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## 维生素 $K_3$ 体外降低 Ehrlich 腹水癌细胞对阿霉素的抗药性[1]

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**关键词** 维生素 K；阿霉素；Ehrlich 瘤癌；瘤抗药性；谷胱甘肽；谷胱甘肽转移酶；膜流动性

**目的**：研究维生素 $K_3$（Men）降低 EAC/Dox 细胞对阿霉素（Dox）的抗药性. **方法**：测定谷胱甘肽（GSH），细胞膜流动性及谷胱甘肽 *S*-转移酶（GST）活性. 细胞存活力以甲基四唑蓝法测定. **结果**：EAC/Dox 细胞 GSH，GST 及膜流动性均较 EAC 细胞增加（$P < 0.01$）. Dox 对 EAC/Dox 细胞 $IC_{50}$ 为 22.3（15.8 - 28.8）$mg \cdot L^{-1}$. Men 5 或 10 $mg \cdot L^{-1}$ 可降低 EAC/Dox 细胞 GSH（$P < 0.01$），1 $mg \cdot L^{-1}$ 对 GSH 无影响（$P > 0.05$），但可降低细胞膜流动性（$P < 0.05$）. Men 1，5 或 10 $mg \cdot L^{-1}$ 可使 Dox $IC_{50}$ 降低到 9.6（7.8 - 11.3），6.0（2.8 - 9.2），或 5.3（3.9 - 6.7）$mg \cdot L^{-1}$（$P < 0.01$）. **结论**：Men 在体外降低 EAC/Dox 细胞对 Dox 抗药性与对 GSH 的耗竭有关.

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# Negative correlation between cyclophilin mRNA level in leukocytes of renal allograft recipients and ciclosporin concentration in whole blood

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**KEY WORDS** cyclosporine; cyclophilin; kidney transplantation; polymerase chain reaction; leukocytes

**AIM:** To study mRNA level of cyclophilin in the white blood cells (WBC) of the renal allograft recipients (RAR) and its correlation with ciclosporin concentration in whole blood. **METHODS:** The cyclophilin mRNA levels and β-actin as controls in the WBC of 47 RAR were measured by quantitative reverse transcription polymerase chain reaction. The blood ciclosporin assay utilized monoclonal antibody fluorescence polarization immunoassay. **RESULTS:** With the increase of ciclosporin concentration in whole blood (from 62 $\mu g \cdot L^{-1}$ to 678 $\mu g \cdot L^{-1}$), relative cyclophilin mRNA level in the WBC of RAR decreased nonlinearly (from 1.1 to 0.03, $r = 0.8195$). **CONCLUSION:** There was a negative correlation between the mRNA level of cyclophilin in the WBC of RAR and the ciclosporin concentration in whole blood.

Ciclosporin (Cic) is an immunosuppressive agent to prevent renal transplant rejection[1]. But often there was a discrepancy between the effects of Cic treatment and its concentration in the whole blood of the renal allograft recipients (RAR). Cyclophilin (CyP) is the receptor of Cic in cytosol, which mediates immunosuppressive action of Cic in the cells. Different expressions of CyP in the cells directly affect the distribution and absorption of Cic *in vivo* as well as the immunosuppressive effect[2]. This paper was to study the mRNA level of CyP in the white blood cells (WBC) of the RAR and its correlation with Cic concentration in whole blood.

## MATERIALS AND METHODS

**Patients and Cic assay** Forty-seven patients (37 M and 10 F) with renal transplantation, aged 17 - 58 ($37 \pm s$ 10) a were given immunosuppressive regimen according to their clinical signs. Blood samples were collected into heparinized tubes at 8:00 before breakfast. The whole blood Cic concentration was measured by the TDX Cic monoclonal antibody whole blood assay (Abbott Laboratory). Serum creatinine

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(Cr) was determined by the automated kinetic Jaffe method.

**Reverse transcription polymerase chain reaction (RT-PCR)** Total RNA was isolated with the acid-guanidine-phenol-chloroform method from 1 mL blood, dissolved in DEPC treated water and quantitated spectrometrically at 260 nm[3].

