Therapeutic drug monitoring of cyclosporin-A following the first heart transplantation in Guangxi Zhuang Autonomous Region of China

Wu Min[1], Liang Chen-fang[1], Wu Hong-wen[1], Zhou Li-fang[2]

Abstract

BACKGROUND: Due to the difference in bioavailability and pharmacokinetics of cyclosporine A (CsA) among different individuals, it is of great importance to carry out blood drug concentration monitoring for safety, effectiveness and reduction of acute immunological rejection.

OBJECTIVE: To develop a method of blood drug concentration determination of CsA after the heart transplantation, and to analyze the correlation between dose, outcome and concentration so as to develop an optimal administration scheme.

DESIGN, TIME AND SETTING: Controlled sample observation was conducted between August and September 2003 at the Clinical Pharmaceutic Laboratory, Fourth Affiliated Hospital, Guangxi Medical University.

PARTICIPANT: Heart transplantation patient in the Guangxi Zhuang Autonomous Region was treated with CsA capsule, prednisone and mycophenolate mofetil.

METHODS: 3 mL venous blood was taken from the receptor before taking medicine in the morning. As an anticoagulant, heparin was added and misced bene. 10 μL CsB, the in internal standard fluid was inserted into a 10 mL glass tube, which was added with 1 mL whole blood and 1 mL NaOH (0.2 mol/L) and misced bene. The supernatant was put into another tube and dried out at 60 ℃, again cooled to room temperature and added with 100 μL solution of 0.05 mol/L acid hydroc and acetonitrile plus 400 μL N-hexane, which was centrifuged for 5 minutes, and 20 μL from lower layer was taken for sample injection. A high efficiency liquid chromatography was used with cyclosporine-B as the internal standard, the patient blood concentration of CsA was detected at 214 nm and the dose was adjusted accordingly.

MAIN OUTCOME MEASURES: Whether patients occurred acute rejection or not, and blood drug concentration of CsA were measured.

RESULTS: High efficiency liquid chromatography showed good resolving power. The absolute recoveries were not lower than 75%. Monitoring results demonstrated that the highest concentration occurred 10 days after transplantation. The dose was regulated according to results. Peak concentration was between 200-300 μg/L 14 days following transplantation, and acute rejection did not occur in recipients.

CONCLUSION: High efficiency liquid chromatography can satisfy the demand of general clinic determination of blood concentration of CsA. Peak concentration between 200-300 μg/L 14 days following transplantation is suitable.

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INTRODUCTION

Heart transplantation is the important therapy for final stage heart diseases, which cannot be cured by internal medical or surgical treatment. A proper immunosuppressive therapy is one of the key factors for a long-term survival after heart transplantation [1]. Cyclosporine-A (CsA) is the most effective immunodepressant at present, and is widely used in organ transplantation, such as liver, kidney and bone marrow. It can significantly suppress immunological rejection and improve the survival rates of the transplanted organs[2]. Due to the difference in bioavailability and pharmacokinetics of CsA among different individuals, it is of great importance to carry out blood drug concentration monitoring for safety, effectiveness and reduction of immunological rejection. This paper referred to relevant reports[3-5] and experimental research, high efficiency liquid chromatography was used with Cyclosporine-B as the internal standard, which is simple, fast and accurate to determine blood drug concentration of CsA. The first case with heart transplantation in Guangxi Zhuang Autonomous Region of China in 2003 was monitored at the Fourth Affiliated Hospital, Guangxi Medical University in this way and the outcome was satisfactory.

SUBJECT AND METHODS

Design

Controlled sample study.

Time and setting

Experiments were conducted between August and September 2003 at the Clinical Pharmaceutic Laboratory, Fourth Affiliated Hospital, Guangxi Medical University.

Subject

The female patient aged 54 years old, received heart transplantation on August 29, 2003. Immunosuppressants included CsA capsule, prednisone and mycophenolate mofetil (MMF), among which, dose of oral CsA capsule was 200-300 mg/d and the lowest concentration was 85.7 μg/L with highest concentration of 618.0 μg/L and mean concentration of 278.3 μg/L. Informed consent was obtained from the subject, which is in accordance with *Administrative Regulations on Medical Institution*, formulated by State Council of the People's Republic of China[6].

