

## RESEARCH ARTICLE

## Correlation between pharmacokinetics and pharmacodynamics of tacrolimus in the treatment of pediatric patients with renal transplant

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### ABSTRACT

**Background.** Tacrolimus is a widely used immunosuppressant in renal transplant patients. Drug monitoring is performed by measuring trough levels. Pharmacodynamic monitoring is used to evaluate the biological effect.

The aim of the study was to determine the relationship between tacrolimus area-under concentration vs. time curve (AUC) and the gene expression of tumor necrosis factor alpha (TNF- $\alpha$ ) in children with renal transplant.

**Methods.** An experimental, cross-sectional study was performed in children with renal transplant at 3 months after transplant. An 8-h pharmacokinetic-pharmacodynamic profile was obtained. Gene expression of TNF- $\alpha$  and internal control of 18s rRNA was performed by real-time PCR. CYP3A5 genotype was obtained by direct sequencing.

**Results.** Six patients were included with a median age of 14.5 years. A negative correlation was found between tacrolimus pharmacokinetics and TNF- $\alpha$  gene expression. There was a trend of low TNF- $\alpha$  with high tacrolimus blood levels.

**Conclusions.** There is a negative correlation between tacrolimus concentration and TNF- $\alpha$  gene expression. TNF- $\alpha$  gene expression was not modified by CYP3A5 genotype.

**Key words:** tacrolimus, pharmacokinetics, pharmacodynamics, TNF- $\alpha$ , renal transplant, immunosuppression.

### INTRODUCTION

Kidney transplantation is the best treatment for pediatric patients with chronic end-stage renal disease (ESRD) because it not only resolves symptoms of uremia but also allows a significant improvement in skeletal growth retardation, sexual maturation, cognitive and psychosocial performance. The quality of life of pediatric patients with functional kidney graft is always superior to any existing method of dialysis.<sup>1</sup>

Transplant treatment provides better survival than dialysis in any age group of pediatric patients. Graft survival is described to be up to 95% at 5 years of the transplant, whereas patients subjected to dialysis have an 80% survival rate.

Tacrolimus is a drug with a narrow therapeutic index used to prevent rejection in solid organ transplantation. It requires careful handling in the adjustment of the dose used to obtain therapeutic levels. Its mechanism of action is by binding to an intracellular protein called the FK binding protein (FKBP-12). Once the FKBP-tacrolimus complex is formed together with calcium, a selective inhibition of calcineurin occurs, an enzyme that normally acts as a phosphatase of certain regulatory nuclear proteins. One is the nuclear factor of activated T cells (NFAT) which, under normal conditions, on being dephosphorylated, passes through the nuclear membrane. In this manner the expression of various genes involved in T-cell activation is inhibited, including the gene

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for interleukin 2 (IL-2), its receptor, IFN- $\gamma$ , GM-CSF, TNF- $\alpha$  and protooncogenes *H-ras* and *c-myc*. At the same time, promotion of TGF- $\beta$  is carried out, which also inhibits IL-2 with a consequent decrease in the proliferation of cytotoxic T lymphocytes and limitation on cytokine production. However, it also causes the development of interstitial fibrosis, which is the main cause of nephrotoxicity associated with calcineurin inhibitors. The degree of inhibition of calcineurin activity and production of IL-2 may reflect the balance between an excessive or deficient immunosuppression.<sup>2,3</sup>

Tacrolimus is metabolized by microsomal enzymatic systems of the cytochrome P-450 IIIA (CYP3A4 and CYP3A5) found in the gastrointestinal tract and liver. Tacrolimus dose required to achieve therapeutic levels depends on the type of polymorphism in the CYP3A5 enzyme. Those patients who express the enzyme require greater doses.<sup>4</sup>

Therapeutic monitoring of tacrolimus is carried out by determining the minimum concentrations, also known as peak levels. However, this has been questioned because some cases of toxicity and rejection present themselves, even when low concentrations are considered to be within acceptable limits. Several studies have shown a good correlation between blood concentration and area under the curve (AUC) of plasma concentration against time. This correlation can be improved by using different sampling times.<sup>2,5-7</sup> It should be noted that drug monitoring does not reflect the biological activity of the drug.

