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The impact of CYP3A5 on the metabolism of cyclosporine A and tacrolimus in the evaluation of efficiency and safety of immunosuppressive treatment in patients after kidney transplantation

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The aim of the study was to determine the impact of CYP3A5 mutation on the serum levels of immunosuppressive drugs (tacrolimus and cyclosporine A), and on the occurrence of acute rejection episodes among patients after kidney transplantation. A limited number of such research in Polish patients was also an important factor encouraging to perform the study. Fifty-two persons were recruited. The tested patients underwent kidney transplantation and were treated either with cyclosporine A (17 persons) or with tacrolimus (35 persons). The group included 21 women and 31 men. DNA was isolated from whole blood and a modified Van Schaik et al. (2002) PCR-RFLP method was used for genotyping. The serum levels were controlled at the 7th, 14th, 30th, 90th, 180th and 360th day after transplantation. The CYP3A5 genotype had no impact on the concentrations of cyclosporine A and tacrolimus at any investigated time point. No correlation between the rate of acute rejection episodes and different genotypes of the CYP3A5 isoenzyme could be proven.

1. Introduction

Modern transplantology has developed in the last few years mainly because of new drugs used in immunosuppressive therapy, which is an inseparable element of effective treatment after each transplantation. In that context, research on the pharmacokinetic parameters of immunosuppressive drugs and their influence on the safety and effectiveness of therapy is needed. As for the huge progress of molecular techniques, modern scientists are aware of the impact of genetic polymorphism on pharmacokinetics and, what comes along with it, the possibility of individualized therapy. The improvement of pharmacotherapy can minimize the incidence of adverse drug reactions (ADR) or acute rejection episodes caused by too low drug serum levels. Cyclosporine A (CyA) and tacrolimus (Tac) are two drugs used in the optimal maintenance immunosuppressive therapy after renal transplantation. They are both calcineurin inhibitors, but show different side effects and toxicities. Tacrolimus may be less nephrotoxic than cyclosporine A. Patients receiving this drug showed better graft function and easier blood pressure control, but a high incidence of post-transplantation diabetes mellitus (Mutschler et al. 2010; Tarchalska-Kryńska 2010). However, despite the long use of these agents in clinical practice, the best dosage regimen is still a matter of intense debate. At present, research has shown that the CYP3A5 genotype modifies their pharmacokinetics (Tang et al. 2010, 2011). CYP3A5 is one of the P450 isoenzymes. Its gene is located at the 7p21. For this gene many single nucleotide polymorphisms (SNP's) were identified. One of them is the A6986G SNP in intron 3. This transition causes a loss of CYP3A5 activity in homozygous for the CYP3A5*3 allele and a decrease in enzyme activity in heterozygotes CYP3A5*1/*3 (Ferraris et al. 2011; Chen et al. 2014). Therefore genotyping may be an attractive option for therapeutic drug monitoring in order to start the dosing of cyclosporine A and tacrolimus.

2. Investigations and results

In the conducted research, first we tried to determine whether there is an impact of CYP3A5*1 and CYP3A5*3 alleles on the serum levels of cyclosporine A and tacrolimus. Secondly, correlations between possible CYP3A5 genotypes and acute rejection episodes rates were established. Thirdly, we tried to find out whether other factors, such as sex or age, may influence the serum levels of CyA and Tac.

The research was carried out with the permission of the Ethics Committee of the Wrocław Medical University (KB-242/2012).

Fifty two patients of the Department and Clinic of Nephrology and Transplantation Medicine of the Wrocław Medical University were included. The recruited patients underwent kidney transplantation in 2000 – 2012. Either cyclosporine A (17 patients) or tacrolimus (35 patients) were administered. The group included 21 women and 31 men. The median age was 49 years and the median weight was 71.5 kg respectively. The patients have been treated with haemodialysis approximately for 2.35 years before the transplantation. The induced therapy complied with the recommendations of the Polish Transplantation Society and the KDIGO (Kidney Disease Improving Global Outcomes Association). The characteristics of patient's diseases that led to an end-stage renal failure and the immunosuppressive regimen are presented in figure 1 and 2 respectively.

We evaluated a patient's protocol which was compliant with the "*Recommendations for Immunosuppressive Therapy after Organ Transplantation"* worked out by the Polish Transplantation Society. The protocol was divided into three parts: biometric, biochemical data and parameters connected with the pharmacokinetics of cyclosporine A and tacrolimus after 7, 14, 30, 90, 180 and 360 days from kidney transplantation.

