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Modulating effect of mitomycin or cisplatin on lymphokine-activated killer cell proliferation and antitumor activity to bladder cancer cell lines *in vitro*[1]

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KEY WORDS mitomycin C; cisplatin; lymphokine-activated killer cells; bladder neoplasms; cell division; interleukin-2; immunologic cytotoxicity; cultured tumor cells

AIM: To study the effect of mitomycin (Mit) or cisplatin (Cis) on the proliferation of lymphokine-activated killer (LAK) cells in patients with transitional cell cancer of bladder and their cytolysis to bladder tumor cells. **METHODS**: LAK cell proliferation was assayed in the presence of Mit or Cis by cell counting. Bladder cancer cell lines BIU-87 and EJ were cultured as target cells and cytotoxicity of LAK cells was determined by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. **RESULTS**: The proliferation of LAK cells induced by recombinant interleukin-2 (IL-2) was inhibited by Cis in a concentration-dependent manner and was decreased to 55.3 % at 100 mg·L^{-1} compared with control at 96 h. The enhanced growth of the LAK cells was observed with Mit 5 - 10 mg·L^{-1} from 48 to 96 h. Cis 10 mg·L^{-1} increased the cytotoxicity against BIU-87 and EJ cells. **CONCLUSION**: Immunomodulatory effect of chemotherapeutic agents on LAK cell proliferation induced by IL-2 in patients with bladder cancer mainly depends on the drug itself.

Although lymphokine-activated killer (LAK) cells exert a potent cytotoxicity on many tumors[1,2] the results using LAK in clinical practice are not very satisfactory. Immunomodulatory effect of chemotherapy on LAK activity is variable and largely depends on the drug itself. Some anticancer agents have stimulatory effects on cytotoxicity against tumor cells and other agents suppress the immune reponse[3,4]. Therefore, it becomes important to identify which chemotherapeutic agent is endowed with an immunostimulatory effect and which agent is more compatible with combined immunochemotherapy.

There is a general tendency in oncology to combine different chemotherapeutic and immunological agents to incease efficacy and minimize side effects. It is not clear what kinds of roles mitomycin (Mit) or cisplatin (Cis) may play in the proliferation and cytotoxicity of LAK cells in patients with bladder cancer. This study was to investigate the roles of Mit or Cis in the modulation of LAK cells in patients with bladder cancer.

MATERIALS AND METHODS

Chemicals RPMI-1640 medium was obtained from Gibco. 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) and *L*-glutamine were obtained from Sigma. Recombinant interleukin-2 (IL-2) was made by Changchun Institute of Biological Products, Ministry of Pubulic Health, China. Fetal calf serum (FCS) was purchased from Sino-American Biotechnology Co, Mit was made by Kyowa Hakko Kogyo Co, Japan. Cis was made by Qilu Pharmaceutical Factory, Ji-nan, China.

Cultivation of LAK cells Peripheral blood mononuclear cells (PBMC) obtained from fresh, heparinized periperal blood of 21 patients with pathologically diagnosed transitional cell carcinoma of bladder were isolated by Ficoll-paque (Shanghai 3rd Chemical Reagents Ltd) density-gradient centrifugation. Interface cells were aspirated and washed 3 times with Hanks' solution. The PBMC were suspended (1×10^9 cells·L^{-1}) in complete medium (CM) consisting of RPMI-1640, benzylpenicillin 100 kU·L^{-1}, streptomycin 100 kU·L^{-1}, gentamycin 50 kU·L^{-1}, *L*-glutamine 2 mmol·L^{-1}, sodium pyruvate 1 mmol·L^{-1} and 15 % heat-inactivated FCS. The cells were allowed to settle in 25-cm^2

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tissue culture flasks at 37 ℃ in 5 % CO_2 for 2 h. The non-adherent PBMC (2×10^8 cells $\cdot L^{-1}$) were transferred into another 25-cm^2 flasks and further cultivated in CM supplemented with IL-2 1 MU $\cdot L^{-1}$ for 96 h.

