Faculdade de Medicina de São José do Rio Preto
Programa de Pós-graduação em Ciências da Saúde

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Polimorfismo das UDP-glucuronosiltransferases e efeitos adversos em indivíduos receptores de transplante renal em terapia com micofenolato mofetil

São José do Rio Preto
2008

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Tese apresentada à Faculdade de Medicina de São José do Rio Preto para obtenção do Título de Doutor no Curso de Pós-Graduação em Ciências da Saúde, Eixo Temático: Medicina Interna.

Orientadora: Dra Érika Cristina Pavarino-Bertelli

São José do Rio Preto
2008

Betônico, Gustavo Navarro
Polimorfismo das UDP-glucuronosiltransferases e efeitos adversos em indivíduos receptores de transplante renal em terapia com micofenolato mofetil.
São José do Rio Preto, 2008
92 p.

Tese (Doutorado) – Faculdade de Medicina de São José do Rio Preto – FAMERP
Eixo Temático: Medicina Interna

Orientador: Profa. Dra. Érika Cristina Pavarino-Bertelli

1. Micofenolato Mofetil; 2. UGT; 3. Transplante renal;
4. Polimorfismo

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São José do Rio Preto, 15/02/2008

Sumário:

DEDICATÓRIA

À minha esposa Carolina,
por ter sempre me apoiado e por ter me mostrado que tudo o que passamos só fez crescer nosso amor.

Aos meus filhos Mariana e Gustavo,
que sempre me receberam com sorrisos inocentes e aconchegantes apesar da ausência que às vezes lhes impus durante esta jornada.

Aos meus pais, Renato e Maria Helena e meu irmão Daniel,
que enquanto eu batalhava neste projeto, batalharam e venceram, juntos, uma guerra inteira pela vida.

Para os erros há perdão;
para os fracassos, chance;
Não deixe que a saudade sufoque,
que a rotina acomode,
que o medo impeça de tentar.
Desconfie do destino e
acredite em você.

Gaste mais horas realizando que sonhando,
fazendo que planejando,
vivendo que esperando
Porque, embora quem quase morre esteja vivo,
quem quase vive já morreu.

Fernando Pessoa

AGRADECIMENTOS

A Deus,

por permitir que eu esteja aqui, ao dar-me a vida e abençoar-me nestas estradas...

À Prof[a]. Dr[a]. Érika Cristina Pavarino Bertelli

pela coragem de acreditar em alguém que não conhecia e assim ter me dado a chance de conhecê-la e assim aprender muito com sua dedicação e competência. Agradeço a orientação, sempre perspicaz e equilibrada, e o tempo que dispensou em meu favor, sempre demonstrando disponibilidade.

Ao Prof. Dr. Mário Abbud Filho

por ter me apresentado à FAMERP, à UPGEM e à Farmacogenética, proporcionando a concretização desta idéia. Agradeço por me instruir, com extrema simplicidade e afeição, no caminho pela pesquisa em transplante renal.

À Prof[a]. Dr[a]. Eny Maria Goloni Bertollo

pelo carinho e competência com que rege a pesquisa nesta instituição e neste laboratório.

À Prof[a]. Dr[a]. Chantal Guillemette e seus colaboradores

Pela disponibilidade de me receberem e me permitido a utilização das dependências do laboratório de farmacogenética da Universidade de Laval, Quebec, Canadá.

Às amigas Mariângela, Maria Paula, Joice e Patrícia

pelo tempo despendido comigo, pelas dicas que me deram, mas acima de tudo pela amizade e companheirismo que demonstraram nestes anos.

Aos meus amigos da Faculdade de Medicina da UNOESTE

pelo incentivo, pela amizade e pela ajuda fundamental nos momentos mais difíceis desta jornada.

Aos meus amigos do serviço de Nefrologia do Hospital Universitário Dr Domingos Leonardo Cerávolo

por entenderem a necessidade das minhas viagens a São José do Rio Preto e por assistirem os nossos doentes na minha ausência.

À direção da Faculdade de Medicina da UNOESTE e do Hospital Universitário Dr Domingos Leonardo Cerávolo

por terem acreditado neste projeto e me incentivado a realizá-lo.

À Prof[a]. Dr[a]. Maria Alice Sperto Ferreira

pela afabilidade, amizade e interesse que demonstrou neste projeto desde o seu início.

Aos amigos de estrada

por fazer do nosso trajeto não mais curto, mas com certeza muito mais agradável.

Aos estagiários e pós-graduandos da UPGEM

pelas conversas, pelo apoio e colaboração de todos.

Ao Prof. Dr. José Antônio Cordeiro

pela assessoria estatística, pelo conhecimento transmitido e pelas críticas tão bem fundamentadas.

Aos funcionários da Secretaria da Pós-graduação, em especial à Fabiana e José Antônio

por terem me dispensado atenção e me auxiliado na resolução de eventuais entraves que surgiram.

Aos funcionários da Biblioteca, do ambulatório e do serviço de diálise da FAMERP

pela disponibilidade e presteza dedicadas a este trabalho.

Aos pacientes transplantados renais

por se disponibilizarem e aceitarem participar deste trabalho. Sem eles este sonho seria irrealizável.

Ao Diretor Geral Prof. Dr. Humberto Liedtke Junior

pelo grande incentivo e contribuição para o desenvolvimento e fortalecimento desta Instituição

Ao Programa de Pós-graduação em Ciências da Saúde da FAMERP

pela excelência em pesquisa e constante dedicação no fortalecimento do curso de pós-graduação da Instituição.

Pouca coisa é necessária para transformar inteiramente uma vida: amor no coração e sorriso nos lábios.

Martin Luther King

Não existe essa de ciências aplicadas, somente aplicações da ciência.

Louis Pasteur

LISTA DE FIGURAS

ARTIGO I

LISTA DE TABELAS

LISTA DE ABREVIATURAS E SÍMBOLOS

AcMPAG	Metabólito acil-glucuronídeo do ácido micofenólico *Mycophenolic acid acyl-glucuronide*
AUC	Área sob a curva *Area under the curve*
AUC_{0-12}	Área sob a curva de 0 a 12 horas *Area under the curve from 0 to 12 hours*
CEP	Comitê de Ética em Pesquisa *Research Ethics Committee*
CINTRANS	Centro de Transplantes de Órgãos e tecidos da FAMERP *Center for Organ and Tissue Transplant- FAMERP*
CONEP	Comissão Nacional de Ética em Pesquisa *National Research Commission*
CYP450	Citocromo P450 *Cytochrome P450*
DNA	Ácido desoxirribonucléico *Desoxirribonucleic acid*
FAMERP	Faculdade de Medicina de São José do Rio Preto *São José do Rio Preto Medical School*
IMPDH	Inosino monofosfato desidrogenase *Inosine monophosphate dehydrogenase*
MDR1/ABCB1	Gene de resistência a múltiplas drogas *Human multidrug resistance gene*
MMF	Micofenolato Mofetil *Mofetil Mycophenolate*
MPA	Ácido Micofenólico *Mycophenolic Acid*
MPAG	Metabólito glucuronídeo do ácido micofenólico *Mycophenolic acid hydroxyphenyl-β-glucuronide*
MPAGI	Metabólito glucosídeo do ácido micofenólico *Mycophenolic acid glucoside*

μmol/L	Micromol / litro
PCR	Reação em Cadeia da Polimerase *Polymerase chain reaction*
SD	*Standard deviation*
SNP	Polimorfismo de nucleotídeo simples *Single nucleotide polymorphism*
TPMT	Tiopurina metiltransferase *Thiopurine s-methyltransferase*
UPGEM	Unidade de Pesquisa em Genética e Biologia Molecular *Genetics and Molecular Biology Research Unit*
UGT	Uridinoglucuronosiltransferase *Uridinoglucuronosyltransferase*

POLIMORFISMO DAS UDP-GLUCURONOSILTRANSFERASES E EFEITOS ADVERSOS EM INDIVÍDUOS RECEPTORES DE TRANSPLANTE RENAL EM TERAPIA COM MICOFENOLATO MOFETIL

RESUMO

Introdução: Estudos na área de farmacogenética têm sido realizados na tentativa de se demonstrar possível influência do padrão genético na variabilidade da resposta aos imunossupressores, criando assim uma nova ferramenta de ajuste na dosagem destes medicamentos. **Objetivo:** Avaliar possível associação dos efeitos adversos, principalmente hematológicos, gastrintestinais, infecciosos e imunológicos apresentados por indivíduos transplantados renais que receberam terapia com micofenolato mofetil (MMF), com os polimorfismos nos genes que codificam enzimas da família das uridino-glucuronosiltransferases (UGTs), responsáveis pela biodisponibilidade do ácido micofenólico, metabólito ativo do MMF. **Métodos:** Foram estudados transplantados renais adultos que receberam, por no mínimo 30 dias, doses de 1 a 2g/dia de MMF como parte do esquema imunossupressor. A genotipagem dos polimorfismos de *UGT1A8* (-999C/T, códon 255A/G, códon 277G/A), *UGT1A9* (-2152C/T, -275T/A, -118T9/10, códon 33T/C) e *UGT2B7* (-79G/A, códon 268C/T) foi realizada por meio de seqüenciamento automático e os cromatogramas gerados foram analisados no programa Staden Gap and PreGap4. Os genótipos obtidos foram comparados com os efeitos adversos eventualmente apresentados e com a necessidade de suspensão ou redução da dose do MMF. A análise estatística foi realizada utilizando-se o teste de qui-quadrado de Pearson. **Resultados:** Foram genotipados 74 indivíduos com 56 ± 41 meses pós-transplante em acompanhamento ambulatorial. Destes, 68,9% eram do sexo masculino, com

