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ORIGINAL ARTICLES

The clinical value of enzyme-multiplied immunoassay technique monitoring the plasma concentrations of cyclosporine A after renal transplantation

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Abstract: The feasibility and the clinical value of the enzyme-multiplied immunoassay technique (EMIT) monitoring of blood concentrations of cyclosporine A (CsA) in patients treated with CsA were investigated after kidney transplantation. The validation method was performed to the EMIT determination of CsA blood concentration, the CsA whole blood trough concentrations (C_0) of patients in different time periods after renal transplantation were monitored, and combined with the clinical complications, the statistical results were analyzed and compared. EMIT was precise, accurate and stable, also with a high quality control. The mean postoperative blood concentration of CsA was as follows: <1 month, (281.4 ± 57.9) ng/mL; 2 - 3 months, (264.5 ± 41.2) ng/mL; 4 - 5 months, (236.4 ± 38.9) ng/mL; 6 - 12 months, (206.5 ± 32.6) ng/mL; >12 months, (185.6 ± 28.1) ng/mL. The toxic reaction rate of CsA blood concentration within the recommended therapeutic concentration was 14.1%, significantly lower than that of the none-recommended dose group (37.2%) ($P<0.05$); the transplantation rejection rate was 4.4%, significantly lower than that of the none-recommended dose group (22.5%) ($P<0.05$). Using EMIT to monitor the blood concentration of CsA as the routine laboratory method is feasible, and is able to reduce the CsA toxicity and rejection significantly, leading to achieving the desired therapeutic effect.

Keywords: enzyme-multiplied immunoassay technique; renal transplantation; cyclosporin A; blood concentration monitoring

1 Introduction

Cyclosporine A (CsA) is an effective immunosuppressant, which is able to inhibit helper T lymphocytes and the activity of B lymphocytes specifically. It is widely used in the immunosuppressive therapy of patients after renal transplantation and the inhibition of allograft rejection. The clinical application of CsA is a new milestone in the modern transplantation [1,2]. CsA's main toxic reactions include liver toxicity and kidney toxicity, and the long-term renal toxicity can lead to chronic allograft nephropathy (CAN), affecting the graft survival [3]. These side effects are closely related to the CsA blood concentration, but the reduction of CsA dosage will lead to rejection, particularly a higher rejection, 4 to 6 months after transplantation [4]. Because of the large individual differences in CsA bioavailability and pharmacokinetics, the therapeutic window is narrow, and to make a distinction between the renal toxicity and the rejection after the renal transplantation is difficult. The regular clinical monitoring of blood trough concentrations of CsA is able to help adjust the dosage to reduce the incidence of acute rejection and the rejection degree and to suggest a toxic dosage, thus reducing the incidence of liver and renal toxicity [5].

Currently, the commonly used determination methods of CsA concentrations in plasma include immunoassay and high performance liquid chromatography (HPLC). HPLC uses chromatographic separation to determine the concentrations of CsA parent drug and metabolites, with advantages like a strong specificity, a high sensitivity and a wide linear range (50 - 1500 ng/mL), etc. Its selectivity, precision and accuracy are high, and it is a reference method for the evaluation of other methods [6]. The main disadvantages are: very tedious and time-consuming sample preparation, a larger sample size (about 1 mL), and a low sample recovery rate, and experienced personnel is needed, and therefore, it is not suitable for determining bulk samples [7]. EMIT is a homogeneous enzyme immunoassay technique, whose determination is based on the antibody's combining sites competed for by the drugs in the sample and the drugs labeled by glucose 6-phosphate dehydrogenase (G_6PDH). Combined with the antibody, the enzyme activity decreases, and based on the activity of enzyme, the concentration of analytes in the sample is determined, and the active enzyme transfers the oxidized nicotine adenine dinucleotide (NAD) into the reduced NAD, resulting in the absorbance changes,

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so the spectrophotometric measurement should be used. The results of 145 CsA samples were compared between Cloned Enzyme Drugs Immunoassay(CEDIA, TDx), Fluorescence Polarization Immunoassay (FPIA, AxSYM), EMIT and HPLC methods. EMIT and HPLC are best correlated. EMIT and HPLC have the minimum deviation [8,9]. EMIT has a high degree of automation, with a short measurement period, which is suitable for determining the therapeutic concentration and the toxicity range of CsA. Therefore, it may be considered to monitor large quantities of clinical CsA samples.

2 Materials and methods

2.1 General information

90 males, with a mean age of (38.4 ± 11.42)years, a mean body weight of (62.3 ± 7.82)kg, and 486 times of monitoring; 72 females, with a mean age of (43.6 ± 10.35) years, a mean body weight of (54.2 ± 8.26)kg, and 269 times of monitoring.

2.2 Medication scheme

The triple immunosuppressive scheme, cyclosporine A (CsA) + prednisone (Pred) + mycophenolate mofetil (MMF), was adopted for the elected patients. In addition, the adjuvant drugs included Hexinshuang, Bailing capsule, liver-protective and treating drugs.