Total RNA 1 μg was heated at 65 ℃ for 5 min and reversely transcribed using oligo-dT as initial primer and reverse transcriptase 50 u (Perkin-Elmer). The mixture was incubated at 42 ℃ for 30 min, heated at 99 ℃ for 5 min, and stored at −20 ℃ until use. Selected sequences in 5-μL aliquots of cDNA were amplified by PCR for 26 cycles using primers for CyP and β-actin as an internal standard in the same tube. The mixtures were cycled at 95 ℃ for 30 s, 53 ℃ for 30 s, 72 ℃ for 30 s, and 72 ℃ for 7 min. The sequences of the primers were: CyP, 5′TCTTCTTGCTGGTCTTGCC and 5′ TCTCCTTTGAGCTGTTTGC, amplified fragment 408 bp; β-Actin, 5′CTTCCTGGGCATGGAGGTC and 5′ GCCGATCCACACGGAGTA, amplified fragment 234 bp. PCR reactants were resolved on 1.5 % agarose gels stained with ethidium bromide and photographed with Lucky film. The film was developed and examined with a densitometer CS-9000 (Shimadzu). The identity of each fragment was confirmed by restriction digestion and sequencing of the fragments[4,5]. The ratio of CyP mRNA to β-actin mRNA (CyP/β-actin) was referred to the relative level of CyP mRNA transcript (expressed as CPH) (Fig 1).

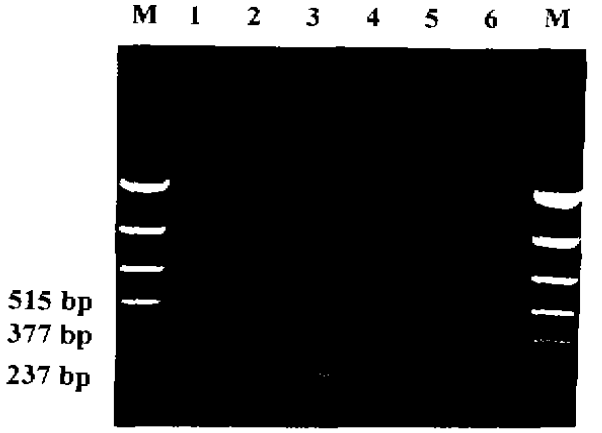


**Fig 1. RT-PCR analysis of CyP and β-actin on 1.5 % agarose gel. M: Marker; 1 – 6: CyP (408 bp) and β-actin (234 bp) amplified fragments from WBC of renal allografts.**

**Data analysis** All data were presented as $\bar{x} \pm s$. Comparisons between groups were made using $t$-test. Correlation between CPH and Cic was analyzed by nonlinear regression with the use of the STATISTICA system (release 4.5, Statsoft, Inc, USA).

## RESULTS

Cic concentration in whole blood of 47 RAR increased from 62 μg · L$^{-1}$ to 678 μg · L$^{-1}$, relative cyclophilin mRNA level (CPH) in the WBC decreased from 1.1 to 0.03. A good correlation was found between CPH and Cic/CPH (Fig 2). After we changed the correlation equation, the regression equation was CPH = 0.08924/[1 − (0.1138/Cic)]. There was a negative correlation between CPH and Cic.

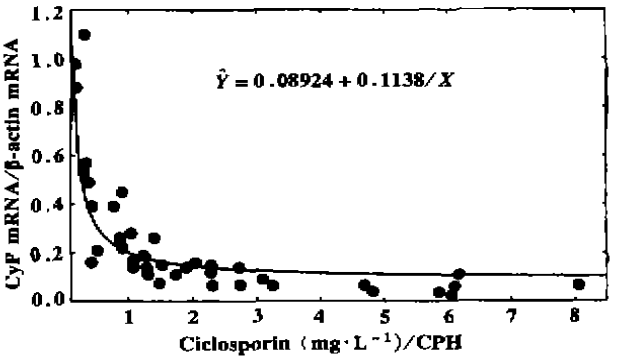


**Fig 2. Correlation between CPH (CyP mRNA/β-actin mRNA) and Cic/CPH.**

Results of renal allografts who survived for 6 months or more were indicated in Tab 1. Group 1 were patients whose Cr were < 120 μmol · L$^{-1}$ while Group 2 were > 120 μmol · L$^{-1}$. There was no significant difference between both groups in CPH and Cic ($P > 0.05$), but a significant difference in CPH/Cic ($P < 0.01$).