média de idade de 42 ± 12 anos. A taxa de filtração glomerular média foi de 46 ± 19 ml/min/1,73m^2 e os diversos esquemas imunossupressores associados ao MMF se basearam em prednisona (98,6%), ciclosporina (39,2%), tacrolimus (35,1%) e sirolimus (28,4%). Foram encontrados todos os polimorfismos citados, em homo ou heterozigose, com exceção do *UGT2B7*-79G/A que só foi encontrado na forma selvagem. Os episódios de infecção foram mais freqüentes em indivíduos que receberam 2g/dia de MMF e eram portadores da variante *UGT1A8* codon 277A (p=0.03), bem como nos portadores do haplótipo *UGT1A8*H5 (-999C/códon 255A/códon 277A) (p=0.02) e do diplótipo *UGT1A8*H2/H5 (-999CC/códon 255AA/códon 277GA) (p=0.02). Também naqueles que receberam 2 g/dia de MMF, a presença do haplótipo *UGT1A9*H4 (-2152T/-275A/-118T9/códon 33T) associou-se com o desenvolvimento de distúrbios hematológicos, principalmente leucopenia (p<0.01) e necessidade de interrupção da medicação (p<0.01). **Conclusão:** A presença de distúrbios hematológicos e infecções em indivíduos transplantados renais que receberam 2g/dia de MMF está associada com variantes dos genes *UGT1A9* e *UGT1A8*. Este estudo sugere que estes polimorfismos influenciam a ocorrência de alguns efeitos colaterais, principalmente infecção e leucopenia em indivíduos recebendo 2 g/dia de MMF como parte da terapia imunossupressora.

ABSTRACT

Introduction: Pharmacogenetic studies have been performed in an attempt to demonstrate possible influences of the genetic pattern on the variable responses to the immunosuppressive medications. This finding could be another tool to tailoring individual immunosuppression. **Objective:** This work aimed to analyze the side effects presented by kidney transplant patients on MMF-based immunosuppression and verify their relation with the genetic polymorphism of uridine glucuronosyltransferases (UGT) enzymes, major responsible for bioavailability of mycophenolic acid (MPA), the active metabolite of MMF. **Methods:** In this study, we retrospectively analyzed 74 kidney transplant patients who had used MMF as part of their immunosuppression regimen. Genotyping of polymorphisms in *UGT1A8* (-999C>T, codon 255A>G, codon 277G>A), *UGT1A9* (-2152C>T, -275T>A, -118T9>10, codon 33T>C) and *UGT2B7* (-79G>A, codon 268C>T) was performed using an automated sequencer and the chromatograms were analyzed on program StadenGap and PreGap4. The genotypes were then compared to the occurrence of eventual side effects, mainly diarrhea, blood disorders and infections. Statistical analyses used Pearson´s chi-square test. **Results:** Seventy-four kidney transplant patients with 56 ± 41 months post-transplant were enrolled, with mean age of 42 ± 12 years. The glomerular filtration rate was 46 ± 19 ml/min/1,73m^2 and the other immunosuppressors were prednisone (98,6%), cyclosporine (39,2%), tacrolimus (35,1%) and sirolimus (28,4%). All polymorphism could be identified on the population, except the *UGT2B7*-79G/A. Data analysis showed that infection episodes were more frequently observed in individuals who carried the variant *UGT1A8* codon 277A (p=0.03) and received 2g/day of MMF. Within individuals receiving this

dosage of the medication, infection could be related to the presence of haplotype *UGT1A8*H5 (-999C/códon 255A/códon 277A) (p=0.02) or diplotype *UGT1A8*H2/H5 (-999CC/códon 255AA/códon 277GA) (p=0.02). Hematological disturbances (p<0.01) and MMF dose reduction (p<0.01) were more frequent in individuals carrying the haplotype *UGT1A9*H4 (-2152T/-275A/-118T9/codon 33T) and receiving 2g/day of MMF. **Conclusions:** The clinical and molecular data of this study suggest that *UGT1A9* e *UGT1A8* polymorphisms seem to be an additional factor influencing the occurrence of side effects, mainly infection and hematologic disturbances, in patients receiving 2g/day of MMF as drug transplant therapy.

Introdução

INTRODUÇÃO

A partir do Projeto Genoma Humano, a avaliação de eficácia e segurança de várias medicações passou a ser relacionada com padrões de metabolismo inatos.[1] Assim, genes que codificam receptores e enzimas podem ter variabilidade de expressão de acordo com o polimorfismo que apresentam, sendo esta influência do padrão genético na farmacodinâmica denominada farmacogenômica.[2]

Farmacogenômica e farmacogenética estudam os fatores genéticos que podem contribuir para a variação na resposta individual dos pacientes às medicações. Usualmente os dois termos são utilizados como sinônimos, porém a farmacogenética está relacionada ao campo da ciência focado no modo como genes isolados modulam o efeito de certa droga, enquanto a farmacogenômica abrange o genoma como um todo, estudando tanto os genes individualmente quanto a interação entre eles.[2]

O papel promissor da farmacogenética e da farmacogenômica, ao elucidar as bases hereditárias das diferenças interindividuais nas respostas aos medicamentos, é a possibilidade de identificar a medicação correta na dose adequada para cada paciente, minimizando efeitos colaterais e maximizando o potencial terapêutico.[3, 4]

Farmacogenética, farmacogenômica e transplantes

Inicialmente, as interações farmacogenéticas dos imunossupressores foram identificadas por meio de análises fenotípicas e uma das primeiras medicações estudadas e com sua farmacogenética definida foi a azatioprina.[2] Nos anos 80, estudos identificaram variabilidade interindividual na atividade da enzima tiopurina metiltransferase (TPMT), responsável pelo metabolismo desta medicação, posteriormente relacionando-a com uma herança autossômica codominante.[5, 6]

Devido a esta variabilidade, indivíduos com atividade enzimática baixa ou não detectável que recebiam doses habituais de azatioprina apresentavam elevadas concentrações dos seus metabólitos ativos e conseqüentemente uma imunossupressão maior. Estes dados traziam implicações terapêuticas, já que até 10% da população caucasiana pode apresentar alelos que aceleram a proteólise desta enzima, reduzindo sua função. Por outro lado cerca de 1% da população possui dois alelos que inativam a TPMT, levando à incapacidade de metabolização da medicação e a efeitos colaterais ainda mais exacerbados.[6, 7, 8] A realização de testes farmacogenéticos antes da utilização da medicação pode identificar pacientes de risco e auxiliar na decisão terapêutica em relação à dose utilizada ou até mesmo na indicação de esquemas imunossupressores alternativos à azatioprina.[9]

Para outros imunossupressores o impacto da variabilidade genética ainda não está bem definido. Para alguns deles, como a ciclosporina, o tacrolimus, o micofenolato mofetil e o sirolimus, sabe-se que há grande variabilidade interindividual em sua farmacocinética, levando com frequência à necessidade de monitorização sérica da medicação a fim de se evitar desfechos terapêuticos indesejáveis.[10]

Inicialmente, a maioria dos estudos em transplantes de órgãos sólidos voltou sua atenção à glicoproteína P e ao sistema do citocromo P450, responsáveis pela metabolização dos inibidores de calcineurina.[10] Para o tacrolimus existem dados na literatura que sugerem associação da variabilidade interindividual com a origem étnica dos receptores estudados, inclusive com sugestão de que esta possa influenciar na prescrição do medicamento.[11] Para a ciclosporina, outro imunossupressor da classe dos inibidores de calcineurina, existe diferença na farmacocinética da

medicação quando avaliados diversos polimorfismos genéticos de *CYP3A5*, mas ao contrário do descrito para tacrolimus, não se demonstrou associação dos polimorfismos com necessidades na mudança das doses da medicação.[12, 13, 14]

Outra classe de imunossupressores que tem trazido discussões crescentes sobre a necessidade de terapêutica controlada por dosagens séricas é a dos agentes antiproliferativos.[15, 16] Esta classe de imunossupressores é utilizada em associação a tacrolimus ou ciclosporina e seus principais representantes são a azatioprina e o micofenolato mofetil (MMF). A azatioprina é utilizada em transplantes há várias décadas e hoje no estudo de sua farmacogenômica, conforme citado acima, sugere-se que o polimorfismo da enzima TPMT pode favorecer a presença de efeitos colaterais como leucopenia e mielotoxicidade.[8] O MMF, outro agente antiproliferativo, é um imunossupressor aprovado para uso na prevenção de rejeição em transplantes de órgãos sólidos assim como em transplante de medula óssea.[17, 18]

Micofenolato mofetil é uma pró-droga de utilização oral, que é rapidamente hidrolisada no nível intestinal por estearases plasmáticas e teciduais, resultando no seu metabólito ativo ácido micofenólico (MPA). Este é um inibidor seletivo da enzima inosino monofosfato desidrogenase (IMPDH), envolvida na síntese de novo das purinas e conseqüentemente na divisão linfocitária, tendo como metabólitos principais o hidroxifenil-β-glucurônico (MPAG) e o acil-glucurônico (AcMPAG), conjugados pelas uridino-glucuronosiltransferases (UGTs). Estes metabólitos têm sido relacionados a efeitos adversos do MMF, principalmente anemia, leucopenia e toxicidade gastrintestinal.[19, 20, 21, 22]

