Chinese Journal of Cancer Research 10(3):194–197,1998.

AUGMENTATION OF IMMUNE FUNCTIONS AND AUTOLOGOUS TUMOR-KILLING ACTIVITY BY KAPPA-SELENOCARRAGEENAN IN MICE BEARING SARCOMA 180

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Objective: To study the enhancement of the immune functions and autologous tumor-killing (ATK) activity by kappa-selenocarrageenan (KSC) in mice bearing sarcoma 180. Methods: To measure the effects of KSC and/or Cyclophosphamide (Cy) on natural killer (NK) activity, lymphokine-activated killer (LAK) activity, the production of interleukin-2 (IL-2), ATK activity and the growth of sarcoma 180 (S_{180}). Results: KSC promoted NK activity, LAK activity and ATK activity *in vivo*, increased IL-2 production at 40 mg/kg/d×9d. It also enhanced the antitumor action of Cy (20 mg/kg/d×9d) and offset the inhibition of Cy on immunocopetent cells. The ATK activity in splenocytes of S_{180}-bearing mice could be induced and increased by recombinant interleukin-2 (rIL-2) *in vitro*. Conclusion: KSC has an up- regulating effect on the immune functions and ATK activity in tumor-bearing mice. It can be used as a biological response modifier (BRM) in cancer biotherapy.

Key words: Kappa-selenocarrageenan, Cyclophosphamide, Natural killer cells, Lymphokine-activated killer cells, Interleukin-2, Autologous tumor-killing activity, Sarcoma 180.

With increasing knowledge of tumor immunity and cancer biotherapy, it had been concentrated that host immune functions markedly affect the occurrence, development, treatment and prognosis of cancers. Autologous tumor-killing (ATK) activity mainly represents the tumoricidal activity of autologous lymphocytes against its own tumor cells in tumor-bearing host, which facilitates a key role in host antitumor mechanism. Previous studies have indicated that the immune functions and ATK activity of a host bearing tumor decreased significantly, or even lacked.[1-3] Selenium had a biological response modifiers (BRM)-like bioactivity and promoted the specific cytotoxicity of host immunocompetent cells.[4-6] This work is to investigate the effects of kappa-selenocarrageenan (KSC), a organic compound of selenium and polysaccharide, on tumor growth, natural killer (NK) cell activity, lymphokine-activated killer (LAK) cell activity, interleukin-2 (IL-2) production and ATK activity in mice bearing sarcoma 180 (S_{180}).

Accepted March 18, 1998

MATERIALS AND METHODS

Agents

KSC, with 7692ppm of Se, was purchased from Beijing Tian ci Fu Biopharmaceutical Co., Ltd; Recombiant IL-2 (rIL-2) from Sinochem Institute of Biotechnology; MTT and ConA from Sigma; Cyclophosphamide (Cy) from Shanghai 12th Pharmaceutical Factory.

Mice

BALB/c male and female mice, 6–8 wk old, 20–24 g, were purchased from Lanzhou Institute of Biological Products.

Cell Lines

S_{180} cell line (murine sarcoma) was obtained from Institute of Oncology of Gansu Province; CTLL-2 cells (IL-2-dependent cells line) was from Shanghai Institute of Biochemistry, Chinese Academy of Sciences; Raji cells (human lymphoma cell line) and K_{562} cells (human leukemia cell line) were preserved in our laboratory.

Murine Sarcoma 180 Model and Treatment

BALB/c mice were injected sc with S_{180} cells (0.2 ml, 1×10^6 cells) into the right axilla on day 0, and then randomly divided into 4 groups after 24 h. The tumor-bearing mice were administrated once-daily ig KSC (40 mg/kg), ip Cy (20 mg/kg) or ig KSC plus ip Cy for 9 days, respectively, normal and tumor control mice were treated with equal volums of normal saline (NS). On day 10 mice were killed, the solid tumor was removed and weighed, and the inhibition rate (IR) was calculated.

NK Cell Activity Assay

Murine splenocytes were harvested as effectors, K_{562} cells were prepared as target cells, ratio of effector and target (E:T) was 50:1, and the cytotoxicity of splenocytic NK cells to K_{562} cells were assessed by MTT coloriassay.[6]

ATK Activity Assay

Lymphocytes were separated from splenocyte suspension by Ficoll-Hypaque density gradient (d=1.089) as effectors, autologous S_{180} cells as target, ATK activity (E:T=50:1) was determined with MTT assay as descripted.[7]

Generation and Activity of LAK Cells

Splenocytic lymphocytes were plated in RPMI 1640 medium containing 20% fetal calf serum and 1000 μ/ml rIL-2 at cell concentration of 4×10^6/ml, and incubated for 3 days at 37°C in fully humidified air plus 5% CO_2 for generation of LAK cells. Raji cells as target to determine cytotoxicity of LAK cells (E:T=20:1); S_{180} cells as target to determine ATK activity of autologous LAK cells (LAK-ATK activity, E:T=20:1).

Production and Quantification of IL-2

IL-2 activity was evaluated by CTLL-2 bioassay.[6] Murine splenocytes were harvested and 5×10^6/ml cells were induced with 10 μg/ml ConA for 24 h, and supernatant was separated by centrifuging at -4°C. Serially diluted supernatants were incubated with 3×10^4 CTLL-2 cells per well in 96 flat microplate for 24 h at 37°C. Recombinant IL-2 was included as an internal standard of IL-2 in each assay. Unites of IL-2 were defined as the reciprocals of the dilution necessary to multiply 50% CTLL-2 target cells.

