytochromes in the liver and 70% in the intestine; cyclosporine is the major substrate for CYP3A4 and to some extent 3A5. CYP3A4 is responsible for the majority of cyclosporine metabolism and hence bioavailability. CYP expression in the liver and intestine is variable; several 3A5 polymorphisms have been discovered that drastically alter 3A5 expression, including CYP3A5*3 and CYP3A5* 1. Further studies have demonstrated a link between the CYP3A4* I and CYP3A5*3 polymorphisms and 40% decreased dose tacrolimus required to achieve therapeutic drug levels. CYP3A polymorphisms have thus been linked to altered metabolism and dosing requirements of cyclosporine in transplant patients. Polymorphisms in the CYP3A genes have been shown to exist in approximately 8.5 -10% of the population. A better understanding of the role of CYP3A polymorphisms in cyclosporine metabolism and dosing might lead to a more individually tailored approach to drug dosing, hopefully with improved drug efficacy and fewer side effects.

B. Study Design and Statistical Analysis

Approximately 1000 patients will be screened for potential enrollment in this study; 137 "normals" (those with wild type CYP 3A) and 137 "mutants" (those hetero- or homozygous for any CYP3A polymorphism) will be required for the study. Normals will be selected preferentially to match with any identified mutant; thus all mutants identified will be continued in the study, but only 137 of the normals who match mutant demographics will be continued out of the likely 863 screened. Demographic information such as age, sex, race, date of transplant, time on hemodialysis, etiology of ESRD, type of donor and rejections will be recorded.

An average dose per kg per blood level will be calculated for each participant, from 3 different values recorded over time with a stable cyclosporine dose and creatinine clearance. As several highly active polymorphisms have already been identified it will be valid to consider them as having a group effect on dosage. These two ranges will be compared. The previously published range of normal values is 0.4-3.0 mg/kg/level with a SD=0.6. I expect to detect 5% of normals and 15% of mutants falling outside this range. This study, with an alpha of 0.05 is powered at 80% via a Chisquare test to detect a difference of 10% in the event rate.

Chi-square sample size with equal group sizes:

Mutant rate of abnormal clearance = P2 = 15% = 0. 15
Control rate of abnormal clearance = pi 5%=0.05
Rate ratio = R = PI/P2 = 0.05/0.15 = 1/3
α= 0.05
1 - β = 0.80
k = 7.85
n = group sample size

$$n=\frac{k((R+1)\,p_2\,(R^2+1))}{p_2(1-R)^2}$$

$$n=\frac{7.85((l/3+1)-0.15((1/3)^2+1))}{0.15(1-1/3)^2}$$

$$n=\frac{7.85(4/3-0.15(119+1))}{0.15(2/3)^2}$$

$$n=\frac{7.85(12/9-0.15(10/9))}{0.15(4/9)}$$

$$n=7.\frac{85(12/9-1.5/9)}{0.6/9}$$

$$n=\frac{7.85(12-1.5)}{0.6}$$

n=137-375

Absolute Risk = 0. 15 - 0.05 = 0. 10
Relative Risk = 0. 15/0.05 3.0
Number Needed to Harm I /AR = 1 /0. 1 10

	Abnormal clearance	Normal clearance	Total
Mutant	0.15(137)=20.55=21	137 - 21 = 116	137
Control	0.05(137)= 6.85 = 7	137 - 7 ~ 130	137

Previous studies in general failed to demonstrate a significant difference in cyclosporine dosing, most likely because they were insufficiently powered to do so. Further analysis will compare each particular polymorphism group to the normal range, although this study is not in particular powered for this analysis. Regression analysis will also be done to identify any differences between the groups that may have otherwise accounted for any positive result garnered.