In the last 6 years, pharmacodynamic monitoring has been proposed as a new strategy to provide information about the specific biological effect exerted by the mechanism of action of tacrolimus.<sup>8</sup>

Regarding pharmacodynamic monitoring, there are several reports for cyclosporine (CsA), another calcineurin inhibitor, and it has been reported that the maximum concentration (C<sub>max</sub>) reached at ~2 h is related to the level of immunosuppression, measured as inhibition of calcineurin in lymphocytes.<sup>9</sup>

Giese et al. showed a correlation between the level of blood CsA and suppression of IL-2, TNF- $\alpha$  and GM-CSF up to 85%, which represents an approach for assessing the biological effectiveness of CsA, allowing for individualization of the immunosuppressive regimen.<sup>10</sup> Pharmacodynamic monitoring is applied as an additional method to provide information on the biological impact of CsA in transplanted patients, such that inhibition of calcineurin in lymphocytes is inversely correlated with blood concentrations of CsA.

Maximal inhibition of calcineurin occurs 2 h following initial dose in 90% of patients.<sup>11-13</sup> Pharmacodynamic studies with tacrolimus have been carried in adult populations, and it has been suggested that the residual expression of genes regulated by NFAT could be a pharmacodynamic method for monitoring renal transplant patients treated with tacrolimus because it can identify low or high immunosuppression based on its relationship with the presence of infection or acute rejection of the graft.<sup>14</sup> The objective of this study was to determine the correlation between the area under the curve (AUC) of plasma concentration of Tac against time and the genetic expression of TNF- $\alpha$  regulated by NFAT in children with renal transplants.

## PATIENTS AND METHODS

An experimental cross-sectional, analytic study was conducted. The study was approved by the research and ethics committees of the Hospital Infantil de México Federico Gómez (HIMFG) (HIM protocol 2011/026, SSA 949). Pediatric patients of both genders and any age group from the HIMFG were invited to participate. Patients were diagnosed with chronic kidney disease and renal transplant at least 6 months progress after the surgical intervention.

### Inclusion criteria

Inclusion criteria included the following: patients of both genders and with ages between 3 and 18 years with 3 months from renal transplantation in the HIMFG; immunosuppression established with tacrolimus; stable graft function (serum creatinine level  $\leq 0.3$  mg/dl at discharge) after renal transplantation without metabolic disorder and clinically stable; no adjustments in immunosuppressive therapy 3 months prior to the taking sample; no apparent infection at the time of the study; voluntary participation in the study with a letter of consent from the parent or guardian and assent of each child.

### Exclusion criteria

Exclusion criteria were as follows: renal transplant patients with administration of tacrolimus in different doses at each time administered or a different administration interval every 12 h; concomitant administration of drugs affecting tacrolimus plasma concentrations with the exception of verapamil; and patients with any type of liver diseases.

### Elimination criteria

Elimination criteria included patients with impaired liver function tests at the time of the study or elevated cholesterol or triglycerides, which could cause alterations in plasma measurement of tacrolimus and other serum parameters; patients with recent infections or suspicion of any kind of infection that could change the level of immunosuppression. We recorded the following variables: age, gender, complete physical examination, regimen and dose of complete immunosuppression in weighted doses, concomitant treatment with antihypertensives, antimicrobial prophylaxis and electrolytes. Regarding the pharmacokinetics and pharmacodynamics, it was conducted at an interval of 8 h in all patients from the initial administration of the medication at 8:00 am until 4:00 pm. Six sampling points were taken at different times (0, 1, 2, 4, 6 and 8 h).

Each sample was processed with 5.4 ml whole blood placed into a tube containing EDTA. For each sample, the plasma concentration of tacrolimus and the gene expression of TNF- $\alpha$  was determined. Additionally, in the first sample the procedure for obtaining DNA from leukocytes was carried out and the subsequent sample was used for CYP3A5 genotyping. The samples were stored at 4°C until processing to determine plasma levels. The remaining samples were used for cell culture. In the 0 sample, serum creatinine and liver function tests were also carried out. Glomerular filtration rate was estimated using the Schwartz formula.<sup>15-17</sup>

### Determination of tacrolimus levels

Determination was performed on whole blood using a commercial kit (Architect System, Abbott Park, IL) for particle enzyme immunoassay (CMIA).