As UGTs são as principais enzimas responsáveis pelo metabolismo do MPA. Encontram-se ligadas à membrana celular e apresentam capacidade para conjugação

de seus substratos, facilitando a excreção biliar ou urinária.[23] As isoformas UGT1A8, 1A9 e 1A10 são as principais responsáveis pela metabolização do MPA, sendo que dentre estas, UGT1A8 e 1A9 têm a maior atividade catalítica.[24, 25] Outra isoforma, UGT2B7, de atividade principalmente hepática, é responsável pela produção do metabólito ativo AcMPAG, que parece estar relacionado com alguns dos efeitos indesejáveis do MMF.[25, 26]

A farmacocinética do MPA possui uma enorme variabilidade interindividual e intraindividual, sendo influenciada pela função do enxerto, concentração sérica de albumina, função hepática, e tipo de imunossupressor associado.[18, 26, 27, 28, 29, 30]. Recentemente, estudos *in vivo* e *in vitro* têm relacionado o polimorfismo dos genes que codificam as UGTs como um dos fatores responsáveis pela variabilidade no perfil farmacocinético desta medicação.[24, 27, 31]

Já foi demonstrado em transplantados renais que indivíduos portadores das variantes UGT1A9 -275T/A e -2152C/T que receberam 2g/dia de MMF apresentam redução significativa na área sob a curva do MPA e conseqüente menor exposição à medicação.[27]. Já aqueles portadores da variante do códon 33T/C apresentam elevação nos níveis séricos do MPA, MPAG e AcMPAG, o que pode levar a efeitos colaterais graves, como por exemplo maior grau de imunossupressão.[31] Em relação à UGT1A8, apesar desta ser uma das isoformas envolvidas na formação do MPAG, ainda não foi demonstrado que a presença de variantes genotípicas pode influenciar no perfil farmacocinético ou na incidência de efeitos adversos.[32, 33] Nos indivíduos portadores de polimorfismo no gene *UGT2B7*, os níveis de MPA também estão elevados, porém os níveis de AcMPAG permanecem pouco alterados, sugerindo que vias alternativas podem ser utilizadas na metabolização do MPA.[31]

Como alguns dos genes que codificam as enzimas responsáveis pelo metabolismo do MMF/MPA já possuem polimorfismos identificados, este trabalho teve como objetivos:

1. identificar os efeitos colaterais do MMF em pacientes submetidos a transplante renal que receberam esta medicação;

2. estimar as freqüências dos haplótipos que codificam as isoformas enzimáticas UGT1A8, UGT1A9, e UGT2B7 nestes indivíduos.

3. investigar associação entre estes polimorfismos e a presença de efeitos colaterais.

Artigo I

PHARMACOGENETICS OF MYCOPHENOLATE MOFETIL: A PROMISING DIFFERENT APPROACH TO TAILORING IMMUNOSUPPRESSION?

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Abstract

Since the Human Genome Project, the evaluation of effectiveness and safety of some medications became partially connected to innate metabolic patterns. Thus, genes that encode receivers and enzymes could play different roles in metabolism, according to their polymorphisms. Especially in organ transplantation, some of the available medications used in immunosuppressive therapy have a narrow therapeutic index, affecting the individual response to these drugs. This review focuses on the polymorphism of genes that encode enzymes of the UDP-glucuronosyltransferase (UGT) family, responsible for the bioavailability of mycophenolic acid, the active metabolite of mycophenolate mofetil (MMF), used as immunosuppressant in solid organ transplantation. The increasing literature data regarding the pharmacogenetics of UGT and MMF suggest that enzyme polymorphism can explain the factors which influence the occurrence of side effects in patients receiving MMF as drug transplant therapy.

Keywords: mycophenolate mofetil, pharmacogenetics, polymorphysm, UGT

PHARMACOGENETICS OF MYCOPHENOLATE MOFETIL: A PROMISING DIFFERENT APPROACH TO TAILORING IMMUNOSUPPRESSION?

Pharmacogenetics and pharmacogenomics

The development of biotechnologies based on information from the Human Genome Project has allowed the identification of a group of genes that defines the individual response to drug therapy, and the effectiveness and safety of some drugs were also linked to innate patterns of metabolism[1, 2, 3]. Thus, genes that encode receivers and enzymes could be expressed in a variety range depending on their polymorphism. The study of this influence of the genetic pattern on the pharmacodynamics is known as Pharmacogenomics. Both Pharmacogenomics and Pharmacogenetics study the genetic factors which influence the individual response to medications. Usually the two terms are used as synonyms, however pharmacogenetics deals with the field of science that focuses on how isolated genes modulate the effect of a certain drug, while pharmacogenomics encompasses the genome as a whole, studying the interaction between genes individually[4].

Absorption, distribution and clearance of endogenous and exogenous substances are controlled by the expression of specific proteins at different sites. Each one of these processes can be affected by genetic variations, resulting in significant clinical impact[5]. It is believed that in the general population genetic factors contribute to variations of 20 up to 95% in the bioavailability of medications, but non-genetic factors, such as organic dysfunction, drug-to-drug interactions and the illness itself, also influence the effect of the drugs[6].

A promising role seems to be in store for Pharmacogenetics and Pharmacogenomics in elucidating the hereditary bases of the differences between drug responses in different individuals. The aim is to identify the correct drug dose for each patient, minimizing the side effects and maximizing the therapeutic potential[5, 7].

Historical background

The description by Kalow, about 40 years ago, of a reduction in the phase I metabolism of succinylcholine related to genetic inheritance was one of the first steps in the development of pharmacogenetics[8]. This first report was a description of patients which were homozygous for a gene that encoded an atypical form of the enzyme butyrylcholinesterase. These patients presented a delayed recovery from muscular paralysis and consequent apnea, after administration of succinylcholine[8]. Immediately after this description, a number of pharmacogenetic findings for the phase II metabolism were reported, relating genetic variations to significant differences in the half-life and the concentration of drugs such as isoniazid, hydralazine and procainamide, with significant clinical consequences[9, 10, 11, 12]. Following these initial findings, there was a significant increment in publications regarding this field, and controversial issues, such as the frequency of some variant alleles in protein coding, and the influence of ethnic and population variability on the frequency of these alleles, started to arise in the literature[8].

Pharmacogenetics, pharmacogenomics and transplantation

The interactions between genes and immunosuppressive drugs had been initially identified through phenotype analyses. One of the first drugs studied for kidney immunosuppression was the purine synthesis inhibitor azathioprine[8]. Over the last 80 years, a great variation between individuals was found regarding the activity of the enzyme thiopurine methyltransferase (TPMT). This enzyme, responsible for the metabolism of azathioprine, is encoded by a gene with autosomal codominant inheritance[13, 14]. Individuals with low or undetectable activity of this enzyme receiving the usual doses of azathioprine showed higher concentrations of its active metabolites, resulting in a stronger immunosuppression effect. These data brought information of significant therapeutic usefulness, since up to 10% of the Caucasian population can present alleles that speed up proteolysis, reducing drug function. On the other hand, less than 1% of this same population carries two TPMT-inactivating alleles, leading to incapacity of drug metabolization, and exacerbating side effects[14, 15, 16]. Performing pharmacogenetic studies before prescribing the medication could help detecting the patients' risk and even assist in the therapeutic decision to use alternative immunosuppressants instead of azathioprine[17]. For other immunosuppressors, the impact of genetic variability has not been completely defined yet. Immunosuppressive drugs, such as cyclosporine, tacrolimus, mycophenolate mofetil and sirolimus, present great inter-individual variability in their pharmacokinetics, and most of them need continuous therapeutic drug monitoring[18]. The majority of studies in pharmacogenetics of solid organ transplantation have turned their attention to P-glycoprotein, a product of gene MDR-1 (Multidrug Resistance 1), and the P450 cytochrome system, an intracellular transporter that is capable of carrying a variety of substances, including cyclosporine

and tacrolimus[19, 18]. For the phase I metabolism, it was already demonstrated that the expression of gene MDR-1/ABCB1 can influence the pharmacokinetics and pharmacodynamics of tacrolimus in kidney transplant patients[20]. Regarding the phase II metabolism, the relationship between gene polymorphism, enzymes CYP3A4 and CYP3A5, and its role in the catalytic activity of tacrolimus was also demonstrated, leading to an increase of the prescribed doses[21, 22, 23]. In a recent review, the association of such findings with the ethnic origin of the transplant recipients was also considered[24]. For cyclosporine, genetic polymorphisms of CYP3A5 showed impaired metabolization in healthy volunteers, but in contrast to the described data for tacrolimus, studies linking genetic polymorphism of CYP3A and P-glycoprotein did not show any correlation with possible changes in cyclosporine dosages[23, 25, 26, 27]. On the other hand, data on another immunosuppressive drug, mycophenolate mofetil, have brought about an increasing discussion concerning the need of blood therapeutic monitoring of its active metabolite, mycophenolic acid[28, 29].

Mycophenolate Mofetil

Mycophenolate mofetil (MMF) is approved for use both in solid organ and bone marrow transplantation[30, 31, 32]. MMF is a pro-drug, and its oral ingestion is quickly followed by hydrolization at the intestinal level by plasma and tissue esterases into an active metabolite, mycophenolic acid (MPA). The metabolism of MMF and MPA is complex and involves a number of enzymes, including esterases, phase-II enzymes and possibly the P450 cytochrome system (figure) [31, 33, 34, 35]. MPA is a selective inhibitor of the enzyme inosine monophosphate dehydrogenase (IMPDH), involved in *de novo* nucleotide biosynthesis and consequently in

lymphocyte proliferation. Through its active metabolite MPA, MMF reduces the proliferation of T and B lymphocytes, induces apoptosis of activated T-lymphocytes, and inhibits the expression of adhesion molecules, as well as the production of nitric oxide [36, 37].

