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Kanglemycin C *vs* ciclosporin on immunosuppression in mice

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KEY WORDS kanglemycin C; cyclosporine; delayed hypersensitivity; homologous transplantation; hemolysin; phagocytosis; immunosuppression

ABSTRACT

AIM: To study inhibitory effects of kanglemycin C (Kan) on the mouse immune system, and compare with the effects of ciclosporin (Cic). **METHODS**: Delayed hypersensitivity (DH) and cyclophosphamide-potentiated DH induced by dinitrofluorobenzene (DNFB); heart allograft and skin allograft; hemolysin; the phagocytosis of the peritoneal macrophage. **RESULTS**: Kan (12.5, 25, 50 mg·kg^{-1}·d^{-1}, ig, 8 d) markedly inhibited DH and cyclophosphamide-potentiated DH induced by DNFB ($P < 0.01$), prolonged survival times of heart and skin allografts ($P < 0.01$), and decreased the content of hemolysin ($P < 0.01$), but had no significant effect on the neutral-red phagocytosis of the peritoneal macrophage ($P > 0.05$). **CONCLUSION**: Kan had marked suppressive effects on cell-mediated and humoral-mediated immune responses, but no effect on phagocytosis of macrophage.

INTRODUCTION

Kanglemycin C (Kan), a benz(α)-anthracene[1], first discovered in China was isolated from the culture filtrate of *Nocardia mediterranei* var knglensis 1747 - 64. In preliminary *in vitro* experiments, Kan possessed a weak action against Gram-positive bacteria and potent immunosuppressive and antitumor activities[2].

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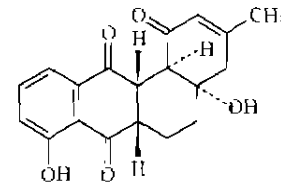

Kanglemycin C
$C_{19}H_{18}O_5$, $M_r = 322$

Kan suppressed the proliferations of human peripheral blood lymphocytes and mouse splenocytes stimulated by mitogens, and the allogenic mixed lymphocyte reaction *in vitro*[2]. Ciclosporin (Cic), a small cyclopeptide in fungal metabolite, possessed a strong immunosuppressive action in many experimental models for both humoral and cell-mediated immunity in several animal species[3]. T-Lymphocyte was the major target of Cic[4] and has been widely used as the primary drug to facilitate human organ transplantations[5]. Dinitrofluorobenzene (DNFB)-induced delayed hypersensitivity (DH) was caused mainly by helper-inducer T (T_h) and cytotoxic T-lymphocyte (T_c)subsets[6]. Cyc can augment function of suppressor-inducer T-lymphocyte (T_s) and potentiat DH[7]. Hemolysin production represented humoral immune response. Allograft rejection reaction was predominantly mediated by T_c subsets about 10 d, and by immunoglobunin and complement from 11 d onways after organ grafted[8]. To discern influence of Kan on immune action, the immunosuppressive activities of Kan *in vivo* was studied in comparison with Cic.

MATERIALS AND METHODS

Drugs and reagents Cic (Sandoz Pharmaceuticals, E Hanover NJ, USA) was kindly given by Dr M A Evans (Indiana University, USA).

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Cic 100 mg was dissolved in 1 mL ethanol and diluted with olive oil to the concentrations needed (as the volume given 10 mL·kg^{-1} and 12.5, 25, 50 mg·kg^{-1}·d^{-1}). Kan (yellow pin-shaped crystal, purity 99.5 %, mp 170 ℃) was isolated by Institute of Medicinal Biotechnology, Chinese Academy of Medical Sciences. Kan 100 mg was dissolved and diluted as Cic. Cyc (Shanghai 12th Pharmacetical Factory) 25 mg was dissolved in 1 mL sterile 0.85 % saline solution.

Mice and treatment Inbred strains BALB/c (Grade Ⅱ, Certificate № 01-3046), C57BL/6j (Grade Ⅱ, Certificate № 01-3044), and LACA (Grade Ⅱ, Certificate № 01-3051) mice, ♀, 8 - 12 wk, 20 - 22 g, and C57BL/6j newborn mice (24 - 48 h old) of both sexes, were purchased from the Department of Experimental Animal, Beijing Medical University. The mice were randomly divided into groups. Cic and Kan 12.5, 25, 50 mg·kg^{-1}·d^{-1} were ig administered from 3 d before mice were immunized or transplanted.

Experiment instrument FKIIA, ECG instrument (Qingdao Fukuda Denshi Co); 721 spectrometer (Shanghai 3rd Analysis Instrument Factory); DG3022A ELISA analyser (Hua-Dong Electronic Tube Factory).

DH and cyclophosphamide-potentiated DH LACA mice were sensitized and challenged with 1 % DNFB. The mean difference in ear weight between the 2 ears was taken as DH[9]. Cyc-potentiated DH was done as DH, except mice were injected ip with Cyc 250 mg·kg^{-1} on d 3.