### Sample preparation

The blood in EDTA tubes was stimulated with 1 ml of RPMI-1640 medium (culture medium) supplemented with 10% FBS and antibiotics plus 100 ng/ml phorbol myristate acetate (PMA) and 5 mg/ml ionomycin (Sigma, St. Louis, MO) for 3 h. Subsequently, it was centrifuged for 15 sec at 10,000 rpm in a CO<sub>2</sub> incubator at a controlled temperature of 37°C. After lysis of red cells and removal of the supernatant, it was stored at -70°C. Washing of WBCs was done twice with sterile saline for later storage in combination with the RNA reagent (Ambion) at -70°C until the time of extraction of ribonucleic acid (RNA). Extraction

of RNA from WBCs was carried out using a commercial kit (RNAeasy mini kit, Qiagen, Hilden, Germany). After this, reverse transcription was then performed to obtain the cDNA. Quantification of the expression of the TNF- $\alpha$  gene and the internal control gene 18s rRNA was carried out using real-time PCR with a RT-PCR 7500 kit (Applied Biosystems, Foster City, CA) with amplification of sequences using commercial probes (Applied Biosystems). The concentration of the transcript for the target gene was calculated from a standard curve.

### Determination of genotype

Genotype determination was performed by direct DNA extraction for subsequent PCR amplification, purification and sequencing as previously described.<sup>4</sup>

### Statistical analysis

Using descriptive statistics, determination of averages and standard deviation of normally distributed data was done. In non-normally distributed variables, calculation of medians and ranges (minimum and maximum) was done. For some variables a calculation of proportions was performed.

For calculation of correlation, Spearman r test was carried out when there was an abnormal distribution of the study population, using the program Graph Pad Prism v.5.0. To determine the existing comparison between genotypes expressing and not expressing the CYP3A5 gene with pharmacokinetic parameters, we used Mann-Whitney U test (SPSS v.16).

## RESULTS

There were six patients evaluated (Table 1). Average age was 14.5 years (range: 9–18 years). The median time after transplantation was 13.5 months (range: 5–29 months). Results of the pharmacokinetic study are shown in Table 2.

The plasma concentration curve of tacrolimus and genetic expression of TNF- $\alpha$  against time (average and standard error) show a tendency to decrease TNF- $\alpha$  levels at the highest levels of tacrolimus (Figure 1). It is noteworthy that the large standard deviation at each point is due to the low number of patients.

Of the patients included in the study, determination of the genotype for CYP3A5 was conducted in only five because in one patient there was no suitable sample available

and an erroneous reading of the sequencer was obtained. In three cases there was no expression of cytochrome (homozygous GG-\*3\*3) observed and, in two cases, the expression thereof (heterozygous AG-\*1\*3). Table 3 shows the distribution of the genotype by groups compared with the pharmacokinetics in each patient (Table 3). No statistically significant differences in the variables studied were found according to the CYP3A5 genotype. However, patients who expressed the protein required higher doses of tacrolimus.

Gene expression was determined by the number of copies per microgram of RNA TNF- $\alpha$ , normalized with the control gene of 18s rRNA. The dispersion of points of the total patients in the log-transformed (LN) TNF- $\alpha$ /18 s rRNA against tacrolimus concentrations at all time-points of the drug taking into consideration the time of administration is shown. There was a negative correlation between the concentration of tacrolimus and TNF- $\alpha$  expression ( $r = -0.07$ ) without reaching statistically significant differences. It was observed that, in effect, the concentration of TNF- $\alpha$  decreases as tacrolimus concentrations are higher with the highest levels at the initiation of the pharmacokinetic.

Taking into consideration the mean concentration of each point of the pharmacokinetics with regards to LN TNF- $\alpha$ /18s rRNA, it was observed that in the dispersion of points there is a negative correlation between the concentration of tacrolimus globally by point of pharmacokinetics with respect to the corresponding average for the same point of LN TNF- $\alpha$ . Applying Spearman  $r$ , an  $r = -0.498$  with  $p = 0.17$  was obtained (Figure 3).