Main medicine, reagent and instrument	Source
CsA control and CsB control	Fujian Microbiogy Research Institute, China
Caustic soda, aether and acidum hydrochloricum are analytical pure	Xinning Chemical Plant, Shantou, Guangdong Province, China
Acetonitrile chromatographic pure	Shanghai Chemical Agent Co., China
LC-10Atvp high efficiency liquid chromatography	SHIMADZU CORPORATION, Japan
SPD-10Atvp detector	Shanghai First Medical College Equipment Factory, China
XW-80 vortex admixer	Shanghai First Medical College Equipment Factory, China
80-2 centrifugation machine	Shanghai Surgical Instrument Factory, China

Methods

Chromatographic conditions: SHIMADZU-C_{18} (10 μm, 250 mm× 4.6 mm) analytical column; Column temperature is 68 ℃; Wavelength is 214 nm; acetonitrile-methanol-water (6:2:2，*V*/*V*); Flow speed is 1.4 mL/min, volume of sample injection quantization tube is 20 μL.

Blood sample management: 3 mL venous blood was taken from the receptor before taking medicine in the morning. As an anticoagulant, heparin was added and misced bene. 10 μL CsB, the in internal standard fluid was inserted into a 10 mL glass tube, which was added with 1 mL whole blood and 1 mL NaOH (0.2 mol/L) and misced bene, kept it still for 5 minutes and then added with 5 mL aether, swung for 1 minute and centrifuged for 10 minutes (3 000 r/min). The supernant was put into another tube and dried out at 60 ℃, again cooled to room temperature and added with 100 μL solution of 0.05 mol/L acid hydroc and acetonitrile (4: 6) plus 400 μL N-hexane, which was centrifuged for 5 minutes and 20 μL from lower layer was taken for sample injection [7-8].

Standard curve: With concentrations of 52, 104, 208, 312, 416, 520, 1 040 μg/L, CsA standard products were added into blank human whole blood samples, and then added with 20 μL internal standard fluids respectively, which were dealt with the above management methods. Linear regression of CsA concentrations was done by using CsA/CsB peak area ratio. Equations of y = 0.002 327x – 0.019 were obtained, with coefficient correlation of r =0.999 5. The lowest detectable concentration was 20 μg/L with this method.

Authentication of the methods: For the precision of calculation, relative standard deviation (RSD) of the inter-day assay (n=5) and intra-day assay (n=5) were lower than 10% among the three different concentrations (low, middle and high).

Main outcome measures

Whether patients occurred acute rejection or not, and blood drug concentration of CsA were measured.

Design, enforcement and evaluation

The first author completed experiment design. The first and the third authors were responsible for implementation, and the second author was responsible for evaluation. All the authors received professional training.

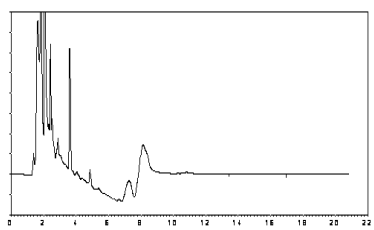
Statistical analysis

The data were analyzed by the first author with statistic software SPSS 10.0. It showed linear regression of correlation between the two variance with sample data calculation of coefficient of differentiation by RSD.

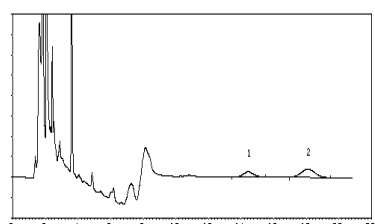
RESULTS

Chromatogram

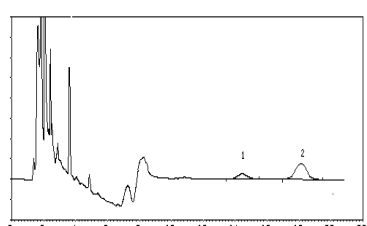
Under this chromatographic condition, chromatogram of the sample and internal standard was shown in Figure 1. The preservation durations for CsB and CsA were 14.4 minutes and 18.08 minutes, respectively, with strong resolving power and good disassociation from the hetero-peaks of the blood.



a: Blank human whole blood



b: Human whole blood with CsA and CsB



c: Blank human whole blood with standard CsA and CsB

Figure 1 High efficiency liquid chromatography (HPLC) chromatograms of Cyclosporine-A (CsA)and Cyclosporine-B (CsB)

Absolute recoveries

Samples with CsA concentrations of 104, 416 and 1 040 μg/L were prepared with blank whole blood, dealt with the above methods. The actual values obtained and the control products with equal concentration of CsA were determined and compared (n = 5). The absolute recoveries were not lower than 75% (Table 1).

Table 1 The absolute recoveries of Cyclosporine-A (CsA) and relative standard deviation (RSD) in human whole blood ($\bar{x}$±s, n=5)

Concentration (μg/L)	Recovery rate (%)	RSD (%)
104	79.4±5.6	7.05
416	84.2±4.4	5.22
1 040	87.3±2.9	3.32

Samples with CsA concentrations of 104, 416 and 1 040 μg/L were prepared with blank whole blood, dealt with the above methods. The results were calculated with standard curve equations to obtain relative recoveries (Table 2).