C. Study Procedures

Study subjects will have one 5mL vial of blood drawn at the time of a routine surveillance blood draw. Cyclosporine dose, trough cyclosporine blood level, patient's weight and creatinine clearance will be recorded at that time. Genomic DNA will be extracted from the sample and the entire CYP3A4 gene sequenced. The sequences will be compared to those previously published; known polymorphisms will be identified and novel ones characterized. Patients will be registered in the database as wild type, homozygous or heterozygous for all identified polymorphisms. A total of 3 data points will be recorded for trough cyclosporine blood level at a stable dose and creatinine clearance; these values will be averaged. A dose per kg per level will be calculated for each patient enrolled in the study. These data will be averaged to calculate an average dose/kg/level for the wild type patients, which will be compared with the average value for all patients having any polymorphism as well as specific genotypes.

D. Study Drugs

Cyclosporine is an FDA approved calcineurin-inhibitor, indicated for the prevention of rejection in renal transplant recipients. It is a small fungal polypeptide which complexes with the cytoplasmic binding protein cyclophilin, and binds with calcineurin. This prevents the calcineurin mediated dephosphorylation of other proteins, blocking the expression of the cytokines responsible for T cell activation. It is administered orally and doses are adjusted per blood level, as is standard practice.

E. Medical Device

not applicable

F. Study Questionnaires

not applicable

G. Study Subjects

Any patient over the age of 18 who has had a renal transplant (cadaveric or living donor) for at least 6 months, with a stable creatinine clearance >30cc/hr by the MDRD calculation and no evidence of acute rejection at the time of enrollment are eligible for participation in this study. Patients with more than one allograft may not participate in this study. Patients who are taking any other medication known to interact with the metabolism of cyclosporine will not be eligible for this study; this includes: phenobarbital, dilantin, rifampin, cimetidine, ketoconazole, macrolide antibiotics, calcium channel blockers, or neur'ontin.

H. Recruitment of Subjects

Patients will be approached regarding their participation in this study by their private nephrologist at a routinely scheduled out-patient appointment. Once the patient has expressed interest in participation, he will be approached by the Principal Investigator regarding consent. There will be no mailing, flyers or advertisement.

Ideally study subjects will be recruited from all 5 major transplant centers in NYC. As approximately 100 renal transplants are performed per center per year, this would make it possible to screen a sufficient number of study subjects in about 2 years. If the other centers do not wish to participate, a sufficient number of subjects could still be enrolled from our center alone, including participants who have had their allograft for several years.

I. Confidentiality of Study Data

All study data will be kept strictly confidential. Study specimens will be given a unique numerical identifier at the time of study entry, and all specimens and data collected about them will use only that number. The masterlist of study participant names and unique identifiers will be kept in a locked cabinet in the Principal Investigator's office. No information of any kind will be shared with insurance companies, the study subjects, their physicians or other Columbia University personnel.

J. Potential Conflict of Interest

none

K. Location of Study

Ideally this will be a multicenter study involving the 5 major transplant centers in NYC. Until the protocol is approved for use in those institutions, the study will take place in the out-patient Renal Transplant Clinic currently located in PH- 12, where the blood specimens will be drawn. DNA extraction and analysis of the blood specimens will take place in the Principal Investigator's laboratory in the Black Building.

L. Potential Risks

The only risks associated with this study are those of having an additional 5mL of blood drawn at the time of routine blood testing for kidney transplant monitoring. There will not be an extra needlestick required for this study.

M. Potential Benefits

Study participants will not directly benefit from participating in this study beyond contributing to the general scientific knowledge. Patients will not be privy to genotyping information.

N. Alternative Therapies

If patients choose not to participate in this study, they will continue to receive all the standard and appropriate care given to renal transplant patients at CUMC.

O. Compensation

Patients will not be compensated for their participation in this study.

P. Costs to Subjects

Patients will not incur any additional cost for participation in this study; the expense of genotyping samples will be paid for by the Principal Investigator's research grant.

Q. Minors as Research Subjects

not applicable

R. Radiation

not applicable

S. References

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