Table 1 shows the percentage of CYP3A5*1/*1, CYP3A5*1/*3 and CYP3A5*3/*3 genotypes in recruited patients and the occurrence of acute rejection episodes.

Average dose and serum levels among different genotypes separately for cyclosporine A and tacrolimus for chosen time points are shown in Tables 2 and 3.

According to the presented results, gender did not influence the frequency of different genotypes. No relation between the concentration of CyA and Tac and genotypes was observed at any time point. Different genotypes of the CYP3A5 isoenzyme had no impact on the

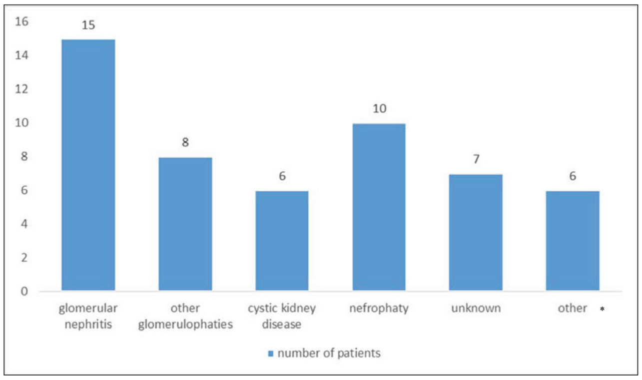

Fig. 1: Primary kidney disease in patients (*Other known diseases that led to an end-stage renal failure)

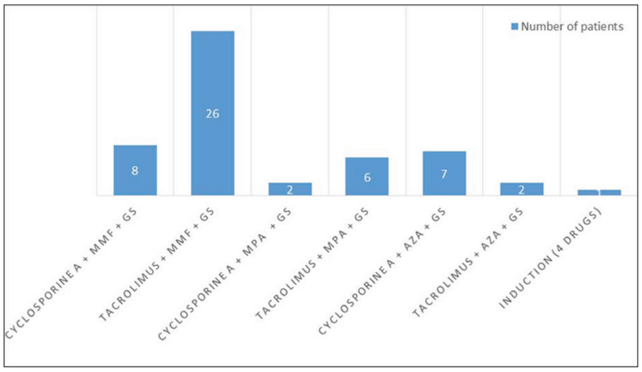

Fig. 2: Immunosuppressive regimen in patients (MMF - mycophenolate mofetil, GS - steroids, AZA - azathioprine, MPA - mycophenolic acid)

number of acute rejection episodes. The concentration of tacrolimus and cyclosporine A wasn't influenced by the age of patients enrolled.

3. Discussion

We examined a total number of 52 patients after renal transplantation. The safety and efficiency of treatment with cyclosporine A and tacrolimus in correlation with the CYP3A5 isoenzyme was established. In the undertaken research we also tried to determine if factors such as genotype, sex, age might influence the serum levels of tacrolimus and cyclosporine A in patients after kidney transplantation. No correlations were found in both groups.

For cyclosporine A the result was consistent with the findings of other authors based on Caucasians after kidney transplantation. In the meta-analysis of Tang et al.(2010) the significant growth of the CyA concentration in blood after the administration of standard doses was observed only among the Asiatic population, whose phenotype and genotype differ from the Caucasian one. This is why adjustments of dosage regimen in that population are needed. In a study carried out with people of Chinese origin a correlation between CyA concentrations and loss of enzymatic activity for the CYP3A5*3/*3 genotype was found. Nevertheless results in this population are ambiguous (Shi et al. 2011). For example in a study of Zhao et al. (2005) the C_0 (through level) of cyclosporine A stands in no relation to the CYP3A5 genotype among Asians. In the Caucasian population an influence of allel *1 and *3, on the metabolism of cyclosporine A wasn't observed which is described in the meta-analysis of Tang et al. (2010) and shown in the research of Anglicheau et al. (2004). In the last study, the influence of the CYP3A5 and MDR1 polymorphism on the pharmacokinetics of CyA in 160 French patients after renal transplantation was examined. The study showed no influence of the CYP3A5 genotype on the drug serum levels. Also in a recent paper of Lunde et al.(2014) no correlation between CYP3A5*1 and CyA exposure was found.

