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Monitoramento terapêutico de tacrolimus *em transplante de pâncreas no Hospital São Lucas*

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key words

Tacrolimus

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abstract

Tacrolimus (FK 506), a potent immunosuppressive drug used in prevention and treatment of rejection of transplanted organs, exhibits efficacy related to its blood levels and has a narrow therapeutic index. These factors require frequent monitoring of patients blood levels, in attempt to adjust the dose to reach the best drug concentration with minimum side effects. In this historic study, the authors evaluated *tacrolimus* blood profile in patients submitted to pancreas transplantation between June 2002 and March 2004. The results show that blood levels were, mostly, within subtherapeutic (39.1%) and toxic (43.4%) ranges. Considering post-transplantation period, subtherapeutic levels were more frequent until three months after the graft receiving (51.1%) and between three and six months (41.9%), whereas toxic levels were more common six months after the transplantation (63%). Patients who received pancreas/kidney transplantation showed a tendency to present toxic levels. The same did not happen with the patients who received isolated pancreas and pancreas after kidney; these patients presented subtherapeutic blood levels in all post-transplantation periods. The results found in this study reassure the importance of therapeutic monitoring to achieve the adequate blood levels of *tacrolimus* following pancreas transplantation.

unitermos

Tacrolimus

Imunossupressão

Pâncreas

Monitoramento terapêutico de fármacos

Transplante

resumo

O tacrolimus *(FK506), um potente imunossupressor utilizado na profilaxia e no tratamento de rejeições pós-transplante, exibe eficácia relacionada com sua concentração sangüínea e possui estreita janela terapêutica. Esses fatores requerem o freqüente monitoramento dos níveis sangüíneos em pacientes que fazem uso do fármaco, tendo como objetivo o ajuste de dose para uma concentração terapêutica ótima com efeitos colaterais mínimos. Este estudo retrospectivo foi realizado através do acesso à base de dados do Laboratório de Patologia Clínica do Hospital São Lucas, da Pontifícia Universidade Católica do Rio Grande do Sul (PUCRS), e teve por objetivo analisar o perfil das concentrações sanguíneas de* tacrolimus *em pacientes transplantados de pâncreas, no período de junho de 2002 a março de 2004. Os resultados mostram que as concentrações se encontravam, em sua maioria, em níveis subterapêuticos (39,1%) e tóxicos (43,4%). Considerando-se o período pós-transplante, níveis subterapêuticos foram mais freqüentes nos períodos de zero a três meses (51,1%) e de três a seis meses (41,9%) após o transplante, enquanto níveis tóxicos (63%) foram mais freqüentes após seis meses. Pacientes que receberam pâncreas/rim simultâneo apresentaram, de maneira geral, mais concentrações em níveis tóxicos; o mesmo não aconteceu em pacientes que receberam pâncreas isolado e pâncreas pós-rim. Os pacientes que receberam pâncreas isolado e pâncreas pós-rim tenderam a apresentar níveis subterapêuticos em todos os períodos pós-transplante considerados. Os resultados obtidos neste trabalho demonstram a importância do monitoramento terapêutico, uma vez que seus resultados orientam o ajuste das doses.*

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Introduction

Therapeutic drug monitoring (TDM) can be defined as the concentration measurement of therapeutic drugs in biologic fluids to improve their efficacy and limit their toxicity[16, 24]. In clinical toxicology practice, TDM provides diagnostic or prognostic information that may assist in guiding treatment[4]. Immunosuppressive drugs are critical-dose drugs, that is, they have a narrow therapeutic index with acceptable tolerability only within a narrow range of blood concentrations. Furthermore, they exhibit a high degree of interindividual and intraindividual pharmacokinetic and pharmacodynamic variability, what increases the possibility of therapeutic failure if these agents are used at uniform doses in all patients[15].

Available since 1994, *tacrolimus* is a potent immunosuppressant drug inhibitor of calcineurin, used in the prophylaxis and treatment of transplant rejection[8, 19]. Its absorption is rapid, variable, and incomplete from the gastrointestinal tract; mean bioavailability of the oral dosage form is 27% (range 5%-65%), and absorption rate decreases in the presence of food[33]. The concentration peak after oral administration occurs in 30 minutes to four hours. From the blood, about 80% is bound to erythrocytes, and variations in red-cell binding account for much of variability in pharmacokinetics[25, 34]. It is extensively metabolized by cytochrome P450 3A in liver enzymes, resulting in at least nine metabolites[5]. Only one metabolite, 31-demethylated *tacrolimus*, has pharmacological activity similar to that of the parent compound[31]. It is eliminated on feaces, in 11.3 hours mainly (range 3.5 to 40.5 hours), and less than 1% of the dose is eliminated unchanged in urine[33].

