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Effects of cantharidin on interleukin-2 and interleukin-1 production in mice *in vivo*[1]

ZHANG Jun-Ping, QIAN Ding-Hua, QI Li-Hong[2]
(*Research Laboratory of Natural and Synthetic Drugs, School of Pharmacy, Second Military Medical University, Shanghai 200433, China*)

ABSTRACT After cantharidin (0.75, 1.5 mg · kg^{-1}) was given ip 3 times every other day in mice, Con A-induced spleen lymphocyte proliferation, as measured by [^{3}H]TdR incorporation assay, was enhanced from 7 978 ± 1 780 to 36 631 ± 8 467 and 29 997 ± 3 788 dpm in both doses. Interleukin-2 and interleukin-1 production were also increased from 11 ± 4 to 52 ± 18, 23 ± 6 U · ml^{-1} and from 7 628 ± 1 477 to 14 532 ± 2 272, 11 515 ± 2 862 dpm, respectively. These results suggest that cantharidin potentiates immune response through the release of interleukin-2 and interleukin-1.

KEY WORDS cantharidin; interleukin-1; interleukin-2; thymidine

Cantharidin (Can) is a cytotoxic anti-tumor drug applied for the treatment of primary liver cancer and viral hepatitis[1]. It is well known that cytotoxic agents cause damage of immunocompetent cells. Recently a number of cytotoxic drugs have been reported to alterate cytokines production such as interleukin-2 (IL-2) and interleukin-1 (IL-1)[2,3]. As Can has not been found to produce immunosuppression activity, it is interesting to determine whether Can has any effects on the IL-2 and IL-1 production.

METHODS AND RESULTS

Drugs and animal treatment Cantharidin (E Merck) was dissolved in methyl sulfoxide / ethanol (1 : 4) as a stock solution (1.5 mg · ml^{-1}) and diluted with saline when test. Concanavalin A (Con A, Sigma) and lipopolysaccharides (LPS) from E coli 0111 : B4 (Sigma) were dissolved in serum free RPMI 1640 medium (Sigma) and sterilized by filtration through 0.22 μm filter.

ACR mice, both sexes and weighing 25 ± *s* 4 g, obtained from the Animal Center of this University, were used in this study. The mice were kept on a standard chow and given tap water *ad lib*.

Mice were injected ip with Can (0.75, 1.5 mg · kg^{-1}) thrice every other day. Control mice were injected with an equal volume of the solvent equivalent to the maximal dose of Can. All mice were killed on d 7. Spleen was removed and prepared as single spleen cell suspension. Peritoneal exudate cells (PEC) were also collected from these mice by washing the peritoneal cavity with phosphate buffer saline (PBS).

Mitogen induced lymphocyte transformation The lymphocyte transformation assay was assessed in triplicate wells in microplates containing 5 × 10^5 spleen cells in 0.2 ml of RPMI 1640 culture medium in the presence of Con A (5 μg · ml^{-1}) for 72 h incubation at 37 ℃ in 5% CO_2 + 95% air. [^{3}H]TdR incorporation into spleen cells of Can group was more than that of control (Tab 1). These results indicated that Can enhanced Con A-induced T lymphocyte proliferation.

IL-2 production One ml of spleen cell 5 × 10^6 / ml suspension was cocultured with Con A 5 μg · ml^{-1} for 24 h in 24-well flat bottom plates. The IL-2 activity in supernatants was determined by a standard

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[2] Now in *Department of Pharmacy, Dong Fang Hospital, Fuzhou 350001, China.*

microassay based on the IL-2 dependent proliferation of CTLL-2[14]. Marked elevation of IL-2 production was seen in Can groups (Tab 1).

IL-1 production PEC were plated in 24-well plates at 2×10^6 / well in 1 ml culture medium. After 2 h incubation, non-adherent cells were removed by extensive washing by PBS. IL-1 production was initiated by the addition of LPS (10 $\mu g \cdot ml^{-1}$) to adherent macrophages for 24 h. IL-1 activity of macrophages supernatants was evaluated by thymocyte proliferation assay[15]. Increased production of IL-1 in the supernatants was seen in Can (0.75 $mg \cdot kg^{-1}$) group. The finding was quite similar to those in lymphocyte transformation assay and IL-2 production.

Tab 1. Effects of cantharidin on spleen lymphocyte transformation, interleukin-2 (in Con A 5 $\mu g \cdot ml^{-1}$ supernatants) and interleukin-1 (in LPS 10 $\mu g \cdot ml^{-1}$ supernatants) production. IL-1 activities were expressed as dpm of [^{3}H]TdR incorporation by thymocytes at 1 : 8 dilution. $n=4$, $\bar{x} \pm s$. $^{**}P<0.05$, $^{***}P<0.01$ *vs* **control.**

Cantharidin / $mg \cdot kg^{-1}$	lymphocyte transformation / dpm	IL-2 activity / $U \cdot ml^{-1}$	IL-1 activity / dpm
Control	7 879 ± 1 780	11 ± 4	7 628 ± 1 744
0.75	36 631 ± 8 467***	52 ± 18***	14 532 ± 2 272***
1.50	29 997 ± 3 788***	23 ± 6***	11 515 ± 2 862**

DISCUSSION

Administration of Can has been shown to have inhibitory effect on some transplanted tumors and produce no immunosuppression activity in mice at the doses of 0.75 and 1.5 $mg \cdot kg^{-1}$ [6]. Our results demonstrated that the same treatment with Can induced significant enhancement of IL-1 and IL-2 production. These effects were not observed at the doses lower than 0.75 $mg \cdot kg^{-1}$ or higher than 1.5 $mg \cdot kg^{-1}$ (data not shown). It has been well documented that IL-1 and IL-2 exerted important roles in regulating the immune response and augmenting the immune defense of the host. Therefore, the potentiation of endogenous IL-1 and IL-2 production may be one of antitumor mechanisms of Can.

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小鼠体内斑蝥素对白细胞介素 2 和白细胞介素 1 产生的影响[1]

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张俊平, 钱定华, 齐荔红[2] (第二军医大学药学院中西药研究室, 上海 200433, 中国)

提要 小鼠隔日腹腔注射斑蝥素(0.75, 1.5 $mg \cdot kg^{-1}$)三次, 两个剂量组可使脾淋巴细胞产生白细胞介素 2 分别从 11 ± 4 ($U \cdot ml^{-1}$)增加到 52 ± 18 和 23 ± 6; 巨噬细胞产生白细胞介素 1 分别从 7 628 ± 1 744 (dpm)增加到 14 532 ± 2 272 和 11 515 ± 2 862. 结果提示斑蝥素增强这些细胞因子的产生可能是其增强机体的免疫功能及发挥抗肿瘤作用的重要机制之一.

关键词 斑蝥素; 白细胞介素 1; 白细胞介素 2; 胸苷