The metabolism of MPA involves its conjugation by UDP-glucuronosyltransferases (UGTs) to an inactive molecule known as hydroxyphenyl-β-glucuronic acid (MPAG). Other four end-products were identified in plasma of individuals receiving MMF: an acyl-glucuronide (AcMPAG), two glucosides (phenolic and acyl), and a demethylated metabolite[31, 34, 38, 39, 40]. The AcMPAG metabolite was known as pharmacologically inactive and its clearance occurs through the urine[38, 41, 42]. However, the literature is challenging the concept of AcMPAG inactivity, and some authors have demonstrated that it has an inhibitory effect on lymphocyte proliferation *in vitro* and could possibly be associated with toxic effects[43, 44]. Thus, it is believed that AcMPAG could be responsible for some of the side effects of the therapy with mycophenolate mofetil or mycophenolic acid, such as leucopenia and gastrointestinal toxicity[45]. The glucoside metabolites of MPA (MPAGIs) do not seem to present any immunosuppressive drug activity; their potential of toxicity was not studied yet[38]. The MMF/MPA metabolites, mainly MPAG, also play a role in the enterohepatic circulation, undergoing biliary excretion followed by intestinal deglucuronidation, and finally being metabolized again into MPA[31, 44]. This enterohepatic cycle is recognized as one of the main factors which are responsible for the undesirable gastrointestinal effects of MMF, since up to 40% of the area under the curve from 0 to 12 hours (AUC_{0-12}) of MPA can be represented by this recirculation[31, 45, 46]. Despite the concept that MMF is a well tolerated

immunosuppressive drug, side effects such as leucopenia, anemia and gastrointestinal disorders like nausea, vomiting, diarrhea, gastritis and ulcers have been described[47, 48, 49]. The occurrence of hematological disorders, mainly leucopenia, can be fully explained by the pharmacological action of MPA, which inhibits IMPDH and plasma levels of MPA, as mainly its area under the curve (AUC) was demonstrated to be related to leucopenia and infections[50]. However, the mechanisms involved in the development of gastrointestinal effects cannot be completely explained by the pharmacological action of MPA[48, 51, 52, 53, 54]. Indeed, it was already demonstrated that anemia can be related to high plasma levels of MPA; however, a more recent study linked the occurrence of anemia to high levels of MPAG and AcMPAG and not to MPA values[55, 56]. Regarding the gastrointestinal effects, a close correlation was established between the prescribed dose of MMF, high levels of AcMPAG and MPAG, and the frequency of symptoms[57, 58, 59].

The pharmacokinetics of MPA presents an enormous inter-individual and intra-individual variability and is influenced by graft function, plasma albumin levels, liver function, and the associated immunosuppressive drug[31, 56, 60, 61, 62, 63, 64]. The association with other immunosuppressants can influence the pharmacokinetics of MMF, due to their interaction in coincident steps of the metabolic pathways. Patients under immunosuppressive therapy combining MMF and cyclosporine are exposed to reduced MPA levels, probably due to the inhibition of its enterohepatic circulation[60, 65]. Moreover, MPA concentrations *in vivo* and immunosuppressive drug activity *in vitro* seem to be higher when MMF is used in association with tacrolimus rather than with cyclosporine, with a reduction of MPAG concentrations[66, 67]. These findings suggest that tacrolimus can inhibit the metabolism of MPA, as demonstrated

in *in vitro* studies[66]. The uridino-glucuronosyltransferases, called UDP-GTs or UGTs, are a superfamily of enzymes with twenty different amino acid sequences already described in humans, and some of the UGT isoforms are directly involved in the MPA metabolism[68].

Pharmacogenetics of UGT

There is a high degree of interindividual variability in the glucuronidation rate of several compounds. This variability can be due to age, type of disease, diet or factors directly related to the individual[68, 69]. However, a number of studies are bringing light to the role of genetic polymorphism in the variability of glucuronidation rates presented by different populations[68, 70, 71, 72, 73]. This genetic variability can occur under different forms, such as small insertions or deletions, and even single nucleotide polymorphisms (SNPs), and can be found in several positions of the gene, including regulatory sequences and codons. Depending on the type of the variation and its position in the gene, the phenotypical consequences can exert different levels of influence on protein expression[74].

MMF and UGT polymorphism

Most of the UGT isoforms involved in MPA glucuronidation have been described, but the whole pathway is not completely understood. UGT1A7, 1A8, 1A9 and 1A10 seem to be able to glucuronidate MPA, but UGT1A7 and 1A10 present weaker catalytic activity[57, 58, 75, 76]. Recently, these findings were confirmed *in vivo* and *in vitro*, with identification of the enzymatic forms, their expression sites and their capacity of glucuronidating MPA[59, 46]. Another isoform, UGT2B7, with liver

and kidney activity, is responsible for the generation of the active metabolite AcMPAG, related to undesirable effects of MMF[56, 59]. Considering that UGT isoforms play a significant role in the metabolism and bioavailability of MMF/MPA, induction or inhibition of their activity could lead to underexposure to the drug or to the induction of side effects, such as anemia, leucopenia and diarrhea[59, 74]. Unfortunately, few clinical data support a correlation between side effects and genetic variations, but increasing data on this issue are being published[46, 77, 78, 79].

Regarding the pharmacokinetic profile of MMF/MPA, a recent publication on healthy individuals showed a lower exposure to MPA when the drug was administrated to carriers of the UGT1A9 -275/-2152 polymorphism, but no changes in MPAG plasma concentration were demonstrated. Differences in MPA, MPAG and AcMPAG profiles were also found in the UGT1A9*3 (M33T) carriers and the levels of free and total plasma MPA were higher among the UGT2B7 mutation carriers[46]. However, in a specific Japanese kidney transplant population, the allelic variations of UGT2B7 did not show significant variability for the plasma MPA concentration[79]. This lack of correlation could be due to the kidney expression of UGT2B7, which is probably impaired in the native kidneys of that Japanese transplant population.

In conclusion, the inter-individual variability of MPA pharmacokinetics is related to several possible interferences in the drug metabolism, including the genetic profile of the recipient. There is increasing interest in finding out whether therapeutic drug monitoring of MMF/MPA is useful and cost-effective in kidney transplantation, and if a pharmacogenetic evaluation of UGT polymorphisms in transplant recipients could be an additional tool to help clinical management.

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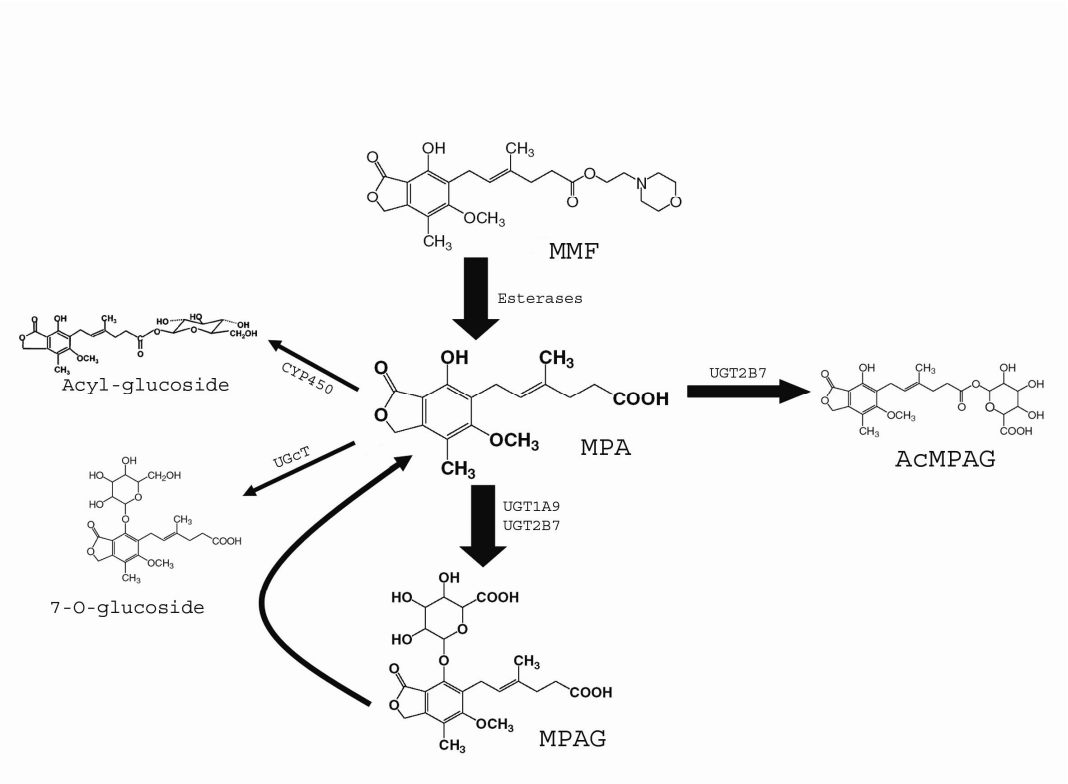

Figure 1: Metabolic pathway of mycophenolate mofetil (MMF). MMF is hydrolyzed to mycophenolic acid (MPA) by plasma and tissue esterases. MPA is converted by glucuronidation to the phenolic MPA glucuronide (MPAG) and acyl glucuronide (AcMPAG). MPAG also presents an enterohepatic recirculation. Two additional glucosides metabolites have also been described. UGT, uridino glucuronosyltransferase; UGcT, uridino glucosyltransferase; CYP450, cytochrome P450.

Artigo II

INFLUENCE OF UDP-GLUCURONOSYLTRANSFERASE POLYMORPHISMS ON MYCOPHENOLATE MOFETIL-INDUCED SIDE EFFECTS IN KIDNEY TRANSPLANT PATIENTS

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Financial Support: This work was supported by the Canadian Institutes of Health Research (MOP-42392) and Canada Research Chair Program (C.G.)