Important side effects are related to *tacrolimus*, such as nephrotoxicity and neurotoxicity[33]. Besides, as with any other immunosuppressant agent, there is an increased risk of developing secondary tumors and opportunistic infections[6, 19]. The incidence of side effects usually diminishes with the reduction of dose[6].

Due to its high interindividual and intraindividual pharmacokinetics variations, the poor correlation between dose and blood concentration, and its toxicity, *tacrolimus* blood levels must be monitored regularly[1, 12, 21]. Studies have shown that pharmacokinetics variability can be a reflex of polymorphisms of P-glycoprotein and CYP3A enzymes[7, 11, 22, 32].

The administration schedules may vary in the different transplantation centers, but the daily dose is generally divided in two takings: in the morning and at night[33]. The administration time does not affect the *tacrolimus* pharmacokinetics[9, 30]. It can be associated to other immunosuppressive drugs, but a dose adjustment is required due to the possibility of interactions [2, 5, 17, 18, 20, 23, 29, 32].

The aim of this historic study was to analyze, through database access, the *tacrolimus* blood concentration profile in pancreas transplanted patients of São Lucas Hospital, a university hospital, between June 2002 and March 2004, and to evaluate TDM, according to the type of transplantation and the period elapsed after it.

Methods

Whole blood samples (approximately 4 ml) were collected from patients under *tacrolimus* therapy after pancreas transplantation, using pneumatic tubes with ethylenediaminetetra-acetic acid (EDTA) and analyzed with Pro-Trac™ II *Tacrolimus* Assay (DiaSorin Inc., Stillwater, USA). The sample was collected 12 hours after the last *tacrolimus* taken, to obtain residual drug levels. The blood levels adopted as therapeutic by the hospital were: to simultaneous pancreas/kidney (SPK) transplantation, 10-12 ng/ml till three months after transplantation; 8-10 ng/ml three to six months after transplantation; and 5-8 ng/ml six months after transplantation. To pancreas transplant alone (PTA) and pancreas after kidney (PAK), therapeutic levels were 12-15 ng/ml until three months after transplantation; 10-12 ng/ml three to six months after transplantation; and 8-10 ng/ml six months after transplantation.

Data collected from patients, such as gender, age, and results, were analyzed on computer with Microsoft Office Excel 2003 and are summarized on **Table 1**. Other pieces of information, such as concomitant pathologies, administration of other drugs, and patient clinical evaluation, were not available through this database. Data are expressed as mean ± standard deviation (SD) and percentage. Fischer exact test was applied using the statistical program SPSS V 13.0. The level of statistical significance was accepted as $p < 0.05$.

Results

Tacrolimus blood levels of twenty-six patients of São Lucas Hospital, in the period between June 2002 and March 2004, were analyzed. From these twenty-six patients, twenty-two were SPK, four were PTA, and two were PAK who had received pancreas and kidney simultaneously in a

Table 1 Patients' data: gender, age, type of transplant and date, number of blood analyses

Patient	Gender	Age (years)	Type of transplant	Transplant date	Number of blood analyses
1	Female	27	SPK	8/13/2002	28
		29	PAK	3/4/2004	15
2	Male	35	SPK	8/17/2002	34
3*	Female	30	SPK	9/8/2002	11
4	Female	29	SPK	9/21/2002	22
5	Male	27	SPK	10/22/2002	37
6*	Male	49	SPK	11/4/2002	5
7	Male	39	SPK	11/27/2002	16
		40	PAK	10/17/2003	8
8	Male	40	SPK	12/9/2003	28
9*	Female	48	SPK	2/4/2003	6
10	Female	32	PTA	2/12/2003	32
11	Female	29	PTA	3/1/2003	19
12	Male	26	SPK	4/18/2003	26
13	Female	21	SPK	5/20/2003	23
14	Female	50	SPK	5/22/2003	12
15*	Male	26	SPK	11/7/2003	7
16*	Female	34	SPK	8/9/2003	3
17	Female	52	SPK	9/16/2003	10
18	Male	36	SPK	9/29/2003	9
19	Male	28	SPK	12/10/2003	27
20*	Female	34	SPK	12/9/2003	3
21	Female	47	SPK	12/28/2003	12
22	Female	23	SPK	1/27/2004	5
23	Male	23	PTA	2/10/2004	11
24	Male	39	SPK	2/20/2004	11
25	Female	33	SPK	2/27/2004	12
26	Male	39	PTA	3/14/2004	13

*SPK: simultaneous pancreas kidney transplant; PAK: pancreas after kidney transplant; PTA: pancreas transplant alone; *died (23.07%).*

past surgery and lost the primary pancreas due to surgical complications. The total of analyses was 445 (Table 1).