## DISCUSSION

The results obtained are similar to those reported by Sommerer et al. in adult studies. In their study they compared the pharmacokinetics with pharmacodynamics of calcineurin inhibitors in terms of an inverse correlation between the administration of CsA and tacrolimus with the determination of inflammatory cytokine gene expression.<sup>11-14</sup> In the present study we did not observe a significant association between parameters. Nor was it possible to collect a sufficient amount of samples to generate normality and statistical significance in the distribution. However, the methodology was adequate for determination of the pharmacokinetics and cell culture for extraction of RNA and subsequent quantification of the gene

**Table 1.** Demographic distribution and clinical and laboratory characteristics in children with renal transplant

Values as median and range or n (%)	
Age (years)	14.5 (9-18)
Gender (male)	3 (50%)
Time after transplant (months)	13.5 (5-29)
Body mass index (kg/m <sup>2</sup> )	19.37 (15.21-27.85)
Serum creatinine (mg/dl)	1 (0.5-1.2)
Total cholesterol (mg/dl)	135 (96-172)
Triglycerides (mg/dl)	122 (45-144)
ALT (mg/dl)	38 (29-45)
AST (mg/dl)	24 (17-27)
GFR (Schwartz)	101.175 (96.83-106.85)
Mycophenolate (mg/m <sup>2</sup> SC/day)	696.015 (675.67-806.45)
Tacrolimus (mg/kg/day)	0.07 (0.03-0.26)
Valley concentraton of tacrolimus (ng/ml)	5.9 (2.8-6.3)
Peak concentration of tacrolimus (ng/ml)	13.5 (5.9-27.1)

ALT, alanine aminotransferase; AST, aspartate aminotransferase; GFR, glomerular filtration rate.

**Table 2.** Pharmacokinetic parameters of tacrolimus and genotyping of CYP3A5 in children with renal transplant

Patient	AUC/dose (mg/kg/day)	$t_{max}$ (h)	$C_{max}$ (ng/ml)	$t_{1/2}$ (h)	$C_0$ (ng/ml)	CYP3A5
1	1349.58	1	27.1	15.59	6.1	*1*3
2	3666.67	8	12.6	2.93	4.4	*3*3
3	1745.00	2	5.9	9.90	3.8	*3*3
4	355.92	1	14.4	5.94	8.2	*1*3
5	1780.00	1	16.5	10.00	6.0	*3*3
6	1101.00	2	10.8	12.98	7.0	ND

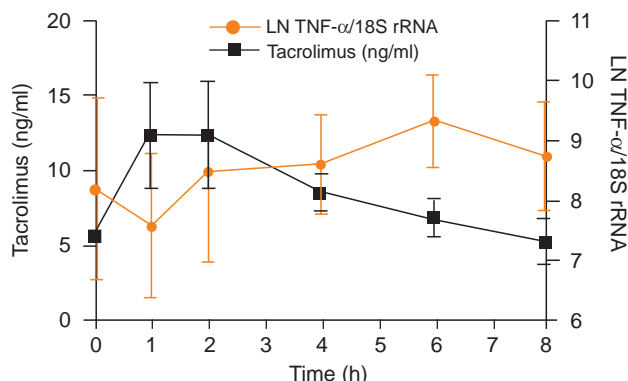
AUC, area under the curve (0-8 h);  $t_{max}$ , time to observe maximum concentration;  $C_{max}$ , maximum concentration reached;  $t_{1/2}$ , half-life;  $C_0$ , initial concentration; ND, not determined.

expression. It is interesting to note that, according to the literature, there is evidence that monitoring of calcineurin inhibitors with isolated plasma levels is not sufficient for individualization of doses per patient and that the genetic factor, with identification of polymorphisms of CYP3A5 and MDR1, in addition to complete pharmacokinetic parameters and genetic expression of genes produced by lymphocytes is essential for the prevention of acute graft rejection, infections, nephrotoxicity and long-term graft dysfunction.