2.3 Determination of plasma concentration

After the patients took the medicine for 3 d after the operation and showed a steady state, 2 mL of venous blood was collected before taking medicine, and Viva detector and Emit 2000 Cyclosporine Specific Assay kit (Dade Behring, Inc., United States) were applied, and following the routine operation, EMIT was used to determine the whole blood CsA trough concentration (C_0). Patients with an acute rejection were excluded.

2.4 Frequency monitoring and sample collection

The monitoring of plasma concentration started one week after the surgery, once every week within 1 month, once every 2 weeks within 2 to 3 months, and a regular monthly monitoring after 3 months. Liver and kidney function or any abnormal clinical manifestations were monitored as needed. Monitoring frequency of long-term survival patients could be lower. 2 mL of venous blood was collected before taking the medicine in the morning, with heparin anticoagulation.

2.5 Statistical analysis

ANOVA and chi-square test were used in the statistical treatment. $P<0.05$ was considered statistically significant. SPSS 13.0 was adopted in the statistical analysis.

3 Results

3.1 Determination of precision and accuracy with EMIT and stability test

The relative standard deviation (RSD) of intra-day and inter-day variation of CsA quality control materials with high, middle and low concentrations was within ± 8%; the recovery rate was around 100% (Table 1). The stability was investigated after the quality control solutions at three concentration levels kept at room temperature for 24 h, and frozen for 7 d at − 30 ℃, repeatedly frozen and thawed once or twice, and the results showed no significant change, RSD <13%.

Table 1 Precision and accuracy of the concentration of CsA by EMIT

No.	C_0 (ng/mL)	Intra-day		Inter-day		Recovery rate (%)
		Measured value (ng/mL)	RSD (%)	Measured value (ng/mL)	RSD (%)	
1	74.0	75.4 ± 2.3	3.05	76.1 ± 5.8	7.62	101.6 ± 2.8
2	168.0	171.3 ± 3.6	2.10	164.3 ± 9.6	5.84	98.4 ± 2.3
3	465.0	464.6 ± 9.5	2.04	484.4 ± 28.5	5.88	99.7 ± 3.2

C_0, the whole blood trough concentration; RSD, the relative standard deviation.

3.2 Determination of CsA whole blood trough concentrations of patients after renal transplantation in different groups

Groups were divided based on the different time lengths after the patients received the renal transplantation, and CsA doses taken by different groups of renal transplant recipients and the mean value and standard deviation of the whole blood trough concentrations were calculated (Table 2).

Table 2 The relationship between CsA dose and concentration at different time after renal transplantation

Time after surgery (month)	Case-times	Dose[mg/(kg·d)]	C_0(ng/mL)
<1	181	4.84 ± 1.03	281.4 ± 57.9
2 − 3	261	4.25 ± 0.89	264.5 ± 41.2
4 − 5	165	3.98 ± 0.45	236.4 ± 38.9
6 − 12	98	3.35 ± 0.32	206.5 ± 32.6
>12	50	3.11 ± 0.29	185.6 ± 28.1

C_0, the whole blood trough concentration.

3.3 The relationship between the whole blood trough concentration of CsA and the rejection

With CsA plasma concentrations of 210, 210, 140, 120 and 100 ng/mL as the reference limits respectively, the rejection in each group was analyzed. When the CsA plasma concentration was lower than the reference limits of rejection, the rejection rates were 32.5%, 18.0%, 18.0%, 26.1% and 66.6%, respectively, with an average rate of 22.5%; when it was higher than the reference limits of the rejection, the mean rejection rate was 4.4% (Table 3).

Table 3 The relationship between CsA concentration and rejection at different time after transplantation

Time after surgery (month)	Case-times	C_0 (ng/mL)	Rejection (n)	No rejection (n)	χ^2	P value
<1	181	≤210	25	57	16.90	<0.05
		>210	7	92		
2-3	261	≤210	16	73	14.12	<0.05
		>210	7	165		
4-5	165	≤140	9	50	4.22	<0.05
		>140	6	100		
6-12	98	≤120	6	17	12.74	<0.05
		>120	1	74		
>12	50	≤100	2	3	5.67	<0.05
		>100	1	44		

C_0, the whole blood trough concentration.

3.4 The relationship between whole blood trough concentration of CsA and liver and kidney toxicity

With CsA blood concentrations of 400, 360, 360, 280 and 250 ng/mL as the reference limits of toxic reactions respectively, the toxic reaction rate of each group was analyzed. When the CsA blood concentration was higher than the reference limits, the toxic reaction rates were 38.2%, 42.9%, 27.9%, 30.8% and 54.5%, respectively, with a mean toxic reaction rate of 37.2% (67 case-times); when lower than the reference limits, the mean toxic reaction incidence rate was 14.1% (81 case-times), as shown in Table 4.