Tacrolimus monitoring frequency per patient varied from three to 37 analyses per patient (mean 15.9 ± 9.9). Age ranges of the patients were between 21 and 52 (mean 34.5 ± 8.8); 12 patients (46.1%) were male and 14 (53.9%) were female. Male age ranges between 23 and 49 (mean 37.4 ± 7.7), and female age ranges were between 21 and 52 (mean 34.5 ± 9.9).

Drug monitoring frequency was between three and 37 tests for SPK patients (mean 15.8 ± 10.5). Among PTA patients, drug monitoring frequency was between 11 and 32 tests per patient (mean 18.7 ± 9.5), and for PAK patients, the frequency was between eight and 15 tests (mean 11.5 ± 4.9).

SPK patients were submitted to more tests (347 tests, 77.9%) than the other categories (SPK, 75 tests, 16.8%; PTA and PAK, 23 tests, 5.3%), as shown in **Table 2**. Table 2 also shows the association between transplant type and blood levels. There was a significative association between type of transplant and *tacrolimus* levels; SPK patients presented toxic levels (47.3%), while PTA patients presented a larger number of tests with subtherapeutic levels (57.3%). PAK patients also presented more tests in subtherapeutic levels (69.6%).

Data analysis shows an association between the period after transplant and blood levels, as shown in **Table 3**. *Tacrolimus* blood levels are usually in the subtherapeutic interval both in the first three months and in the period between three and six months after the graft receiving. Toxic levels are more commonly found six months after the transplantation.

There is an association between blood levels, transplant type and period after the transplant for patients who received SPK and PTA. In both situations, most of the analyses were found in subtherapeutic levels in the first three months after transplantation, and toxic after six months. Patients who received PAK, however, had more results in subtherapeutic levels. There was no association between SPK transplantation and blood levels and period after transplant. It is important to say that there was no association between the period of three to six months and blood levels or transplant type, as shown in **Table 4**.

Table 2 Analyses distribution associating transplant type and blood levels

Transplant type	Subtherapeutic levels		Therapeutic levels		Toxic levels		Total	
	n	%	*n*	%	*n*	%	*n*	%
SPK	115	33.1	68	19.6	164	47.3	347	100
PTA	43	57.3	8	10.7	24	32	75	100
PAK	16	69.6	2	8.7	5	21.7	23	100
Total	174	39.1	78	17.5	193	43.4	445	100

SPK: simultaneous pancreas kidney transplant; PAK: pancreas after kidney transplant; PTA: pancreas transplant alone; p < 0.001.

Table 3 Analysis distribution associating blood levels with period after transplant

Post-transplant period	Subtherapeutic levels		Therapeutic levels		Toxic levels		Total	
	n	%	*n*	%	*n*	%	*n*	%
0-3 months	119	51.1	34	14.6	80	34.3	233	100
3-6 months	31	41.9	17	23	26	35.1	74	100
After 6 months	24	17.4	27	19.6	87	63	138	100

p < 0.001.

Table 4 Analyses distribution associating blood levels, transplant type and post-transplant period

Transplant type	Post-transplant period	Subtherapeutic levels		Therapeutic levels		Toxic levels		Total	
		n	%	*n*	%	*n*	%	*n*	%
SPK ($p < 0{,}001$)	0-3 months	81	44.7	32	17.7	68	37.6	181	100
	3-6 months	25	39.1	16	25	23	35.9	64	100
	After 6 months	9	8.8	20	19.6	73	71.6	102	100
	Total	115	33.1	68	19.6	164	47.3	347	100
PTA ($p = 0{,}007$)	0-3 months	28	77.8	1	2.8	7	19.4	36	100
	3-6 months	4	50	1	12.5	3	37.5	8	100
	After 6 months	11	35.5	6	19.3	14	45.2	31	100
	Total	43	57.3	8	10.7	24	32	75	100
PAK ($p = 0{,}579$)	0-3 months	10	62.5	1	6.3	5	31.2	16	100
	3-6 months	2	100	-	-	-	-	2	100
	After 6 months	4	80	1	20	-	-	5	100
	Total	16	69.6	2	8.7	5	21.7	23	100

SPK: simultaneous pancreas kidney transplant; PAK: pancreas after kidney transplant; PTA: pancreas transplant alone.