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Abstract

Mycophenolate mofetil (MMF) is an immunosuppressive prodrug approved for use in transplantation and its active metabolite, mycophenolic acid (MPA), is mainly metabolized by UDP-glucuronosyltransferase (UGT) enzymes. In this study, we retrospectively analyzed 74 kidney transplant patients who had used MMF as part of their immunosuppression regimen. Polymorphisms in *UGT1A8* (-999C>T, codon 255A>G, codon 277G>A) were analyzed and correlated with the occurrence of side effects such as diarrhea, blood disorders and infections. The infection episodes were more frequently observed in individuals receiving 2g/day of MMF and carrying the variant *UGT1A8* codon 277A (p=0.031), the haplotype *UGT1A8*H5 (-999C/codon 255A/codon 277A) (p=0.02) and the diplotype *UGT1A8*H2/H5 (-999CC/codon 255AA/codon 277GA) (p=0.015). Molecular data of this study suggest that *UGT* polymorphisms may be a factor influencing clinical outcomes in patients receiving MMF as drug transplant therapy; however, larger studies are warranted.

Keywords: mycophenolate mofetil, polymorphism, renal transplantation, UGT

INFLUENCE OF UDP-GLUCURONOSYLTRANSFERASE POLYMORPHISMS ON MYCOPHENOLATE MOFETIL-INDUCED SIDE EFFECTS IN KIDNEY TRANSPLANT PATIENTS

Introduction

Mycophenolate mofetil (MMF) is an immunosuppressive prodrug approved for use in the prevention of rejection after solid organ or bone marrow transplantation. This prodrug is rapidly hydrolyzed at the intestinal level by tissue and plasmatic esterases into its active metabolite, mycophenolic acid (MPA). The metabolism of MMF is complex and involves several enzymatic steps, including esterases, phase II enzymes and possibly the P450 cytochrome system[1]. The metabolism of MPA mainly involves its conjugation into MPA phenolic glucuronide (MPAG) and acyl-glucuronide (AcMPAG) through UDP-glucuronosyltransferase (UGT) enzymes[1]. Although MMF is usually well-tolerated, side effects such as infections and hematological and gastrointestinal disturbances have been reported. The complete pathophysiology of these side effects is unclear, but it has been demonstrated that they could be related to high plasma levels of MPA and maybe its metabolites MPAG and AcMPAG[1].

The pharmacokinetics of MPA are characterized by a large inter- and intraindividual variability and the genetic variability in MPA-metabolizing enzymes is one of the recently studied causal factors associated to this interpatient variability. MPA is conjugated into its major metabolites, namely MPAG and AcMPAG, mainly by three UGT isoforms: 1A8, 1A9 and 2B7[2,3]. In this retrospective study, we correlated the presence of *UGT1A8* genetic variations with the side effects presented by kidney transplant individuals on MMF therapy.

Methods

Approval was obtained from the Research Ethics Committee of FAMERP - Sao Jose do Rio Preto Medical School (Sao Paulo, Brazil), from the National Ethics Research Commission (CONEP) and from the Institutional Review Board (CHUL Research Center and Laval University, Quebec, Canada). All medical records from kidney transplant patients who received an organ between January 1995 and December 2005 at the Institute for Urology and Nephrology and CINTRANS – Organ and Tissue Transplant Center - FAMERP - Sao José do Rio Preto – Sao Paulo – Brazil were reviewed. Seventy-four patients who received MMF for at least 30 days as part of their immunosuppressive regimen were selected for genetic analyses. Other data such as possible side effects and the eventual adjustment on MMF prescription were obtained from the files.

UGT1A8 genotyping. Genomic DNA samples were obtained from peripheral blood lymphocytes and then amplified using polymerase chain reaction[6]. The amplified products were sequenced to identify wild type and variant *UGT* alleles -999C/T, codon 255A/G and codon 277G/A. The sequences were analyzed using Staden preGap and Gap4 programs (Open Source Technology Group, http://staden.courceforge.net/) in order to identify the variant sequence.

Statistical analysis. The Hardy-Weinberg equilibrium was tested by χ^2 analysis and *UGT1A8* haplotypes were inferred by a Bayesian algorithm using Phase 2.1 software (University of Washington, Seattle, WA). Statistical comparisons were implemented using Pearson's χ^2 test. A p-value < 0.05 was considered statistically significant.

Results

From the 74 kidney transplant recipients with mean age of 42 ± 12 years-old, 51 were male. Average time since transplantation was 56 ± 41 months and the associated immunosuppressive drugs consisted of prednisone, cyclosporine, tacrolimus, and sirolimus. The patients received from 1 to 2 g/day of MMF.

UGT polymorphism and haplotype analysis. There was a higher occurrence of infections in individuals receiving 2 g/day of MMF and carrying the *UGT1A8* 277A polymorphism (p=0,031) when compared to 277G carriers. All other polymorphisms in *UGT1A8* did not show any correlation with adverse reactions. When only the group of individuals receiving 2 g/day of MMF was analyzed, the carriers of haplotype *UGT1A8*H5 (-999C/codon 255A/codon 277A) or diplotype *UGT1A8*H2/H5 (-999CC/codon 255AA/codon 277GA) presented more infection episodes ($p<0.02$).

Discussion

MMF prescription has been recently reviewed and new clinical and experimental data suggest that individualized dosages could be more appropriate to obtain improved outcomes[4]. Furthermore, the MPA pharmacokinetic profile can be influenced by several factors, such as concomitant immunosuppressive drug and individual intrinsic factors[5]. A better knowledge and understanding of the differences in pharmacokinetics of MMF is essential in order to minimize risks for the development of acute rejection, to prevent toxicity, and to support dose adjustment.

The isoform UGT1A8 is one of the described UGT enzymes responsible for MPA metabolism, generating mainly the MPAG metabolite, specifically on *in vitro* studies[2,3]. In an attempt to bring some clinical relevance on that experimental data, we have focused our attention on polymorphisms in *UGT1A8* gene. The individuals

carrying *UGT1A8*H5 haplotype (inferred frequency 0.01; n = 2 patients) or *UGT1A8*H2/H5 (inferred frequency 0.01; n = 2 patients) and receiving 2g/day of MMF presented more infection episodes, but no differences on gastrointestinal or hematological disturbances could be identified. This finding might be related to increased MPA exposition, since the mutated enzyme could present an impaired glucuronidation into MPAG, leading to increased on plasma MPA levels and augmented immunosuppression. This is consistent with *in vitro* findings since haplotype 5 presents the codon 277 polymorphism which yields an inactive enzyme. Previous studies with *UGT1A8* variation on MMF pharmacological profile could not show similar results, since one was performed with healthy volunteers receiving a single-dose of 1,5g of MMF and the other did not studied the codon 277[6,7]. The fact that only transplant patients taking 2g/day of MMF showed more infection episodes corroborates the dose-dependent immunosuppression of this drug.

In conclusion, this study could identify an association between *UGT1A8* polymorphisms and the incidence of infection episodes in Brazilian kidney transplant patients, mainly respiratory diseases. However, a small number of patients carried haplotype 5 and additional larger studies could bring important information on *UGT* variation in relation to clinical outcomes, since recent studies support that these enzymes play an important role in MMF pharmacokinetic profiles.

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Artigo III

UDP-GLUCURONOSYLTRANSFERASE (UGT) POLYMORPHISMS AND SIDE EFFECTS IN MYCOPHENOLATE MOFETIL-BASED IMMUNOSUPPRESSIVE THERAPY OF ADULT KIDNEY TRANSPLANT RECIPIENTS

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Abstract

Mycophenolate mofetil (MMF) is an immunosuppressive prodrug approved for use in transplantation and its active metabolite, mycophenolic acid (MPA), is mainly metabolized by UDP-glucuronosyltransferase (UGT) enzymes. In this study, we retrospectively analyzed 74 kidney transplant patients who had used MMF as part of their immunosuppression regimen. Polymorphisms in *UGT1A8* (-999C>T, codon 255A>G, codon 277G>A), *UGT1A9* (-2152C>T, -275T>A, -118T9>10, codon 33T>C) and *UGT2B7* (-79G>A, codon 268C>T) were analyzed and correlated with the occurrence of side effects, mainly diarrhea, blood disorders and infections. The statistical analysis showed that infection episodes were more frequently observed in individuals taking 2g/day of MMF who carried the variant *UGT1A8* codon 277A (p=0.031) or the haplotype UGT1A8 -999C/255A/277A (p=0.02). The individuals carrying the variant haplotype of *UGT1A9* -2152T/-275A/-118T9/codon 33T and receiving 2g/day of MMF presented more leucopenia (p=0.005) and higher rates of MMF dose reduction (p=0.0009). Diarrhea and/or the need of MMF dose reduction were associated with higher age (p=0.029), and elevated plasma creatinine (p=0.04). These data support the idea that UGT polymorphisms operate as an additional factor influencing the occurrence of side effects in patients receiving 2g/day of MMF as drug transplant therapy.