Table 1: Results of genotyping and the percentage of acute rejection episodes

Recruited patients group				
Genotype	Women	Men	Average value in the whole group	Acute rejection episodes
*1/*1	9%	13%	11.5%	5.76%
*1/*3	0%	6%	4%	1.92%
*3/*3	91%	81%	84.5%	26.92%

Table 2: Average dose (D) and serum levels (C0) among different genotypes in chosen time points for cyclosporine A

Genotype	**Days after transplantation**											
	7		14		30		90		180		360	
	D (mg)	C_0 (µg/ml)	D (mg)	C_0 (µg/ml)	D (mg)	C_0 (µg/ml)	D (mg)	C_0 (µg/ml)	D (mg)	C_0 (µg/ml)	D (mg)	C_0 (µg/ml)
***3/*3**	443.75	228.50	384.61	325.74	335.71	267.72	250.78	258.92	206.67	213.24	161.81	158.07
***1/*3**	-	-	-	-	-	-	-	-	-	-	-	-
***1/*1**	400.00	308.75	366.67	315.47	250.00	252.95	237.50	296.00	237.50	224.40	152.50	180.70

Table 3: Average dose (D) and serum levels (C0) among different genotypes in chosen time points for tacrolimus

Days after transplantation												
Genotype	7		14		30		90		180		360	
	D (mg)	C_0 (ng/ml)	D (mg)	C_0 (ng/ml)	D (mg)	C_0 (ng/ml)	D (mg)	C_0 (ng/ml)	D (mg)	C_0 (ng/ml)	D (mg)	C_0 (ng/ml)
***3/*3**	11.48	15.21	10.51	14.40	9.59	12.61	7.29	11.60	5.65	8.18	4.27	7.98
***1/*3**	12.50	10.30	10.00	11.05	14.00	8.00	8.00	8.10	7.50	7.90	-	-
***1/*1**	9.17	11.67	11.50	10.57	9.75	13.85	6.75	10.62	5.62	7.27	4.00	6.70

Table 4: Methodology used for CYP3A5 genotyping

CYP3A5 allele	Primer sequences	PCR conditions	PCR product size (base pair)	Restriction enzyme	Restriction fragment size (base pair)		Used marker
					Wild type	Variant allele	
*3	5'-CATCAGTTAGTAGACAGATGA-3' 5'-GGTCCAAACAGGGAAGAAATA-3'	7 min at 94°C; 35 cycles of 1 min. at 94°C, 1 min. at 55°C, 1 min. at 72°C; and 7 min. at 72°C	293	SspI	148, 125, 20	168, 125	DNA M1 Marker

As for tacrolimus, there is a lot of research on the drug and the impact of genotypes of CYP3A5 isoenzyme on its serum concentrations in renal transplantation recipients. The study of Quteineh et al. (2008) including French patients shows the influence of CYP3A5 polymorphism on the concentrations and daily dosage regimen of tacrolimus. Homozygous *3/*3 patients needed lower doses of tacrolimus during a year after transplantation, than the wild-type homozygotes and heterozygotes (the frequencies of the CYP3A5 genotypes were 15%, 10% and 75% for *1/*3, *1/*1 and *3/*3 respectively). The higher tacrolimus C_0 levels in homozygotes *3/*3 were connected with lower enzymatic activity. In a study conducted in the Netherlands, a correlation between C_0 levels and the genotype was also observed, but only on day three after transplantation. Then, the serum levels were similar among different genotypes (the frequencies of the CYP3A5 genotypes in this group of patients were 15%, 4% and 81% for *1/*3, *1/*1 and *3/*3 respectively). The homozygotes *3/*3 needed lower doses of tacrolimus during the first year after transplantation (Hesselink et al. 2008). A Spanish study also states the impact of CYP3A5 genotype on the dosage regimen of Tac (the frequencies of the CYP3A5 genotypes is this study were 9%, 1% and 90% for *1/*3, *1/*1 and *3/*3 respectively) (Gervasini et al. 2012). In the meta-analysis done by Tang et al. (2011), the difference between serum levels in relation to CYP3A5 genotypes varies in different ethnic groups. In Caucasians a lower dependency of tacrolimus blood concentration and the CYP3A5 genotype, than in the Chinese and Japanese population was observed. Lately, new research results among a Polish population were published. In a study, where 241 Polish-Caucasian patients were tested (the frequencies of the CYP3A5 genotypes were 7.2%, 0% and 92.5% for *1/*3, *1/*1 and *3/*3 respectively) the CYP3A5 expressers showed significantly lower tacrolimus C_0 at all-time points investigated - 7, 14, 90, 180, 360 days after transplantation (Kurzawski et al. 2014). In our research no impact between genotypes of CYP3A5 and tacrolimus concentration or dosage was found. This can be due to the limited amount of patients, the influence of genetic polymorphisms of other isoforms of the CYP3A subfamily and other genes influencing the CYP3A phenotype such as PPARA (peroxisome proliferatoractivated receptor alpha). Recent studies demonstrated that the CYP3A4*22 allele may affect the clearance of tacrolimus and the final dose regimen of the drug, PPARA polymorphism is associated with lower Tac C_0/D ratio (Lunde et al. 2014).

