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丝裂霉素或顺铂对离体淋巴因子激活的杀伤细胞增殖和抗膀胱癌细胞系活性的调节作用[1]

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关键词 丝裂霉素 C；顺铂；淋巴因子激活的杀伤细胞；膀胱癌；细胞分裂；白细胞介素-2；免疫细胞毒性；培养的肿瘤细胞

目的：研究丝裂霉素（Mit）或顺铂（Cis）对膀胱癌患者 LAK 细胞增殖和对膀胱癌细胞系的细胞毒作用的影响．**方法**：用细胞计数和 MTT 法测定 LAK 细胞的增殖和细胞毒作用．**结果**：Cis 浓度依赖性抑制 LAK 细胞增殖，一定浓度的 Mit（5－10 mg·L^{-1}）却可加强 IL-2 对 LAK 细胞的刺激作用．Cis 10 mg·L^{-1}增强 LAK 细胞对膀胱癌细胞系 BIU-87 和 EJ 的杀伤作用，Mit 则对细胞毒无明显影响．**结论**：化疗药物对 LAK 细胞增殖和抗肿瘤免疫功能的调节依药物的不同而不同，这主要取决于药物本身．

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Effects of ciclosporin on whole blood chemiluminescence of renal transplant patients

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KEY WORDS kidney transplantation; chemiluminescence; cyclosporine; neutrophils; peritoneal macrophages

AIM: To examine the possible inhibitory role of ciclosporin (Cic) on luminol-dependent chemiluminescence (CL) of whole blood in renal transplant patients. **METHODS**: Luminol-dependent CL was used to measure active oxygen species generation in respiratory burst of whole blood stimulated by zymosan A. Fluorescence polarization immunoassay was used to monitor the blood concentration of Cic. **RESULTS**: CL values of Cic group ($n = 50$) decreased in comparison with those of normal group ($n = 10$) ($P < 0.01$). The blood concentration of Cic was negatively related to CL value ($P < 0.01$). The serum of renal transplant patients directly inhibited respiratory burst of peritoneal macrophages of rats in a concentration-dependent manner. **CONCLUSION**: Cic inhibits the phagocytic activity of neutrophils in renal transplant patients.

Ciclosporin (Cic), a cyclic undecapeptide of fungal origin, is a potent immunosuppressive drug that is effective combating tissue rejection following organ transplantation. Compared with other commonly used immunosuppressants, eg, corticosteroids (Cor) and azathioprine (Aza), Cic yields greatly improved graft survival in renal transplantation. We did some researches about the methods to measure Cic concentration[1] and the effects of Cic on T-lymphocyte subset[2]. Neutrophils and macrophages, nonspecific immunocytes, are important in defending the invasion of microorganisms. Renal transplant patients took immunosuppressants that inhibited tissue rejection on allograft kidney which had negative effects on body defense. Cic at the treatment concentration exacerbated infections, but had no influence on morphology of neutrophils[3]. Its effects on function of neutrophils were uncertain. We raised the possibility that the immunosuppressant effect of Cic was related to its inhibiting phagocytic activity. The present study was to confirm the effects of Cic on phagocytosis of neutrophils in renal transplant patients.

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MATERIALS AND METHODS

Drugs Ciclosporin (Sandoz, Switzer-

land), whole blood monoclonal antibody ciclosporin kit (Abbott, USA), luminol, zymosan A (Zym), and hepes were purchased from Sigma Chemical Co, USA. Other reagents were of AR grade.

Apparatus FT662 Biochemistry Luminometer (Beijing Nuclear Apparatus Factory), Z360k refrigerated centrifuge (Hermile, Germany). Fluorescence polarization immunoassay (FPIA) analyzer (Abbott, USA).

Patients Fifty renal transplant patients (30 M, 20 F), aged 42 ± *s* 12 a, and weighing 52 ± *s* 8 (32 - 60) kg, received all primary cadaver renal allografts at our hospital. Characteristics of the donor-recipient combination such as ABC and HLA matching, and T and B cell sensitization were also analyzed. The donators (without renal diseases) (50 M), aged 26 ± *s* 5 a, and weighing 68 ± *s* 7 (55 - 80) kg. All kidney were preserved with pulsatile machine perfusion using a metabolically active perfusate solution. Mean preservation time was 30.5 h. During the operation, venous injection of Cic was initiated, and then oral Cic, along with prednisone (Pre) and Aza therapy had been maintained for 1 month. Then the patients were divided into 3 groups: (1) only oral Cic group ($n = 20$), (2) Cic + Pre group ($n = 15$), (3) Cic + Pre + Aza group ($n = 15$). Cic was orally taken every 8 h to maintain a constant concentration in the blood. Cic levels were monitored with serial 24-h whole-blood FPIA-determined.

Rats Twenty Sprague-Dawley rats (♂, 3 - 4-month old, 243 ± *s* 32 g) were provided by Animal Center of First Military Medical University (Certificate № 96A19). Rats were kept under light from 6:00 to 18:00 daily at 22 ± 2 ℃ with free access to food and tap water.