Keywords: mycophenolate mofetil, polymorphism, renal transplantation, UGT

UDP-GLUCURONOSYLTRANSFERASE (UGT) POLYMORPHISMS AND SIDE EFFECTS IN MYCOPHENOLATE MOFETIL-BASED IMMUNOSUPPRESSIVE THERAPY OF ADULT KIDNEY TRANSPLANT RECIPIENTS

Mycophenolate mofetil (MMF) is an immunosuppressive drug approved for use in the prevention of rejection after solid organ or bone marrow transplantation[1]. This pro-drug is quickly hydrolyzed at the intestinal level by tissue and plasmatic esterases into its active metabolite, mycophenolic acid (MPA), which is a selective inhibitor of the enzyme inosine monophosphate dehydrogenase (IMPDH), involved in *de novo* synthesis of purines and consequently lymphocyte proliferation[1]. The metabolism of MMF is complex and involves several enzymatic steps, including esterases, phase II enzymes and possibly the P450 cytochrome system[2,3]. The metabolism of MPA mainly involves its conjugation into the inactive product phenolic MPA glucuronide (MPAG) through UDP-glucuronosyltransferase (UGT) enzymes. There are four additional end products identified in the plasma of individuals receiving MMF: an acyl-glucuronide (AcMPAG), two glucosides (phenolic and acyl) and a desmethylated metabolite[4-8]. MPAG, which presents an enterohepatic circulation responsible for 10 to 60% of the area under the curve from 0 for 12 hours (AUC_{0-12}) of MPA[4,9]. This enterohepatic cycle consists in the intestinal reabsorption of MPA after a bacterial deconjugation of MPAG, generating a second peak on the pharmacokinetic profile, which occurs 6 to 8 hours after oral ingestion[4]. Although MMF is usually well-tolerated, side effects such as infections and hematological and gastrointestinal disturbances are reported[10,11]. The occurrence of infections and leucopenia can be explained by the pharmacological action of MPA, since

it has immunosuppressive effects through the inhibition of IMPDH. Thus, high plasma levels of MPA, mainly when related to its area under the curve (AUC) have been associated with severe infections and leucopenia[12]. However, it is still controversial if the mechanisms involved in the development of anemia and gastrointestinal disturbances in patients receiving MMF involve directly exposition to MPA[13-17]. It has been demonstrated that anemia could be related to high plasma levels of MPA[18], however, a more recent study associated anemia with high levels of MPAG and AcMPAG and not MPA levels[19]. Gastrointestinal complications related to immunosuppressive therapy include nausea, vomiting, gastritis, ulceration of the gastrointestinal mucosa, esophagitis, and diarrhea[10,11]. Although not life-threatening, gastrointestinal disturbances in renal transplant recipients were reported to have an impact on quality of life and are influenced by various factors, including the recipient's characteristics, the immunosuppressive drug used and the environment[20-22]. An association between the frequency of hematological symptoms, the prescribed dose of MMF and high circulating levels of MPA has been suggested, but the exact role of MPA and its metabolites on MMF-induced diarrhea has been poorly studied[17, 18]. Recently, a multi-center clinical study could not demonstrate a role of MPA acyl and phenolic metabolites on MMF-induced diarrhea[23].

The pharmacokinetics of MPA are characterized by a large inter- and intraindividual variability, influenced mainly by graft function, hepatic function and class of associated immunosuppressive drug[19,24-27]. Furthermore, pharmacogenomic findings strengthen the concept that genes encoding drug-metabolizing enzymes, transporters and drug targets have an important role in the clinical drug response. The

genetic variability in MPA-metabolizing enzymes is one of the recently studied causal factors associated to the interpatient variability during MMF therapy. MPA is conjugated to MPAG and AcMPAG mainly by three UGT isoforms: 1A8, 1A9 and 2B7. UGT1A9 and UGT2B7 are expressed in hepatic and extrahepatic tissues, mediating MPAG and AcMPAG formation, respectively. The UGT1A8 isoform, expressed in the gastrointestinal tract, is involved in the formation of MPAG and, on to a lesser extent, of AcMPAG[28-30].

Considering that UGT isoforms play a significant role in the metabolism and bioavailability of MPA and its metabolites, variation in their activity could lead to an under- or over-exposition to the drug or its metabolites, and thereby to the appearance of side effects[2,31]. To our knowledge, up to know, four *in vivo* pharmacokinetic studies on *UGT1A8* and *UGT2B7* SNPs have been published, the first in healthy volunteers and the others in populations of kidney transplant recipients, but they diverge in their conclusions[30,32-34]. Unfortunately, currently, few clinical data are available regarding a potential relationship between side effects and UGT genetic variations. One study reported that the *UGT1A9* -275T>A and -2152C>T single-nucleotide polymorphisms (SNPs) is associated with significantly lower MPA concentrations in a Caucasian population but without interfering on clinical symptoms[34]. In this retrospective study, we explored the relationship between the presence of *UGT* genetic variations and the occurrence of side effects on long-term kidney transplant patients.

Methods

Study population

Approval was obtained from the Research Ethics Committee of FAMERP - Sao Jose do Rio Preto Medical School (Sao Paolo), from the National Ethics Research Commission (CONEP). All medical records from kidney transplant patients who received an organ between January 1995 and December 2005 at the Institute for Urology and Nephrology and CINTRANS – Organ and Tissue Transplant Center (FAMERP - Sao José do Rio Preto – Sao Paulo – Brazil) were reviewed. Seventy-four patients who received MMF for at least 30 days as part of their immunosuppressive regimen were selected for genetic analyses. The minimum age for inclusion was 18 years and either live or cadaveric first kidney transplant recipients were eligible. Exclusion criteria included previous gastrointestinal disease or surgery and a post-transplant interval of less than three months. Each patient gave their written informed consent prior to enrollment in the study. Clinical and demographic data such as age, gender, weight, glomerular filtration rate (GFR), other associated immunosuppressive drugs, MMF doses, and rejection or infection episodes were extracted from the patients' medical records and from a brief interview to confront files-acquired data before blood sampling. The clinical side effects such as gastrointestinal and hematological disturbances and the eventual adjustment on MMF prescription were retrospectively analyzed from the patients' hospital records.

UGT1A8, UGT1A9 and UGT2B7genotyping

Genomic DNA samples were obtained from peripheral blood lymphocytes and the genomic DNA was amplified on Perkin-Elmer Life Sciences GeneAmp 9700 (Applied Biosystems, Foster City, CA) thermocycler and products were sequenced to identify reference and variant *UGT* sequences.

Briefly, a solution containing 25 ng of genomic DNA, 100 μM of dNTP, 20 pmol of each primer, buffer 10XPCR [67 mM of buffer Tris-HCL (pH 8,8), 16,6mM $(NH_4)_2SO_4$, Triton X-100 0,45% and gelatin 0,02%] and 2 U of Taq polymerase was prepared. The primers and annealing conditions are presented in table 1. The PCR products were sequenced using an ABI 3700 automated sequencer. The sequences were analyzed using Staden preGap and Gap4 programs (Open Source Technology Group, http://staden.sourceforge.net/) and compared to the reference sequence (GenBank AF297093 and AC111000) identify genetic variations.

Statistical analysis

Hardy-Weinberg equilibrium was tested by χ^2 analysis and *UGT1A8*, *UGT1A9* and *UGT2B7* haplotypes were inferred by a Bayesian algorithm using Phase 2.1 software (University of Washington, Seattle, WA)[35].

Statistical comparisons were implemented using Pearson's χ^2 test. A *p*-value < 0.05 was considered statistically significant.

Results

Demographic and transplantation-related data

Recipient demographic data and relevant clinical findings from patients' files are shown in Table 2.

Frequency distribution of UGT1A8 polymorphisms

This study population presented a frequency of 35.1% of heterozygous individuals for the tightly linked codon 173 and promoter gene region -999C>T polymorphisms and 1.3% for homozygous individuals. The codon 255A>G variation occurred in 18/74 (24.3%) individuals and 2 of them were homozygous. The codon 277G>A variation was less frequent and present only in 2.7% individuals, none of them homozygous.

Frequency distribution of UGT1A9 polymorphisms

The -2152C>T promoter region variation occurred in 5/74 (6.7%) individuals, and all were heterozygous. The study population presented 9/74 (12.2%) heterozygous carriers of the -275T>A variation and no homozygous variants were found. The -118T9>10 variation occurred in 33/74 (44.6%) heterozygous individuals and 16/74 (21.6%) homozygous individuals, whereas the codon 33T>C variation was not found in this population.

Frequency distribution of UGT2B7 polymorphisms

Thirty-three individuals (44.6%) were heterozygous for the codon 268C>T variation and 12/74 (16.2%) were homozygous. The -79G>A promoter region variation was found in 4/74 (5.4%) individuals, all of them heterozygous.

Haplotype analysis

For the *UGT1A8* gene, six haplotypes were inferred in the population studied. The three most common haplotypes totalized more than 98% of all variants for this gene. Seven haplotypes were inferred for the *UGT1A9* gene. Two of them were together found in more than 90% of the individuals. Four haplotypes were inferred for the *UGT2B7* gene and among them the *UGT2B7*H1 and *UGT2B7*H2 were the most frequent in the kidney transplant patients studied, corresponding to more than 95% of the subjects (Table 3).

Adverse effects and molecular analysis

The occurrence of side effects did not vary significantly between groups stratified by concomitant immunosuppressive drug (Table 4).

Diarrhea was directly related to patients' age (p=0.029) and to serum creatinine (p=0.04). Interruption of MMF prescription correlated with diarrhea (p<0.0001), infection (p=0.0002) and biopsy-proven rejection (p=0.01). In order to assess the influence of *UGT1A8*, *UGT1A9* and *UGT2B7* polymorphisms on the occurrence of side effects, variants and haplotypes were analyzed in relation to the incidence of diarrhea, infection and hematological disorders, MMF dosing reduction or even changing to a MMF-free regimen.

There was a higher occurrence of infections in individuals receiving 2 g/day of MMF and carrying the *UGT1A8* 277A polymorphism (p=0.031) when compared to 277G carriers (Table 5). All other polymorphisms in *UGT1A8*, *1A9* or *2B7* did not show significant association with adverse reactions.