Skin and heart allograft Skin allograft was modified as the method[10]. Skin graft beds (8 mm × 8 mm) in BALB/c mice were prepared on the right thoracic wall. Full-thickness square grafts (8 mm × 8 mm) were prepared from the trunk-skin of C57BL/6j. The graft was positioned on the graft bed and covered with protective tape. The inspection of skin grafts was done every day.

Heart allograft[11]: BALB/c mice were anesthetized ip with sodium pentobarbital 50 mg·kg^{-1}. The dorsum of the mouse ear was dissected with small curved forceps. The donor half-heart was excised from C57BL/6j newborn mice and inserted into the earpouch. From d 6, heart grafts in the ears of anesthetized mice were monitored with ECG instrument every other day.

Hemolysin assay[9] Splenocytes were prepared from LACA mice immunized with SRBC or unimmunized. The hemolysin production of the splenocytes, the absorbance (A) was measured at 413 nm with 721 spectrometer.

Phagocytosis assay BALB/c mouse peritoneal macrophages were prepared in a general way. The phagocytosis was measured bt neutral-red assay[12]. The absorbance A was measured at 540 nm with ELISA analyzer.

Statistical methods Results were expressed as $\bar{x} \pm s$ and analyzed by t-test to compare the difference between the groups with the Software of Statistica for Windows (4.5, Statsoft Inc 1993).

RESULTS

DH and cyclophosphamide-potentiated DH Kan (12.5, 25, 50 mg·kg^{-1}·d^{-1}, ig, 8 d) markedly decreased the ear weight difference ($P < 0.01$) as Cic (Tab 1).

Cic strengthened DNFB-contacting dermatitis and made the challenged ear-swelling aggravation. Cic-potentiated DH induced by DNFB was inhibited by Kan. In suppressive potencities there was no difference between Kan and Cic at the same dose. Compared with control, however, there were differences from groups treated with Kan in the ear weight difference (Tab 1).

Survival of skin and heart allografts As Cic, Kan (12.5, 25, 50 mg·kg^{-1}·d^{-1}, ig, 8 d) markedly prolonged the mouse skin allograft survival time ($P < 0.05$, $P < 0.01$) and prolonged heart allograft survival time in a dose-dependent manner($P < 0.01$) (Tab 2).

SRBC hemolysin The immunized control and the immunized vehicle, like the Kan- and

Tab 1. Effect of Kan on DH and cyclophosphamide-potentiated DH induced by dinitrofluorobenzene in mice. Cyc 250 mg·kg^{-1} was injected ip on d_{-3}, other reagents were administered on d_{-3} – d_0 – d_4. (n) = number of mice. $x \pm s$. $^bP < 0.01$ *vs* control. $^fP < 0.01$ *vs* Cyc group.

Groups (mg·kg^{-1}·d^{-1}, ig × d)		Ear weight (mg) – Cyc	+ Cyc
Control		22.0 ± 2.6 (11)	
Vehicle		21.5 ± 2.6 (10)	
Cyc			27.4 ± 3.8 (9)
Cic	12.5 × 8	16.6 ± 4.4 (11)^b	17.7 ± 1.6 (10)^b
	25.0 × 8	12.2 ± 3.9 (10)	12.6 ± 1.2 (10)^b
	50.0 × 8	5.6 ± 2.8 (9)	7.2 ± 1.5 (9)^b
Kan	12.5 × 8	17.3 ± 3.8 (10)^b	18.5 ± 2.1 (10)^f
	25.0 × 8	14.6 ± 2.6 (9)^b	12.8 ± 1.6 (9)^b
	50.0 × 8	6.2 ± 2.3 (9)^b	8.7 ± 2.1 (10)^b

d_{-3}: 3 d before immunization; d_0: the day of immunization; d_4: 4 d after immunization; – Cyc: without given cyclophosphamide; + Cyc: given cyclophosphamide.

Tab 2. Effect of Kan on survival time of mouse skin and heart allograft. The reagents were given on d_{-3} – d_0 – d_4. (n) = number of mice. $\bar{x} \pm s$. $^aP > 0.05$, $^bP < 0.05$, $^cP < 0.01$ *vs* olive oil.

Groups (mg·kg^{-1}·d^{-1}, ig × d)		Survival time (d) Skin allograft	Cardiac allograft
Control		8.3 ± 1.3 (9)	10.6 ± 1.9 (10)
Olive oil		8.7 ± 1.1 (10)	10.2 ± 1.1 (11)
Cic	12.5 × 8	10.1 ± 1.4 (9)^b	
	25 × 8	11.9 ± 2.1 (8)	14.3 ± 1.9 (8)^c
	50 × 8	12.8 ± 1.7 (9)^c	15.5 ± 0.9 (8)^c
Kan	12.5 × 8	9.5 ± 1.5 (9)^a	
	25 × 8	11.1 ± 2.0 (9)^c	14.0 ± 2.0 (9)^c
	50 × 8	12.5 ± 2.2 (9)^c	14.9 ± 1.8 (9)^c

Cic-treated groups, were challenged with SRBC on d 0. SRBC hemolysin of the immunized control and the immunized vehicle were markedly increased compared with control ($P < 0.01$). Kan (12.5, 25, 50 mg·kg^{-1}·d^{-1}, ig, 8 d) suppressed SRBC hemolysin production of the mouse immunized splenocyte. There was difference between the suppressive potencities of Kan and Cic of the same dose (Tab 3).