We also investigated the influence of CYP3A5 genotype on acute rejection episodes rate. Different genotypes have not been connected with the number of acute rejection episodes neither in the group treated with cyclosporine A nor in the one with tacrolimus. A meta-analysis by Tang et al. (2010) showed that the risk of acute rejection was similar between different genotypes of CYP3A5 isoenzyme and CyA pharmacotherapy especially in the Caucasian population. The scientists also signalize that there was an insufficient number of patients that underwent examination to confirm the finding. Our research was also performed with a small number of patients. In the meta-analysis of Tang et al. (2011) where tacrolimus was examined the findings were similar. Nevertheless in the first month after transplantation acute rejection episodes may occur more often. This is connected with the fact that patients having minimum one copy of the *1 allel show higher enzymatic activity, which can lead to a lack of target concentration achievement. The Spanish studies and the research done in the Netherlands found no correlation between genotype and acute rejection episode rates similarly to the findings of our research (Hesselink et al. 2008; Gervasini et al. 2012). Only one study carried out in France stated that the CYP3A5 genotype influenced the number of acute rejection episodes in patients treated with tacrolimus during the first month after transplantation. This finding stands in no conflict with those of Tang et al. mentioned above (Quteineh et al. 2008; Tang et al. 2011).

The influence of age on the pharmacokinetics of tacrolimus and cyclosporine A was also tested in the conducted research. There was no correlation between the variables, which complies with bibliographical data. In the study undertaken by Fanta et al.(2008) no impact of age on the pharmacokinetics of CyA was determined. Tirelli et al. (2008) showed no correlation between age and serum levels of tacrolimus. Sex did not affect either tacrolimus or CyA concentration.

In conclusion, further research needs to be conducted, on a larger number of patients to define the influence of the CYP3A5 genotype on the cyclosporine A and tacrolimus pharmacokinetics and acute rejection episodes.

4. Experimental

4.1. Materials

The material used for the genetic research was whole blood, drawn on the anticoagulant ethylenediaminetetraacetic acid (EDTA).

4.2. DNA isolation and genotyping

We isolated DNA using the QIAamp® DNA Blood Mini Kit, according to the manufacturer's instruction in a laminar flow cabinet.

We used a modified PCR-RFLP method previously adapted to the requirements of the Pharmacogenetics and Pharmacogenomics Laboratory of the Department of Clinical Pharmacology for genotyping (Van Schaik et al. 2002). Most important information on the methodology are gathered in Table 4.

4.3. Drug concentration measurement

The determination of drug serum levels was carried out during routine control visits after 7, 14, 30, 90, 180 and 360 days after the transplantation by the diagnostic laboratory of Wrocław Medical University Hospital. Till 2008 FPIA (*Fluorescence polarization immunoassay*) (Abbott, TDx® Analyzer) was the method used for cyclosporine A. Later the method was changed to ACMIA (*Affinity Column – mediated Immunoassay*), which was used from 2008 to 2012 (Dimension® Xpand® Plus Integrated Chemistry System, Siemens Healthcare Diagnostics Inc.). Tacrolimus concentrations were measured using the MEIA (*Microparticle Enzyme Immunoassay*) method (Abbott, IMx® Analyzer).

4.4. Statistical methods

Statistical analysis was performed with the SPSS statistical software (SPSS 16, Chicago Il.). The groups were compared with nonparametric tests. For comparison of two groups the Wilcoxone test for several groups the Kruskal-Wallis test was used. P values less than 0.05 were considered statistically significant. To determine the influence of different variables such as sex, age, genotype on the concentration values of Tac and CyA the χ^2 test was used.

Conflict of interest: None declared.

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