When the group of individuals receiving 2 g/day of MMF was analyzed, both the carriers of haplotype *UGT1A8*H5 (-999C/255A/277A) and diplotype *UGT1A8*H2/H5 (-999CC/255AA/277GA) had more infection episodes (p=0.02). Leucopenia occurred more frequently in individuals receiving 2g/day of MMF and carrying the haplotype *UGT1A9*H4 (-2152T/-275A/-118T9/codon 33T) (p=0.005).

Discussion

MMF prescription has been reviewed and new clinical and experimental data suggest that individualized dosages could be more appropriate to obtain better outcomes[36-38]. In kidney transplantation, the pharmacokinetic profile, such as 12h MPA-AUC was shown to be a predictor of rejection episodes or even adverse effects; however, MPA serum levels assessment is still not a routine in clinical practice[3]. A better knowledge and understanding of the differences in MMF pharmacokinetics is essential in order to minimize risks of development of acute rejection, to prevent toxicity, and to support dose adjustment. In this work, we analyzed the incidence of side effects in stable long-term kidney transplant individuals receiving MMF-based immunosuppression, focusing on their relation with the patients' genetic profile. Experimental data suggests that UGT1A8, UGT1A9 and UGT2B7 are the most important enzymes in MPA metabolism[28,29]. Previous data suggest that individuals carrying a polymorphic UGT1A9 at -275/-2152 position can have an increased MPAG formation, theoretically influencing MPA exposure and thereby possibly increasing the risk of rejection or adverse effects[30,34,39].

There are reports relating a major influence of other immunosuppressive agents on MPA plasma concentrations. The impact of calcineurin inhibitors including cyclosporine and tacrolimus on MPA exposure is clearly demonstrated[40,41,42]. In the present study, the retrospective analysis of side effects and concomitant immunosuppression could not show any association with adverse effects incidence or with modification on drug prescription. This lack of correlation could be due to the small

sample size or even to the study design. Indeed, the analysis of treatment effect in retrospective studies should be treated with caution because adverse symptoms may influence treatment choice.

However, this work could demonstrate an association of clinical parameters with the modification of MMF prescription. Renal function and recipient's age were related to adverse effects such as diarrhea. The occurrence of infection and diarrhea was associated to MMF dose reduction or even to the drug suspension. These results confirm previous findings that impaired renal function leads to reduced renal excretion of MPAG, causing concentrations to become markedly elevated[43]. This impaired renal MPAG excretion could also theoretically lead to increased biliary excretion, and possibly increased enterohepatic recycling. The accumulated MPAG theoretically competes with and displaces MPA from protein binding sites[12,44]. Higher MPAG concentrations induce a progressive increase in MPA free fraction and consequently a more aggressive immunosuppressive state, explaining the higher infection rates and probably the higher incidence of diarrhea.

Indeed, diarrhea, infections and biopsy-proven rejection were independent factors leading to MMF interruption, despite the fact that MMF alone was not a risk factor for any of the complications cited above. On the other side, it is remarkable in our data that interruption of MMF prescription was related to its dosage, suggesting a possible influence of adverse effects on prescription. According to the literature, MMF prescription and dosage are frequently modified, especially during the first year post-transplant, increasing the risk of acute rejection[45].

In this work, we also analyzed the incidence of gastrointestinal effects possibly induced by MMF on long-term stable kidney transplant individuals, focusing on their possible relation with UGT genetic profile. The present study was able to identify most of the frequent polymorphisms of *UGT1A8*, *UGT1A9* and *UGT2B7* described in the literature[20,30]. Dose reduction and hematological disturbances occurred more frequently in individuals receiving 2g/day of MMF who carried the *UGT1A9*H4 haplotype, characterized by the presence of -2152/-275 variations. It has already been demonstrated that healthy volunteers taking a single MMF dose (1,5g) who carry one allele of the *UGT1A9* -275/-2152 polymorphism have reduced MPA exposure. Unfortunately, this pharmacokinetic study was not meant to reveal correlations between the genetic profile and clinical events[30]. It was previously suggested that anemia can be related to high plasma levels of MPA[18]; however another study links the occurrence of anemia to high levels of MPAG and AcMPAG and not to MPA values[19]. Despite the probable reduction of MPA exposure in patients carrying *UGT1A9*H4, our data could not show an increase in rejection rates for these individuals. The effect of the *UGT1A9*H4 haplotype was not recognized on individuals receiving 1.0 or 1.5 g/day of MMF probably due to the sample size or even to a possible dose-dependent effect as already suggested[34].

The individuals carrying *UGT1A8*H5 haplotype (*3 variant) or *UGT1A8*H2/H5 and receiving 2g/day of MMF presented more infection episodes, but no differences on gastrointestinal or hematological disturbances could be identified. An *in vitro* study demonstrated that polymorphic *UGT1A8* at codon 277 present an impaired capacity of generating MPAG and AcMPAG, leading to higher levels of MPA and consequently potentially higher immunosuppression[29].

Based on *in vitro* data, UGT2B7 plays an important role in MPA metabolism[28], which was later confirmed as the formation of AcMPAG and MPAG were significant increased in healthy *UGT2B7*H2/H2 subjects who received a single-dose of MMF[30]. In this present study, the codon 268 polymorphism did not influence the incidence of rejection, infections or gastrointestinal and hematological disturbances. We believe that this possible lack of correlation of *UGT2B7* polymorphism with clinical symptoms in kidney transplant individuals may be related to the studied population particularities. The UGT2B7 enzyme is expressed mainly in kidney and liver tissue, and the residual function of the topic kidneys might not play an important role in MPA glucuronidation.

Conclusion

In conclusion, this study could identify the association of *UGT1A8* and *UGT1A9* polymorphism to the incidence of infection and hematological disturbances in Brazilian kidney transplant patients receiving 2 g/day of MMF. The *UGT2B7* polymorphism did not show any influence on occurrence of side effects. Additional studies with larger sample size are clearly needed to establish if *UGT* polymorphisms are among factors influencing clinical outcomes in patients receiving MMF as drug transplant therapy.

Acknowledgements

The authors wish to thank Lyne Villeneuve and Maria Paula Sanches Alvarenga for their technical assistance. This work was supported by the Canadian Institutes of Health Research (MOP-42392) and Canada Research Chair Program (C.G.). M.O.B.B. was supported by a Canada Graduate Scholarship Doctoral Research Award from the CIHR and by the Canadian Federation of University Women Dr. Marion Elder Grant

Fellowship, funded by CFUW Wolfville. C.G. is the holder of the Canada Research Chair in Pharmacogenomics.

Table 1: Primers and annealing protocols used for DNA amplification

		Sense	Sequence	Annealing Time	Annealing Temperature
UGT1A8	-999C>T	F	5'-ctggaccgggaattcatgga-3'	40s	60°C
		R	5'-gtggctgtagagatcatatgct-3'		
	Codon 255A>G	F	5'-ttcgccaggggaatag-3'	40s	54°C
		R	5'-atttgctctagggggtc-3'		
	Codon 277G>A	F	5'-ttcgccaggggaatag-3'	40s	54°C
		R	5'-atttgctctagggggtc-3'		
UGT1A9	-2152C>T	F	5'-gtaggtcttttacatttccc-3'	40s	53°C
		R	5'-cctgaaacagcaaaaccaa-3'		
	-275T>A	F	5'-gagcccaatttaggaggtta-3'	30s	58°C
		R	5'-cagtaggtgggagaaatacca-3'		
	-118T9>10	F	5'-gtaggtcttttacatttccc-3'	30s	58°C
		R	5'-cctgaaacagcaaaaccaa-3'		
	Codon 33T>C	F	5'-gtgctggtatttctccc-3'	40s	54°C
		R	5'-gtcaaaaatgtcattgtatgaacc-3'		
UGT2B7	-79G>A	F	5'-gacaatggggaaagctgacg-3'	40s	54°C
		R	5'-gtttggcaggtttgcagtg-3'		
	Codon 268C>T	F	5'-gtaattatcttgtgtcatc-3'	40s	52°C
		R	5'-gactatagaatcatttctactg-3'		

F, forward; R, reverse. All amplifications were performed for 35 cycles, except -999C>T (40 cycles).

Table 2: Recipients demographic and clinical data (n=74)

Parameter	*Value*
Gender (n) male/female	51/23
Age (y)	42 ± 12*
Body weight (kg)	64 ± 15*
Glomerular filtration rate (ml/min)	46 ± 19*
Associated immunosuppressive drug (n)	
Prednisone	74
Cyclosporine	29
Tacrolimus	26
Sirolimus	21
Time since transplantation (mo)	56 ± 41*
Type of donation (n) cadaver/live	28/46
MMF daily doses (n)	
1.0g	8
1.5g	19
2.0g	47

*Mean ± SD

Table 3: *UGT1A8*, *UGT1A9* and *UGT2B7* haplotype analysis

Haplotypes					Inferred frequency
UGT1A8	-999/codon 173*	Codon 255	Codon 277		
H1	C	G	G		0.13
H2	C	A	G		0.65
H3	C	G	A		0.0003
H4	T	G	G		0.0004
H5	C	A	A		0.01
H6	T	A	G		0.20
UGT1A9	-2152	-275	-118	Codon 33	
H1	C	T	9	T	0.52
H2	C	T	10	T	0.41
H3	C	A	9	T	0.001
H4	T	A	9	T	0.02
H5	T	T	9	T	0.01
H6	C	A	10	T	0.03
H7	T	A	10	T	0.0004
UGT2B7	-79	Codon 268			
H1	G	T			0.39
H2	G	C			0.58
H3	A	T			0.004
H4	A	C			0.02

All haplotypes were inferred by a Bayesian algorithm using Phase 2.1 software. N = 74. * Polymorphism at position -999 of *UGT1A8* is in complete linkage with codon 173 variation (*UGT1A8**2).