It is important to consider that, in the present study, the number of patients included was low; therefore, the differences were not statistically significant. However, there was a tendency to an inverse correlation with re-

spect to tacrolimus levels compared with the expression of TNF- $\alpha$ .

It is necessary to carry out these types of studies in Mexican populations with a higher number of pediatric patients to determine the potential for the quantification

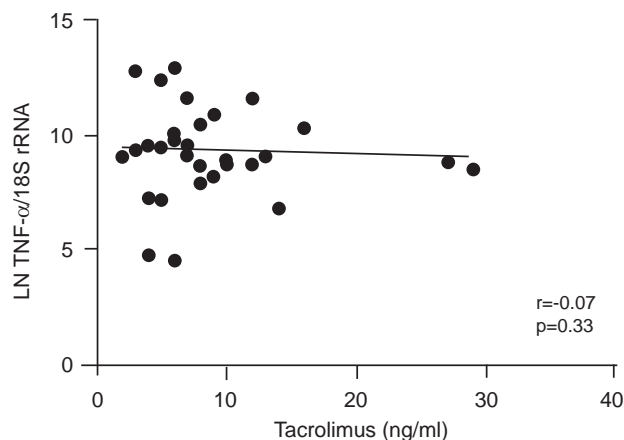


**Figure 1.** Curve of plasma concentration of tacrolimus and gene expression of TNF- $\alpha$ /18S rRNA vs. time. Values expressed as average and standard error.

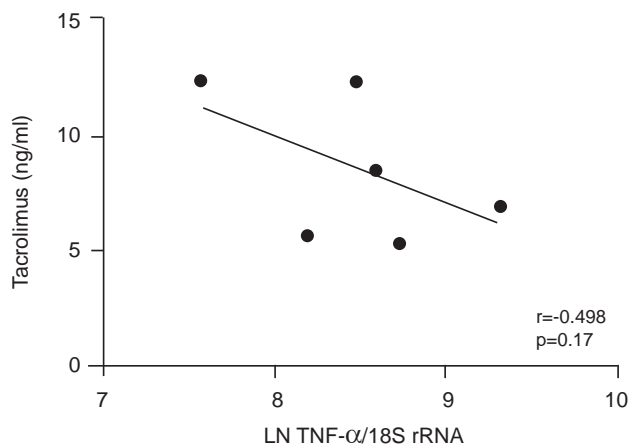
**Table 3.** Comparison of the pharmacokinetic parameters of tacrolimus with genotyping in children with renal transplant

	CYP3A5 *1*3 (Expressed) n=2	CYP3A5 *3*3 (Not expressed) n=3	P
Dose (mg/kg/day)	0.19 (0.12-0.26)	0.03 (0.03-0.06)	0.2
C <sub>0</sub> (ng/ml)	7.15 (6.1-8.2)	4.4 (3.8-6)	0.4
C <sub>max</sub> /dose (ng*ml/ mg*kg*day)	140.61 (55.38-225.83)	275 (196.67-420)	0.4
t <sub>1/2</sub> (h)	10.77 (5.94-15.59)	9.9 (2.93-10)	0.8
t <sub>max</sub> (h)	1 (1-1)	2 (1-8)	0.4
AUC <sub>0-8</sub> (h*ng/ml)	127.25 (92.54-161.95)	106.8 (52.35-110)	0.8
AUC <sub>0-8</sub> /dose (h*ng*ml/ mg*kg*day)	852.75 (355.92-1349.58)	1780 (1745-3666.67)	0.2

C<sub>0</sub>: initial concentration; C<sub>max</sub>: maximum concentration reached; t<sub>1/2</sub>: half-life; t<sub>max</sub>: time until maximum concentration is observed; AUC, area under the curve of plasma concentration against time (0-8 h).  
Value of p obtained with Mann-Whitney U test.



**Figure 2.** Correlation between gene expression of TNF- $\alpha$ /18S rRNA in peripheral blood leukocytes and serum levels of tacrolimus.



**Figure 3.** Correlation between concentration media of tacrolimus according to pharmacokinetic point and gene expression of TNF- $\alpha$ /18S rRNA.

of gene expression as a pharmacodynamic parameter of tacrolimus surveillance in order to achieve the individualization of immunosuppression.

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