Phagocytosis of mouse peritoneal macrophages Kan (50 mg·kg^{-1} × 5 d) had no significant effect on phagocytosis of neutral-red by peritoneal macrophages (Tab 4).

Tab 3. Effect of Kan on SRBC hemolysin production of the mouse immunized-splenocyte. Mice were immunized on d_0. $\bar{x} \pm s$. $^aP > 0.05$, $^cP < 0.01$ *vs* control. $^fP < 0.01$ *vs* immunized control. $^jP < 0.01$ *vs* the same dose of Cic.

Groups (mg·kg^{-1}·d^{-1}, ig × d)		Mice	Hemolysin ($A_{413\,nm}$)
Control		11	0.19 ± 0.04
Vehicle		9	0.21 ± 0.03^a
Immunized control		9	0.64 ± 0.05^c
Immunized vehicle		8	0.60 ± 0.04
Ciclosporin	12.5 × 8	10	0.52 ± 0.03
	25 × 8	10	0.47 ± 0.04^c
	50 × 8	10	0.40 ± 0.06^j
Kanglemycin C	12.5 × 8	9	0.44 ± 0.06^j
	25 × 8	10	0.37 ± 0.04^{fj}
	50 × 8	9	0.28 ± 0.04^{fj}

Tab 4. Effect of Kan on phagocytosis of mouse peritoneal macrophage. n = 10 mice. $\bar{x} \pm s$. $^aP > 0.05$ *vs* control. The reagents were given on d_{-3} – d_0 – d_1. Starch was injected ip on d_0.

Groups (mg·kg^{-1}·d^{-1} × d)		$A_{451\,nm}$
Control		0.40 ± 0.14
Olive oil		0.42 ± 0.10^a
Ciclosporin	50 × 5	0.34 ± 0.13^a
Kanglemycin C	50 × 5	0.36 ± 0.08^a

DISCUSSION

Immune response to antigen comprised of humoral and cell-mediated reactions. The results recorded here demonstrated that Kan like Cic inhibited ear swelling reaction of mouse allergic contact dermatitis to DNFB and cyclophosphamide-potentiated DH, indicating that Kan inhibited T_h and T_c cell action in normal and elevated immune states. It consisted with the previous *in vitro* results[2,3]. Kan prolonged the alograft survival time to 15 d, suggesting that Kan suppressed humoral and cell-mediated immunity. Furthermore, SRBC hemolysin (anti-

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SRBC-antibody) production of the mouse immunized splenocyte was suppressed by Kan and Cic, indicating that Kan interfered in antibody mediated-immune response. Compared with the same doses of Cic, the effect of Kan on humoral mediated-immune reaction was a bit more potentiated. The current study showed that Kan had no effect on phagotosis of neutral-red by mouse peritoneal macrophage, even though at the larger dose, suggesting that phagocytotosis of antigen by antigen presented cell was not interfered by Kan. So, immunosuppressive effect of Kan was mainly through inhibition of T- and B-lymphocyte action.

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康乐霉素 C 与环孢素对小鼠免疫抑制作用的比较

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关键词 康乐霉素 C；环孢素；迟发性超敏感性；同种移植；溶血素；吞噬作用；免疫抑制

目的：与环孢素(Cic)比较，研究康乐霉素 C (Kan)对小鼠免疫系统的影响。**方法**：皮肤迟发性超敏反应(DH)和环磷酰胺(Cyc)增强型 DH；同种异型皮肤和心脏移植；脾细胞溶血素测定；腹腔巨噬细胞吞噬中性红。**结果**：Kan (12.5, 25, 50 mg·kg^{-1}·d^{-1}, ig, 8 d) 与 Cic 相似，抑制小鼠皮肤 DH 和 Cyc 增强型 DH ($P < 0.01$)；延长小鼠同种异型皮肤和心脏移植物存活时间($P < 0.01$)；对溶血素产生也有抑制作用($P < 0.01$)；Kan 50 mg·kg^{-1}·d^{-1}, ig, 5 d 与 Cyc 相似，对小鼠腹腔巨噬细胞吞噬中性红无明显作用($P > 0.05$)。**结论**：Kan 显著抑制小鼠细胞免疫和体液免疫，但不影响巨噬细胞的吞噬。

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