Table 4: Side effects frequency and conversion rates related to the immunosuppressive drugs used in combination with MMF.

	Diarrhea	Anemia/leucopenia	Total side effects	Conversion
Prednisone* (n=73)	10 (13.5)	5 (6.7)	15 (20.2)	15 (20.2)
Cyclosporin (n=29)	3 (10.3) p = 0.522	1 (3.4) p = 0.363	4 (13.7) p = 0.128	3 (10.3) p = 0.088
Tacrolimus (n=26)	5 (19.2) p = 0.290	3 (11.5) p = 0.228	8 (30.7) p = 0.098	8 (30.7) p = 0.098
Sirolimus (n=21)	2 (9.5) p = 0.527	1 (4.8) p = 0.667	3 (14.3) p = 0.695	4 (19.0) p = 0.930

* Statistical test could not be performed because there was only one individual not receiving prednisone. Data presented as n (%). Conversion rates mean MMF dose reduction or suspension.

Table 5: UGT polymorphisms and incidence of side effects.

	Diarrhea	p value	Anemia/ Leucopenia	p value	Infection	p value	Changes on MMF prescription	p value
UGT1A8								
-999C>T								
cc (n=44)	7 (15.9)	0.573	3 (6.8)	0.925	3 (6.8)	0.528	9 (20.4)	0.859
ct/tt (n=27)	3 (11.5)		2 (7.7)		3 (11.1)		6 (22.2)	
Codon 255A>G								
aa (n=56)	9 (16.1)	0.256	3 (5.4)	0.398	4 (7.1)	0.592	11 (19.6)	0.813
ag/gg (n=16)	1 (6.3)		2 (12.6)		2 (12.5)		4 (22.2)	
Codon 277G>A								
gg (n=72)	10 (13.8)	N/D	5 (6.9)	N/D	5 (6.9)	0.031*	6 (24.0)	N/D
ga/aa (n=2)	0 (0.0)		0 (0.0)		1 (50.0)		1 (50.0)	
UGT1A9								
-2152C>T								
cc (n=68)	10 (14.7)	1	4 (5.9)	0.352	5 (7.3)	N/D	14 (20.6)	0.975
ct/tt (n=5)	0 (0.0)		1 (20.0)		0 (0.0)		4 (80.0)	
-275T>A								
tt (n=65)	10 (15.4)	0.644	5 (7.7)	1	5 (7.7)	N/D	15 (23.8)	0.107
ta/aa (n=9)	0 (0.0)		0 (0.0)		1 (11.1)		0 (0.0)	
-118T9>10								
99 (n=25)	6 (24.0)	0.460	2 (8.0)	0.356	2 (8.0%)	0.741	6 (24.0)	0.662
910/1010 (n=48)	5 (18.7)		3 (15.6))		4 (8.3%)		9 (40.15)	
Codon 33								
tt (n=73)	10 (13.5)	N/D	5 (6.7)	N/D	6 (8.2)	N/D	11 (15.06)	N/D
tc/cc (n=0)	N/D		N/D		N/D		N/D	
UGT2B7								
-79G>A								
gg (n=70)	10 (14.3)	N/D	5 (7.1)	N/D	6 (8.6)	N/D	20 (28.7)	N/D
ga/aa (n=4)	0 (0.0)		0 (0.0)		0 (0.0)		0 (0.0)	
Codon 268								
cc (n=29)	4 (13.8)	1	3 (10.3)	0.465	1 (3.4)	N/D	6 (20.7)	0.943
ct/tt (n=45)	6 (23.5)		2 (11.3)		5 (11.1)		9 (20.0)	

N/D = not done. *Pearson's chi-square test. Values are expressed as number of individuals (%).

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Conclusões

Conclusões

A partir dos dados encontrados, podemos concluir que:

1. Transtornos gastrintestinais (principalmente diarréia) e hematológicos (anemia e leucopenia) são os efeitos colaterais mais freqüentes em pacientes submetidos a transplante renal em terapia com MMF. São observados também, em menor freqüência os episódios de infecção, principalmente as respiratórias.

2 Os haplótipos *UGT1A8H2*, *UGT1A9H1* e *UGT2B*7H2 são os mais representativos na população estudada.

3. Os polimorfismos de *UGT1A8* e *UGT1A9* estão associados à maior ocorrência de infecção e transtornos hematológicos, respectivamente, nos indivíduos em uso de 2g/dia de MMF.

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FACULDADE DE MEDICINA DE SÃO JOSÉ DO RIO PRETO
Autarquia Estadual - Lei n.° 8899 de 27/09/94
(Reconhecida pelo Decreto Federal n.° 74.179 de 14/06/74)

Parecer n.° 238/2005

COMITÊ DE ÉTICA EM PESQUISA

O Protocolo n.° ***5642/2005*** sob a responsabilidade de Gustavo Navarro Betonico, com o título "Influência do polimorfismo de UDP-glucuronosiltransferases na ocorrência de efeitos colaterais induzidos por micofenolato mofetil em transplantes renais", está de acordo com a Resolução CNS 196/96 e foi aprovado por esse CEP. Aguardar aprovação da CONEP para início do estudo.

Lembramos ao senhor(a) pesquisador(a) que, no cumprimento da Resolução 251/97, o Comitê de Ética em Pesquisa (CEP) deverá receber relatórios semestrais sobre o andamento do Estudo, bem como a qualquer tempo e a critério do pesquisador nos casos de relevância, além do envio dos relatos de eventos adversos, para conhecimento deste Comitê. Salientamos ainda, a necessidade de relatório completo ao final do Estudo.

São José do Rio Preto, 10 de outubro de 2005.

Prof. Dr. Antonio Carlos Pires
Coordenador do CEP/FAMERP

Av. Brigadeiro Faria Lima, 5416 - 15090-000 - São José do Rio Preto - SP - Brasil
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MINISTÉRIO DA SAÚDE
Conselho Nacional de Saúde
Comissão Nacional de Ética em Pesquisa - CONEP

PARECER Nº 310/2006

Registro CONEP: 12508 (Este nº deve ser citado nas correspondências referentes a este projeto)

CAAE – 0977.0.140.000-05 **Processo nº 25000.181815/2005-24**
Projeto de Pesquisa: *"Influência do polimorfismo de UDP-glucuronosiltransferase na ocorrência de efeitos colaterais induzido por micofenolato mofetil (MMF) em transplante renais".*
Pesquisador Responsável: Dr. Gustavo Navarro Betônio
Instituição: Faculdade de Medicina de São José do Rio Preto – FAMERP
Patrocinador: Não se aplica
Área Temática Especial: Genética humana/cooperação estrangeira

Ao se proceder à análise do projeto de pesquisa em questão, em resposta ao Parecer CONEP nº 081/2006, cabem as seguintes considerações:

a) O pesquisador informou como fará o recrutamento dos sujeitos da pesquisa com aprovação do CEP e adequou o Termo de Consentimento Livre e Esclarecido com as alterações solicitadas.
b) As instituições parceiras apresentaram declaração de anuência com o estudo.
c) A participação estrangeira se dará em forma de colaboração científica, e estão consignados os compromissos com as Res. CNS 292/1999, 340/2004 e 347/2005.
d) As informações enviadas atendem aos aspectos fundamentais da Res. CNS 196/96 sobre diretrizes e normas regulamentadoras de pesquisas envolvendo seres humanos.
e) O projeto foi aprovado pelo Comitê de Ética em Pesquisa – CEP da instituição supracitada.

Diante do exposto, a Comissão Nacional de Ética em Pesquisa – CONEP, de acordo com as atribuições definidas na Res. CNS 196/96, manifesta-se pela aprovação do projeto de pesquisa proposto.

Situação: Protocolo aprovado

Brasília, 17 de abril de 2006.

WILLIAM SAAD HOSSNE
Coordenador da CONEP/CNS/MS

----- Original Message -----
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TRANSPLANTATION PROCEEDINGS

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Aug 3, 2007

Dear MÁRIO ABBUD FILHO:

As you may know, Transplantation Proceedings is the Official Journal of the Brazilian, Luso-Brazilian, Latin American, and Carribean Transplantation Societies. It is with great pleasure that I invite you to submit a manuscript as part of the proceedings of the joint VI Luso-Brazilian Transplantation Congress, XIX Latin American and Carribbean Transplantation Congress, and X Brazilian Transplantation Congress to be held in Florianopolis, Brazil from September 1-5, 2007. The outcome of your efforts, I am certain, will be very useful to our field and we sincerely hope you will submit a manuscript for consideration.

Both oral and poster presenters of this meeting are invited and encouraged to submit manuscripts. We sincerely hope that you elect to submit a manuscript for the following presentation:

Influence of UDP-glucuronosyltransferase polymorphisms on mycophenolate mofetil-induced side effects in kidney transplant patients

We are pleased to announce that Transplantation Proceedings staff will be on-site in Florianoplolis on September 1-4 to personally receive your manuscripts and answer any questions you may have. All manuscripts submitted to the journal will be reviewed once by the Guest Editor and again by the Transplantation Proceedings peer review panel.

All manuscripts must be received before the end of the meeting unless special permission is granted to you personally by either the Editorial Office staff or the meeting organizers. Please visit the on-site Editorial Office for instructions as we will not accept manuscripts for any reason following the meeting unless prior arrangements are made. Staff will be available in Florianopolis on September 1 from 12:00pm- 3:00pm and on September 2-4 from 9 am - 5 pm.

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Sincerely,

Barry D. Kahan, PhD, MD