RESULTS

Enhancement of KSC on NK Activity, LAK Activity and IL-2 Production in S_{180}-bearing Mice

The cytotoxicities of NK cells and ATK cells induced *in vitro*, and IL-2 production in tumor-bearing mice was suppressed markedly ($P<0.05$–0.01), and a further decrease was caused by Cy-chemotherapy alone. KSC significantly enhanced the functions of the immunocompetent cells, NK activity, LAK activity and the production of IL-2 were increased to 225%, 150 and 227%, respectively. KSC also offsetted the inhibition of Cy on immunocompetent cells (Table 1).

Augmentation of ATK activity in Tumor-bearing Mice by KSC

Cy-chemotherapy decreased TAK activity of S_{180}-bearing mice ($P<0.05$), but if the splenocytic lymphocytes were stimulated induced with rIL-2 *in vitro*, ATK activity could partly be induced or increased (LAK-ATK activity). KSC administrated *in vivo* promoted ATK activity and LAK-ATK activity of the mice bearing S_{180} to 239% and 142%, respectively. KSC in combination with Cy the ATK activity and LAK-ATK activity increased by 283% and 69% compared with Cy treatment alone (Table 2).

Antitumor Action of KSC against Sarcoma 180 *in Vivo*

Cy and KSC both exerted an inhibitory effect on S_{180} tumor growth, the inhibition ratios (IR) were 60.2% and 39.9%, respectively; The best effect was seen in combination of KSC and Cy, having IR 81.2% (Table 3).

DISCUSSION

The immune functions and ATK activity of tumor-bearing host represent its abilities of defense and resistance to tumor, which are one of the key factors influencing on the efficacy of cancer biotherapy.[1-3] Especially ATK activity is closely related to the clinical process of cancers, it really represents the host autologous antitumor ability.[2] Therefore it is very important to study tumor-bearing host immune function and ATK activity for judging its antitumor ability and forecasting its prognosis.

Table 1. The effects of KSC on NK activity, LAK activity and IL-2 production in S_{180}-bearing mice ($\bar{x}\pm s$)

Group	NK activity (%)	LAK activity (%)	IL-2 (U/ml)
Normal mice	21.12±3.44	52.60±7.38	60.64±7.26
Tumor control	10.98±4.30*	34.19±10.47*	30.12±4.68**
Cy	10.06±2.75	30.84±8.16	16.88±2.54**
KSC	24.65±4.55**	51.25±4.65*	68.43±8.03**
KSC+Cy	19.27±5.03**	54.48±9.04**	58.90±5.54**

N=5 *P<0.05 **P<0.01 vs normal mice *P<0.01 **P<0.01 vs tumor control

Table 2. The effect of KSC on ATK activity in S_{180}-bearing mice ($\bar{x}\pm s$)

Group	ATK activity (%)	LAK-ATK activity (%)
Control	15.09±5.70	45.15±6.35
Cy	8.32±3.16*	40.50±7.08
KSC	36.09±4.82**	64.23±9.94**
KSC+Cy	31.86±5.19**	68.30±8.01**

N=5 *P<0.05 **P<0.01 vs control

Table 3. The inhibition of KSC on tumor growth in S_{180}-bearing mice ($\bar{x}\pm s$)

Group	Tumor weight (g)	IR (%)
Control	1.38±0.68	
Cy	0.55±0.26**	60.15
KSC	0.83±0.40*	39.86
Cy+KSC	0.26±0.10**	81.16

N=10 *P<0.05 **P<0.01 vs control

The previous reports showed that due to the inhibition of tumor cells and tumor-derived immuno-suppresive factors (TDSF) cancer was accompanied with hypoimmunity, and ATK activity was reduced, even lacked in most cases. Using a biotherapeutic way or various biological response modifiers (BRM) to enhance the immune functions and to induce or promote ATK activity before and after cancer surgery, radiotherapy or chemotherapy, was helpful to raise host antitumor ability.[2,3] Selenium had the cancer-preventing, antitumor and immuno-potentiating actions.[4,5] Kappa-selenocarrageenan (KSC) is a seleno-compound of natural sulfocarrageenan, it has the double effects of selenium and polysaccharide, and play dual functions of tumor cell-inhibitory and immunostimulating efficacy. Therefore it may serve as a novel immuno-cytotoxic anticarcinogenic drug and a useful BRM.[6,8] The present study in the treatment of S_{180}-bearing mice demonstrated that KSC promoted NK cell activity, LAK cell activity and splenocytic IL-2 production, and raised ATK activity in tumor-bearing mice, it also inhibited the growth of sarcoma 180 and enhanced the antitumor action of Cy *in vivo*, the cellular immune functions of S_{180}-bearing mice were recovered close to those of normal mice. In the same time our work found that ATK activity in mice bearing S_{180} tumor was much low, but if their splenocytes were stimulated with rIL-2 *in vitro*, a higher ATK activity (LAK-ATK) could be induced,

and the *in vivo* administration of KSC could enhance the effect. Taking all these together, it is considered that the decrease or lack of host ATK activity may be due to the inhibition of TDSF which causes immunoactive cells to be in the state of immunosuppression or immune tolerance. Therefore reducing host cancer load, or using an appropriate method of biotherapy could spur ATK activity on to recover or induce enhancement. These findings suggest that the inhibition of KSC on tumor growth is mediated through elevating NK activity and LAK activity, stimulating IL-2 production and enhancing ATK activity.

In summary, the *in vivo* induction of ATK activity by using BRMs, or the transfer of autologous lymphocytes after *ex vivo* induction or augmentation of ATK activity may become established as a potent beneficial biotherapy for human cancers.

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