Discussion and conclusions

Tacrolimus results show that the tests were, mainly, in subtherapeutic (39.1%) and toxic (43.4%) levels, without considering the type of transplant. Only 17.5% of the results were within the level referred to by the hospital as therapeutic. When considering the post-transplant period, subtherapeutic levels were more frequent till three months after the transplant (51.1%) and between three and six months (41.9%), while toxic levels (63%) were more common after six months.

Patients who received SPK usually had a larger number of analyses in toxic levels (47.3%), although in the period from zero to three months (44.7%) and three to six months (39.1%) after the transplant subtherapeutic levels were more common. The same did not happen with patients who received PTA and PAK. Patients who received PTA had a tendency to present *tacrolimus* blood levels in subtherapeutic range (57.3%). If we consider the post-transplant period, the periods of zero to three months (77.8%) and three to six months (50%) after transplant have a larger number of analyses in subtherapeutic range, but toxic levels (45.2%) were observed six months after graft receiving. Patients who received PAK presented subtherapeutic levels in all post-transplant periods considered: 62.5% till three months, 100% between three and six months, and 80% after six months (generally 69.3%).

In the evaluation of *tacrolimus* levels, the reference method is liquid chromatographic-tandem mass spectrometry (LC/MS/MS)[1, 27, 28]. However, in daily practice, immunoassays are preferred for their simplicity and cost. The most commonly used are Imx® *Tacrolimus* Assay (Abbot Laboratories), a MEIA assay, and Pro-Trac™ II *Tacrolimus* Assay (DiaSorin Inc.), an enzyme-linked immunosorbent assay (ELISA). Both methods use the same monoclonal antibody that has been shown to cross-react with several metabolites of *tacrolimus*[1, 26]. At São Lucas Hospital, Pro-Trac™ II *Tacrolimus* Assay is used because of its better performance in subtherapeutic and low therapeutic ranges[21]; the estimated detectable concentration is 0,18 ng/ml[13, 21], which is about 10-fold lower than the detection limit of the Imx® *Tacrolimus* II MEIA. It is possible that some toxic results found in this study are due to cross-reactivity of *tacrolimus* metabolites, causing an overestimation of *tacrolimus* blood levels.

For TDM, the blood sample should be taken immediately before next dose is administered. This specific moment is convenient and enhances reproducibility, without undue influences from absorption and distribution rates that cause fluctuations; also, the trough concentration is often linearly related to the area under the curve of the drug, thus allowing it to serve as an index to the overall exposure to the drug[14]. All samples of this study were collected at the appropriated moment, once this was the orientation to patients and hospital staff; however, the possibility of errors cannot be rejected.

The therapeutic range of *tacrolimus* is not clearly defined, but target 12-h trough whole blood concentrations are 5 to 20 ng/ml early post-transplant[26]. There are proposals that the best *tacrolimus* residual dose is between 10 and 20 ng/ml in the three first months after the transplant and 5 and 15 ng/ml after this period, for general transplantation[31]. However, therapeutic ranges vary according to the different protocols adopted for each different transplantation center and type of transplant. The results found in this study differ from other studies published by other institutions[16], where most of the tests were within therapeutic ranges. Nevertheless, the therapeutic range adopted by these institutions was wider than the one used at the hospital in study. If these levels were considered therapeutic, the number of analyses found above or below the therapeutic range would sensibly diminish.

The low number of tests within the therapeutic range demonstrated in this study shows the need of TDM for *tacrolimus*; besides, it shows the importance of the frequency in which tests are performed. It was not possible to compare the results of *tacrolimus* monitoring with dose adjustments or drug interactions[2, 29], genetic factors[3, 7, 11, 22], and other concomitant pathologies[19], once this data were not available.

The success of organ transplantation is intimately related to selection of adequate immunosuppressant in appropriate doses. Choosing the best dose of *tacrolimus* is a complicated procedure due to the high degree of interindividual and intraindividual pharmacokinetic variation and pharmacodynamic variability and the narrow therapeutic index of the drug[3, 27, 28]. TDM is the best tool available to help define the best administered dose.

Tacrolimus levels cannot be considered as isolated indicators of dose adjustment. It is important to keep in mind that TDM is not only a laboratory test; the drug blood levels should be analyzed considering patient individual characteristics, as gender, age, weight, use of other drugs, dose regimen, type of transplant and post-transplant period, plus biochemical and graft function tests[16]. Unfortunately, these data were not available for this study. A prospective study could help to evaluate the role

of these characteristics in *tacrolimus* therapeutic monitoring results. Drug monitoring is a multidisciplinary activity, where precise blood concentrations with clinical relevant information can only be obtained with collaboration of physicians, pharmacists and nurses, and an excellent communication among these professionals is needed to reach the purposes[9]. A prospective study would help this communication.

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