LAK cell proliferation assay The nonadherent PBMC (15×10^3 cells/well in 300 μL CM + IL-2) were placed in 96-well plates in the presence of Mit (0 – 20 mg $\cdot L^{-1}$) or Cis (0 – 100 mg $\cdot L^{-1}$). Each concentration contained 6 wells. The cells were serially counted in a hemocytometer chamber and their activity was estimated with MTT assay. The tests were performed for 4 independent times.

Cytotoxicity assay Human bladder transitional cell carcinoma cell lines BIU87 or EJ cells used as the target cells were placed in 96-well plates 8×10^3 cells/well in 150 μL CM and cultured for 24 h. After removing the medium, the LAK cells treated with IL-2 alone for 48 h and washed with Hanks' solution for 3 times were added to target cells in effector/target (E/T) ratios = 40:1 in 250 μL CM. The cell mixtures were then incubated at 37 ℃ in the presence of Mit 5 mg $\cdot L^{-1}$ or Cis 10 mg $\cdot L^{-1}$ for 4 h. The cytotoxicity of LAK was determined by MTT assay[5]. The cytotoxicity assay was repeated for 4 independent experiments (4 wells each). The absorbance (A) at 570 nm in each well was determined with microplate autoreader (Nanjing, China). The % of lysis = [1-(A of traget cell plus LAK-A of LAK cell)/A of target cell] × 100 %.

Statistical analysis LAK cell proliferation experiments were analyzed with ANOVA. LAK cell cytotoxicity against tumor cells was analyzed by u test.

RESULTS

Proliferation of Cis-treated LAK cells Cis (10, 50, 100 mg $\cdot L^{-1}$) inhibited the proliferation of LAK cells from 24 h. Cis 100 mg $\cdot L^{-1}$ inhibited the LAK cells to 55.3 % of control at 96 h (Fig 1).

Effect of Mit on LAK cell growth Mit 5 – 10 mg $\cdot L^{-1}$ enhanced growth of LAK cells from 48 to 96 h. The strongest stimulation was achieved at Mit 5 mg $\cdot L^{-1}$. In the lower or higher concentration groups, there was no stimulation (Fig 1).