Blood Cic concentration The Cic monoclonal whole blood assay was an *in vitro* reagent system for the quantitative measurement of Cic in human whole blood utilizing FPIA[4].

Whole blood chemiluminescence (CL)[5] The reaction mixtures including 0.5 mL of Hanks' balance saline solution (HBSS) supplemented to 0.4 mL of luminol (0.1 mmol $\cdot L^{-1}$ in Me_2SO), 0.1 mL of whole blood were added. The reaction was initiated by the addition of 0.1 mL of Zym (50 mg $\cdot L^{-1}$). The resulting output was recorded as mV and counted per 60 s. The reaction temperature was 37 ℃.

Rat peritoneal macrophage and CL[6] Rat peritoneal macrophage (PMØ) suspensions 500 μL, $2 \times 10^9 \cdot L^{-1}$ were incubated at 37 ℃ for 20 min. They were placed in the measuring chamber of a CL analyzer, and were added 100 μL of Zym particles 5 g $\cdot L^{-1}$, 100 μL of freshly prepared luminol 0.1 mmol $\cdot L^{-1}$, and 20 μL of patient serum in which Cic concentration was already determined.

Statistics Results were expressed as $\bar{x} \pm s$ and analyzed by two-tail *t* test. Correlation between the variables was determined by linear regression.

RESULTS

Whole blood CL Healthy human control showed CL-values of 4168 ± 1402 mV. The production of radicals oxygen species (ROS) by phagocytes from renal transplant patients was decreased in comparison with those of normal group (Fig 1).

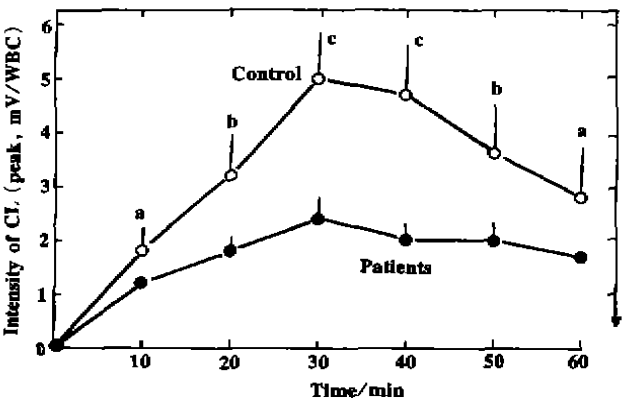

Fig 1. Whole blood chemiluminescence in healthy control ($n = 10$) and renal transplant patients ($n = 50$), $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* renal transplantation patients.

Whole blood CL values of healthy human control were higher than those of renal transplant patient group ($P < 0.01$), reached peak at 30 min. and decreased slowly. Whole blood CL values (mV) in 3 treated groups were 2164 ± 666, 1502 ± 367, and 1458 ± 617, respectively (Fig 2).

CL and white blood cell (WBC) counts Positive correlation was shown in healthy

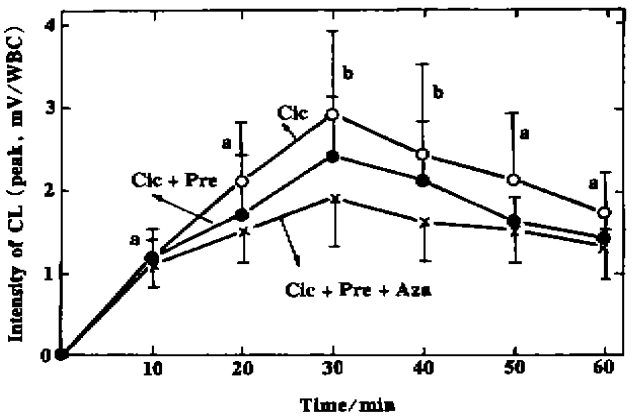

Fig 2. Whole blood chemiluminescence in renal transplant patients. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$ *vs* Cic + Pre + Aza group.

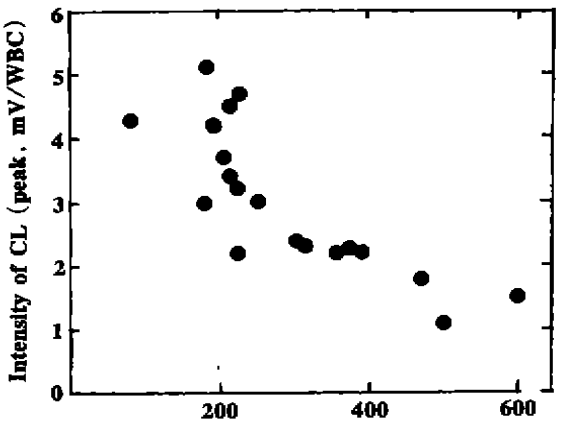

Fig 3. Blood ciclosporin concentration and whole blood CL-values.

volunteers. High CL-values paralleled with WBC counts ($P < 0.01$). But in patients there was no such relationship. The total CL-values and CL-values per WBC in patients were lower than those of the healthy control ($P < 0.01$) (Tab 1).