**Tab 1. Serum creatinine (Cr), ciclosporin (Cic) concentration in whole blood, and relative cyclophilin mRNA level (CPH) in WBC of RAR. $\bar{x} \pm s$.**
**$^aP > 0.05$, $^cP < 0.01$ *vs* group 1.**

| Group | 1 | 2 |
|---|---|---|
| $n$ | 9 | 10 |
| Cr (μmol·L$^{-1}$) | 95 ± 18 | 172 ± 46$^c$ |
| Cic (μg·L$^{-1}$) | 340 ± 169 | 197 ± 84$^a$ |
| CPH | 0.14 ± 0.12 | 0.39 ± 0.33$^a$ |
| CPH/Cic (L·ng$^{-1}$) | 0.5 ± 0.4 | 2.2 ± 1.8$^c$ |

## DISCUSSION

This study demonstrated that there was a significant inverse correlation between CPH in the WBC and the whole blood level of Cic in RAR. The dosage of Cic is complicated by different distribution and absorption in the tissues and the interindividual variability in the pharmacokinetics of Cic[1]. Although Cic mainly binds erythrocytes in the whole blood while some bind WBC, the immunosuppressive action of Cic should rely on its receptor — CyP in the cells, especially in the WBC. CyP also acts as chaperones for folding, assembly, and trafficking of proteins. It assists diverse posttranslational modifications such as glycosylation, phosohorylation, and so on[6]. Hence, CyP plays an important role in the course of mature of protein and generation of various biological functions[2]. The CyP mRNA level may indicate the state of protein synthesization in the cells somehow. It could partly reflect the immunosuppressive effect of Cic. If Cic blood concentration was very low and CyP level of the WBC was very high, it might suggest that the immunosuppression of Cic be not so good that the transplanted kidney would be rejected. If Cic blood concentration was very high and CyP level was very low, the elementary metabolism of the cells would be so immunosuppressed that nephrotoxicity and other complications would be caused. The determination of CyP mRNA level could be conducive to the rationalization of the individual dosage of Cic.

We also investigated the serum creatinine of 19 RAR who survived for more than six months. The result showed the significant difference in the ratio of CPH to Cic between 2 groups. It might suggest that CPH/Cic could be a useful indicator for predicting the kidney function after renal transplantation. Whether CPH/Cic is helpful to prevent Cic-induced nephrotoxicity needs to be established.

Successful renal transplantation depends on many factors. The most important factor is the therapeutic control of the transplanted kidney rejection. In this study, there was a negative correlation between the mRNA level of cyclophilin in the WBC of RAR and the Cic concentration in whole blood. This should contribute to find the way to assess individual variation of the drug sensitivity in Cic-treated RAR and to obtain a more rational individualization of Cic dosage in RAR.

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## 肾移植病人白细胞中亲环蛋白表达水平与全血环孢素浓度呈负相关


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**关键词** 环孢素；亲环蛋白；肾移植；聚合酶链反应；白细胞



**目的**：研究肾移植病人白细胞中亲环蛋白(CyP)表达水平及其与环孢素(Cic)血药浓度的相关性. **方法**：应用逆转录聚合酶链反应(RT-PCR)以 β-actin 为内标，测定了 47 例肾移植病人白细胞中 CyP mRNA 的水平，同时应用单克隆抗体荧光偏振免疫法测定了他们血中 Cic 浓度. **结果**：随着 Cic 全血浓度由 62 $\mu g \cdot L^{-1}$ 升高至 678 $\mu g \cdot L^{-1}$，异体肾移植病人白细胞中 CyP 表达水平由 1.1 非线性地降低至 0.03 ($r = 0.8195$). **结论**：肾移植病人白细胞中 CyP 表达水平与其 Cic 血药浓度呈负相关.