Table 4 The relationship between CsA concentration and toxicity at different time after transplantation

Time after surgery (month)	Case-times	C_0 (ng/mL)	Toxicity (times)	No toxicity (times)	χ^2	P value
<1	181	≥400	13	21	7.50	<0.05
		<400	25	122		
2-3	261	≥360	18	24	14.99	<0.05
		<360	36	183		
4-5	165	≥360	12	31	6.47	<0.05
		<360	14	108		
6-12	98	≥280	12	27	14.37	<0.05
		<280	2	57		
>12	50	≥250	12	10	9.18	<0.05
		<250	4	24		

C_0, the whole blood trough concentration.

4 Discussion

After renal transplantation, the superiority of the triple-based immunosuppressive medication scheme with CsA as the chief drug has been widely recognized by transplantation specialists, but because of the different initial doses and the different reduction schemes in different units, there has been no uniform therapeutic window concentration at home and abroad. In this study, the whole blood trough concentration values of CsA of 162 renal transplanted recipients who had received CsA immunosuppressive therapy were determined for 755 case-times. The CsA trough concentrations in different periods were lower than those reported in the literature, which may be related to the different determination methods in different centers, but the rejection and toxicity of patients with CsA therapy after renal transplantation in our center were lower than those reported at home and abroad [10-13], indicating that the therapeutic window set in our center is reasonable.

The most widely used determination method of plasma concentration is HPLC [14], but because of its complicated procedures and long determining period, and high technical requirements, it is difficult to be widely used in clinic. In the EMIT used in our center, VIVA automatic immune analyzer was used, with an automatic sampling system, a good temperature control system and a data processing and storage device. It has the advantages like high degree of automation, fewer samples and no need of sample pretreatment, so it is suitable for emergency cases and the analysis and determination of a large sample [15]. We performed a method validation for CsA determination with EMIT, and the results showed that, as long as the operating rules were followed, the quality control standards analyzed, and the precision, accuracy and stability met the measurement requirements, the quality control results would be good. EMIT for routine clinical testing of CsA blood concentration is feasible.

The individual differences of CsA plasma concentration are huge, and different patients show different effective plasma concentrations. It is prone to have rejection and toxic reactions, which is related to such influencing factors as the medication time period after transplantation, physical condition, age, liver and kidney function, food, and combined drug therapy [16]. CsA nephrotoxicity is dose-dependent, and < 400 ng/mL whole blood concentration may effectively prevent the incidence of renal toxicity. Therefore, ≥ 400 ng/mL whole blood CsA concentration is generally considered as the boundary of toxic and non-toxic. <100 ng/mL whole blood CsA levels are considered as under-dose, and 100 - 400 ng/mL whole blood CsA concentrations are defined as treatment doses. In the group of 755 case-times of monitoring plasma concentrations, the vast majority of the patients taken medicine for longer than 2 months after renal transplantation showed a more stable

plasma concentration. If the plasma concentration of CsA was adjusted to the recommended therapeutic concentration range, the toxic reaction rate was 14.1%, and the rejection rate was 4.4%. In the non-regular group with a concentration higher than the recommended therapeutic range, the toxic reaction rate was 37.2%, while with a concentration lower than the range, the rejection rate was 22.5%. The clinical manifestations of toxic effects include gingival swelling, and an increase of alanine aminotransferase, creatinine, urea nitrogen, and uric acid in the laboratory tests. In this group, through the timely adjustment of the dosage, all were reduced to or reached the effective concentration, with no new rejection nor severe poisoning phenomenon. CsA was significantly time- and dose-dependent, and in this group of patients, with the prolonging of the postoperative time, CsA dose was gradually reduced, usually 6 – 8 mg/(kg·d) within 6 months after the operation, 3 – 6 mg/(kg·d), 12 months, and 2 – 5 mg/(kg·d), more than one year. Basically, C_0 also showed a downward trend with the prolonging of the postoperative time, with no more change, >6 months. The ideal concentration range of CsA treatment after renal transplantation in the triple immunosuppressive therapeutic scheme recommended by our center is : <1 month: 210 – 400 ng/mL; 2 – 3 months: 210 – 360 ng/mL; 4 – 5 months: 140 – 360 ng/mL; 6 – 12 months: 120 – 280 ng/mL; >12 months: 100 – 250 ng/mL. In this group, the mean CsA plasma concentration of the patients in each period was within the effective concentration range, which ensured the satisfying immunosuppressive effects, and also reduced the incidence of the toxic effect and rejection reaction of CsA, indicating that the application of regular monitoring of CsA in the renal transplant recipients and the blood trough concentration monitoring in our center are reasonable.

In conclusion, the regular monitoring of plasma concentration of CsA and the adjustment of clinical dose according to individual patients show important clinical significance in improving kidney transplant success rate and the life quality of the patients. The routine laboratory determination of CsA plasma concentration with EMIT is practicable. Medication of CsA based on the monitoring results and the recommended therapeutic concentration range is able to reduce CsA toxicity and rejection reaction significantly, achieving a more satisfactory therapeutic effect.

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