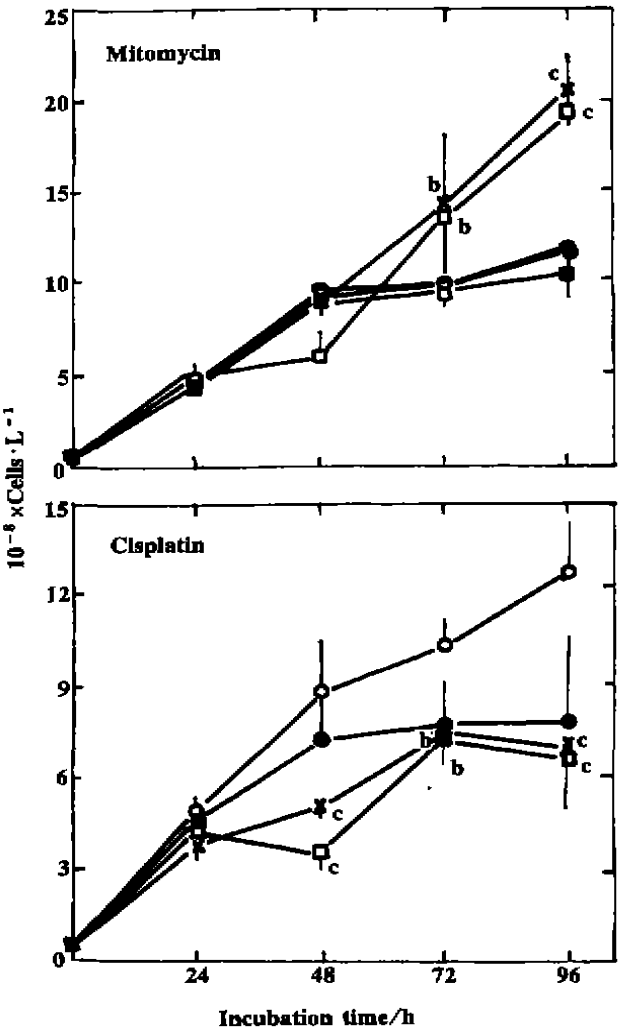

Fig 1. Effect of cisplatin (Cis) or mitomycin (Mit) on LAK cell proliferation induced by IL-2. The LAK cells were treated with IL-2 alone (○), IL-2 + Cis 10 or Mit 2.5 (●), Cis 50 or Mit 5 (×), Cis 100 or Mit 10 (□), and Mit 20 mg $\cdot L^{-1}$ (■) mg $\cdot L^{-1}$. n = 6 wells and repeated for 4 (Cis) or 3 (Mit) independent experiments. $^bP < 0.05$, $^cP < 0.01$ *vs* IL-2 alone.

Cytotoxicity of LAK cells against tumor cells treated with Mit or Cis Cis 10 mg $\cdot L^{-1}$ increased the cytotoxicity against BIU-87 and EJ cells ($P < 0.05$). However, the cytotoxicity of LAK cells treated with Mit was not statistically significant. Incubation of BIU87 or EJ cells with CM containing Mit 5 mg $\cdot L^{-1}$ or Cis 10 mg $\cdot L^{-1}$ for 4 h did not affect A (Tab 1).

DISCUSSION

Previous studies mostly dealt with chemo-

Tab 1. Effect of Mit and Cis on the cytolysis of LAK cells to bladder cancer cells. $n = 4$ wells/group and repeated for 4 independent times, $\bar{x} \pm s$.
$^aP > 0.05$, $^bP < 0.05$ vs BIU-87 treated with CM.
$^dP > 0.05$, $^eP < 0.05$ vs EJ treated with CM.

Tumor cells	Specific cytolysis (%)		
	Complete medium	Mitomycin (5 mg·L^{-1})	Cisplatin (10 mg·L^{-1})
BIU-87	39.1 ± 1.3	42.6 ± 2.2^a	59.5 ± 2.3^b
EJ	37.2 ± 1.7	41.4 ± 3.3^d	47.6 ± 1.9^e

therapeutic agents and immunologic cytotoxicity and there is few report about the effects of chemotherapeutic agents on LAK cell proliferation[6]. The current study provides evidence that Mit treatment of LAK cells from patients with bladder cancer enhances their proliferation induced by IL-2 and Cis treatment inhibits their proliferation. These results indicate that immunomodulatory effect of chemotherapeutic agents on LAK cell proliferation induced by IL-2 in patients with bladder cancer is variable and mainly depends on the drug itself.

Potentially beneficial cellular immunomodulating effects of chemotherapeutic drugs could arise because of differential sensitivities of different components of the immune system[7]. Treatment of PBMC with Cis in the presence of IL-2 resulted in a significant increase in LAK activity against NK resistant Raji and Daudi cells[8]. Present study demonstrated that Mit did not significantly influence the cytotoxicity but incubation of LAK cells and bladder tumor cells with Cis 10 mg·L^{-1} for 4 h increased cytotoxicity activity against BIU-87 and EJ cells. These results indicate that although Cis 10 mg·L^{-1} may inhibit LAK cell proliferation, the cytotoxicity is increased under the synergic effects of Cis on LAK cells and bladder tumor cells and modulation of Cis on different parts of the immune system is complicated or variable.

Alternating chemotherapeutic and immunotherapeutic instillations improved efficacy and reduced toxicity in patients with carcinoma *in situ* of bladder[9]. However, whether combined immunotherapy and chemotherapy for the management of bladder cancer will ever be of clinical use remains to be established. Mit and Cis have been commonly used in postoperation treatment in patients with bladder cancer. The concentrations of Mit or Cis used in the tests are levels that were detected in blood or urine of patients who were treated with Mit or Cis by intravenous or local arterial injection or intravesical instillation[3,10,11]. These studies are a necessary forerunner to possible use of combining immunotherapy with chemotherapy in bladder cancer.

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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-