Cic effects on whole blood CL in renal transplant patients Negative correlation between whole blood CL-values and blood Cic concentration was found in Cic-treated group ($n = 20$) ($P < 0.01$) (Fig 3).

Cic effects on CL of rat PMØ The inhibitory effects of Cic on respiratory burst of rat PMØ was disclosed in a dose-dependent manner (Tab 2).

DISCUSSION

In this study we found that in the healthy control, the total whole blood CL was positively linear relative to circular white blood cells. It was because that in healthy volunteers the CL of each WBC was certain and almost the same. In the patients receiving Cic the total CL depended not only on the number of circular white blood cells but also on the Cic concentration, the CL per WBC. According to this study, Cic had no effect on the number of circular white blood cells, but greatly depressed the total CL and CL per WBC (60 % of that of control) in renal transplantation patients, so we couldn't see any pure linear relationship between total CL and the number of WBC like that in the healthy control. Combination with the Pre and Aza produced limited but no statistic significant depression. In the case of Cic effects on the CL of the rat PMØ, there was a negative correlation between whole blood CL and Cic concentration. While Cic concentration was between 1 – 200 $\mu g \cdot L^{-1}$, the inhibitory rate of CL was about 21.1 % ($P < 0.05$) and increased with the increasing Cic concentration. When Cic concentration was > 400 $\mu g \cdot L^{-1}$, the CL was significantly decreased from that at 1 – 200 $\mu g \cdot L^{-1}$. In clinical practice we take the therapeutic window of Cic at 200 – 400 $\mu g \cdot L^{-1}$ within 6 months after renal transplantation. We don't know whether it is just a coincidence or not.

According to other report[7-8] about Cic

Tab 1. Correlation analysis of whole blood CL and WBC. $\bar{x} \pm s$. $^cP < 0.01$ *vs* healthy control (r value), $^fP < 0.01$ *vs* WBC.

Groups	n	WBC	Peak CL (mV)	Peak CL/WBC
Health control	10	8 036 ± 2 118	4 168 ± 1 402^f(0.83)	5.2 ± 0.9^f(0.87)
Renal transplant control	50	7 091 ± 783	1 708 ± 395^c(0.32)	2.5 ± 0.6^c(− 0.38)
Cic	20	7 390 ± 1 792	2 164 ± 666^c(0.42)	3.0 ± 1.0^c(− 0.49)
Cic + Pre	15	6 203 ± 1 088	1 502 ± 367^c(0.14)	2.5 ± 0.7^c(− 0.56)
Cic + Pre + Aza	15	7 682 ± 1 939	1 458 ± 617^c(0.54)	1.9 ± 0.6^c(− 0.18)

Tab 2. Effects of Cic on CL of rat PMØ. $\bar{x} \pm s$. $^bP < 0.05$, $^cP < 0.01$ *vs* **healthy control.** $^fP < 0.01$ *vs* **Cic 1 – 200 μg·L^{-1}.**

Cic/ μg·L^{-1}	*n*	Intensity of CL/mV	Inhibitory rate/%
0	10	117 ± 18	–
1 – 200	8	92 ± 11^b	21
200 – 400	32	67 ± 15^c	43
> 400	10	49 ± 10^d	58

with CL during respiratory burst, the obtained CL data were correlated to corresponding serum or plasma levels of Cic. Comparing with the healthy control no differences were seen in median CL values, but there was a significant ($P = 0.05$) negative correlation between Cic blood concentration and maximum CL values of PMNC. Such inhibition of CL could be calculated for Zym but not for phorbolmyristate acetate (PMA); suggesting that the Cic-mediated inhibition of granulocyte function may be only partial and restricted to phagocytosis[9]. Similarly in our study, 30 min after Zym stimulation the CL reached its peak, and then went downward. Only the peak values presented significant differences.

In conclusion, Cic inhibited the respiratory burst of white blood cells after Zym stimulation, in other words Cic depressed the phagocyte function in a concentration-dependent manner. We would do more work to evaluate the relationship between this inhibition and clinical occurrence of infection, cancer, and allograft.

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环孢素对肾移植病人全血化学发光的影响

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关键词 肾移植; 化学发光; 环孢素; 嗜中性白细胞; 腹腔巨噬细胞

目的: 研究环孢素(Cic)对50例肾移植病人体内鲁米那依赖性化学发光的影响. **方法**: 用化学发光仪测定酵母多糖刺激引起的全血白细胞呼吸氧爆发; 用免疫荧光偏振法测定全血Cic浓度. **结果**: 与正常对照组相比, Cic用药组病人的全血化学发光显著下降($P < 0.01$); Cic浓度与全血化学发光呈负相关($r = -0.81$, $P < 0.01$); 移植病人血清直接抑制大鼠腹腔巨噬细胞的化学发光强度. **结论**: Cic抑制白细胞的吞噬功能.