Table 2 The relative recoveries of Cyclosporine-A (CsA) and relative standard deviation (RSD) in human whole blood (*n*=5)

Concentration (μg/L)	Recovery rate (%)	RSD (%)
104	94.7±3.9	4.12
416	103.6±5.7	5.50
1 040	99.2±2.8	2.82

Medicine concentration

The lowest concentration was due to poor conditions after surgery and the medicines could not be absorbed well; the highest concentration occurred 10 days after surgery due to the patient being given medicines more frequently.

Administration dose and concentration of CsA within 2 weeks following transplantation were as follows.

Date	Use of immunosuppressant	Cyclosporine-A (CsA) minimum concentration (μg/L)
08-30	CsA 100 mg, Bid; prednisone 25 mg, Bid; mycophenolate mofetil (MMF) 0.5 g, Tid	134.9
08-31	As above	85.7
09-01	CsA 150 mg, Bid; prednisone 25 mg, Bid; MMF 0.5 g, Tid	237.6
09-02	CsA 100 mg, Bid; prednisone 20 mg, Bid; MMF 0.5 g, Tid	472.7
09-03	As above	411.9
09-04	CsA 100 mg, Bid; prednisone 15 mg, Bid; MMF 0.5 g, Tid	133.1
09-05	As above	208.7
09-06	CsA 150 mg, Bid; prednisone 15 mg, Bid; MMF 0.5 g, Tid	116.0
09-07	As above	355.9
09-08	CsA 100 mg (morning), 150 mg (evening); prednisone 15 mg, Bid; MMF 0.5 g, Tid	618.0
09-09	As above	562.8
09-10	As above	177.0
09-11	As above	292.4
09-12	As above	240.8
09-13	CsA 125mg, Bid; prednisone 15 mg, Bid; MMF 0.5 g, Tid	204.4
09-14	As above	200.9

DISCUSSION

The best flow, acetonitrile-methanol-water (6:2:2) to disassociate CsA and CsB with high efficiency liquid chromatography [9-11]. The higher of column temperature, the better of peak shape was. In order to protect the chromato bar and ensure stability of the baseline, we made the column temperature at 68 ℃. In the study of the alkalinized blood sample, it was found that the higher concentration of the NaOH (0.1-1.0 mol/L), the higher the absolute recoveries, but with many hetero-peaks interfering with the main peak. It showed that 0.2 mol/L was the best [12-15]. This method required single extraction and the reagents were easy to obtain. The cycle of determination was short with CsB as internal standard, which is rarely reported. It is much easier, faster and more accurate and reliable compared to the other methods, which can effectively guide clinical application of CsA and improve medical quality.

At present, immunosuppressants are often used against heart rejection. In this hospital, the first case with heart transplantation received CsA, prednisone and MMF before, during and after surgery to make a successful surgery and improve the patient's life quality [16-18]. Meanwhile, daily whole blood drug concentration was monitored and the dose was adjusted accordingly and the drug concentration was kept between 200-300 μg/L at day 14 after surgery. No acute rejection occurred on the receptor. Combining relevant statistics, we hold that whole blood drug concentration of CsA should be kept at 200-300 μg/L within three months after surgery.

REFERENCES

[1] Li JT. Linchuang Yailixue. Beijing:The Publishing House of People's Health. 1998:1327
[2] Chen XD, Jin YY. Xinbian Yaowuxue. Beijing: The Publishing House of People's Health. 2007:765
[3] Chen JS, Du QY, Huang HL, et al. Determination of Cyclosporin A Concentration in Human Whole Blood by Single-step Extraction RP-HPLC Method. Jiefangjun Yaoxue Xuebao. 2004;20(03):357-359.
[4] Ren B, Li SX, Chen X, et al. Determination of cyclosporine A in human whole blood by RP-HPLC. Zhongguo Yiyuan Yaoxue Zazhi. 2001;21(8):407.
[5] Wang QB, Tu ZL, Wang F, et al. Determination of cyclosporine A in the whole blood of organ transplantation patients by HPLC/electrospray ionization-MS(HPLC/ESI+-MS). Zhongguo Yiyuan Yaoxue Zazhi. 2003;23(4):217.
[6] State Council of the People's Republic of China. Administrative Regulations on Medical Institution. 1994-09-01.
[7] Nam HS, McAnulty JF, Kwak HH, et al. Gingival overgrowth in dogs associated with clinically relevant cyclosporine blood levels: observations in a canine renal transplantation model. Vet Surg. 2008;37(3):247-253.
[8] Izumi N, Furukawa T, Sato N, et al. Risk factors for acute graft-versus-host disease after allogeneic hematopoietic stem cell transplantation: retrospective analysis of 73 patients who received cyclosporin A. Bone Marrow Transplant. 2007;40(9):875-880.
[9] Seger C, Tentschert K, Stöggl W, et al. A rapid HPLC-MS/MS method for the simultaneous quantification of cyclosporine A, tacrolimus, sirolimus and everolimus in human blood samples. Nat Protoc. 2009;4(4):526-534.
[10] Sahin G, Akay OM, Kus E, et al. Effects of immunosuppressive drugs on platelet aggregation and soluble P-selectin levels in renal transplant patients. Ren Fail. 2009;31(2):111-117.
[11] Wang SM, Lai MK, Chueh SC, et al. Optimal C2 concentration of cyclosporin corrected with good efficacy and safety in Asian kidney transplant recipients. Transplant Proc. 2008;40(7):2243-2244.
[12] Ventura E, Bonardet A, Pageaux GP, et al. Calcineurin Inhibitor Determination in Whole Blood With the RXL Dimension Analyzer: A Useful Tool for Immunosuppressive Drug Monitoring. Transplant Proc. 2009;41(2):707-709.
[13] Brunstein CG, Cantero S, Cao Q, et al. Promising progression-free survival for patients low and intermediate grade lymphoid malignancies after nonmyeloablative umbilical cord blood transplantation. Biol Blood Marrow Transplant. 2009;15(2):214-222.
[14] Huang XS, Yang GF, Pan YC. Effect of berberin hydrochloride on blood concentration of cyclosporine A in cardiac transplanted recipients. Zhongguo Zhong Xiyi Jiehe Zazhi. 2008;28(8):702-704.
[15] Böhler T, Nolting J, Kamar N, et al. Validation of immunological biomarkers for the pharmacodynamic monitoring of immunosuppressive drugs in humans. Ther Drug Monit. 2007;29(1):77-86.
[16] Barten MJ, Rahmel A, Boldt A, et al. Pharmacodynamic monitoring of the immunosuppressive therapy in patients after heart transplantation: whole blood flow cytometric analysis of lymphocyte function. Comput Biol Med. 2007;37(10):1367-1373.

[17] Wada K, Takada M, Ueda T, et al. Pharmacokinetic study and limited sampling strategy of cyclosporine in Japanese heart transplant recipients. Circ J. 2006;70(10):1307-1311.
[18] Hoda MR, Grimm M, Laufer G. Prediction of cyclosporine blood levels in heart transplantation patients using a pharmacokinetic model identified by evolutionary algorithms. J Heart Lung Transplant. 2005;24(11):1855-1862.

广西首例心脏移植患者术后环孢素 A 的药物监测

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摘要

背景：口服环孢素 A 的生物利用度和药代动力学个体差异大，故进行药物血药浓度监测，对安全、有效地用药和减少急性排斥反应具有重要临床意义。

目的：建立心脏移植后环孢素 A 血药浓度监测方法，分析药物浓度与剂量、疗效的关系，建立最佳给药方案。

设计、时间及地点：样本对照观察，2003-08/09 在广西医科大学第四附属医院药剂科临床药学实验室完成。

对象：广西壮族自治区首例心脏移植患者，环孢素 A 胶囊、强的松、霉酚酸酯三联用药治疗。

方法：受者早上服药前取静脉血 3 mL，肝素抗凝，混匀。在 10 mL 具塞玻璃试管内加入内标环孢素 B 贮备液 10 μL，加入 1 mL 全血，再加入 0.2 mol/L NaOH 溶液 1 mL，混匀。取上清液加入另一试管，于 60 ℃水浴挥干，冷却至室温，残渣加入 0.05 mol/L 盐酸与乙腈混合液 100 μL 溶解，再加入 400 μL 正己烷洗涤，离心 5 min，取下层液 20 μL 进样。运用高效液相色谱法以环孢素 B 为内标、214 nm 处紫外检测，对心脏移植受者进行环孢素 A 血药浓度监测，并及时给予用药剂量调整。

主要观察指标：患者是否出现急性排斥反应，环孢素 A 血药谷浓度。

结果：受者高效液相色谱法分析有很好的分离度，环孢素 A 绝对回收率均不低于 75%。监测结果显示环孢素 A 药物最高浓度产生在移植后第 10 天，根据结果及时调整用药剂量，移植后 14 d 谷浓度维持在 200~300 μg/L，受者未出现急性排斥反应。

结论：高效液相色谱法能满足心脏移植后环孢素 A 的血药浓度监测需求，心脏移植后短期环孢素 A 血药谷浓度维持在 200~300 μg/L 较适合。

关键词：心脏移植；环孢素 A；药物浓度监测；高效液相色谱法

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