EFFECT OF CIANIDANOL (KB-53) ON THE ACTIVITY OF MOUSE NATURAL KILLER CELLS

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The effect of cianidanol (KB-53) on the mouse natural killer (NK) cell activity of the splenic cells was investigated using YAC-1 cells as target cells. The oral administration of KB-53 at a dose of 500 mg/kg augmented significantly the NK cell activity. The activity was observed maximally 3 d after the administration, and significant difference from the control was observed even at 7 d after the administration of KB-53. The oral administration of KB-53, at a dose range of 125–500 mg/kg, augmented the NK cell activity in a dose-dependent manner at 3 d after the administration.

Keywords — cianidanol (KB-53); (+)-catechin; (+)-cyanidanol-3; natural killer cell (NK cell); mouse; splenic cell

INTRODUCTION

Cianidanol (KB-53, Fig. 1), which can also be called (+)-catechin or (+)-cyanidanol-3, is a flavonoid compound that has been shown to be of therapeutic value against viral hepatitis.1–30 In a previous paper,41 we reported that KB-53 augmented the activity of mouse cytotoxic-T-lymphocytes. In this study, an effect of KB-53 on the activity of mouse natural killer (NK) cell was investigated.

![Chemical Structure of Cianidanol (KB-53)](image)

FIG. 1. Chemical Structure of Cianidanol (KB-53)

MATERIALS AND METHODS

Animals — Six-week-old male mice of ddY strain were obtained from the Shizuoka Agricultural Co-operative Association for Laboratory Animals (Hamamatsu) and were given a pellet diet CE-2 (CLEA Japan, Inc., Tokyo) and water ad libitum.

Materials — KB-53 obtained from Zyma S.A. (Nyon, Switzerland).51 Cr-sodium chromate (Na₂₅₁ CrO₄·380 mCi/mg Cr) was purchased from New England Nuclear (Boston, Mass., USA). Fetal calf serum (FCS) and RPMI 1640 were purchased from Grand Island Biological CO. (N.Y., USA).

Target Cells — YAC-1, a tissue culture cell line of YAC, a Moloney virus-induced lymphoma of A/Sn origin, was used as the target cell. It was maintained in a suspension culture in a RPMI 1640 medium containing 10% FCS (10% FCS-RPMI 1640) in an 5% CO₂ incubator at 37°C.

Assay of NK Cell Activity — The assay of NK cell activity was carried out according to the method of Fuyama et al.51 Briefly, 3 ml of a target cell suspension which contained 1 × 10⁷ cells was incubated at 37°C for 1 h in the presence of 100 μCi of ⁵¹Cr-sodium chromate. Cells were washed three times with RPMI 1640, and adjusted to 2 × 10⁹/ml with 10% FCS-RPMI.
The splenic cells \((4 \times 10^6)\) in 0.1 ml and the target cells \((2 \times 10^4)\) in 0.1 ml were added to the wells of a V-bottom microtiter plate (Dynatech Laboratories, Inc., Virginia, USA). After incubation for 4 h in a 37°C humidified 5% CO\(_2\) incubator, the plates were centrifuged at 800 rpm for 5 min, 0.1 ml of the supernatant was collected from each well, and the radioactivity was counted by a well-type gamma counter (Model 5210, Packard). Percentage of specific lysis was calculated using the following formula:

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\% \text{ of specific lysis} = \frac{\text{experimental release (cpm)} - \text{spontaneous release (cpm)}}{\text{maximal release (cpm)} - \text{spontaneous release (cpm)}} \times 100
\]

Maximal release was determined by adding 0.1 ml of 1 N HCl to the target cell. Spontaneous release was determined by incubating the mixture of target cells and medium alone at 37°C for 4 h.

RESULTS

1. Kinetics of Effect of KB-53 on NK Cell Activity

The effect of KB-53 on NK cell activity was investigated at 1, 3, 5 and 7 d after oral administration. As shown in Fig. 2, KB-53, at a dose of 500 mg/kg, augmented significantly the NK cell activity at 1, 3, 5 and 7 d after the administration, the maximal augmentation was observed at 3 d after the administration. Significant augmentation of NK cell activity induced by KB-53 was recognized even at 7 d after the administration of KB-53.

2. Effect of Different Doses of KB-53 on NK Cell Activity

KB-53 was administered orally at doses of 125, 250 and 500 mg/kg, and NK cell activity was measured 3 d after the administration. As shown in Fig. 3, KB-53 augmented the NK cell activity in a dose-dependent manner.

DISCUSSION

Recently, attention is called to the NK cell

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**FIG. 2. Kinetics of Effect of KB-53 on NK Cell Activity in Mice**

a) \(p < 0.05\), b) \(p < 0.01\), significantly different from control.

**FIG. 3. Effect of Different Doses of KB-53 on NK Cell Activity in Mice**

a) \(p < 0.05\), b) \(p < 0.01\), significantly different from control.
which shows nonspecific cytotoxicity against tumor cells or virus infected cells. It is reported that the interferon (IFN) accelerate the removal of virus and augmented the NK cell activity in patients with chronic active hepatitis. From these results, it is suggested that the NK cell activity related closely to the therapy of viral hepatitis.

In this study, we investigated an effect of KB-53 on the activity of mouse NK cells. The oral administration of KB-53 at a dose of 500 mg/kg augmented significantly NK cell activity from 1 d after the administration, and the augmentation activity continued for 7 d. The maximal effect was observed 3 d after the administration. The effect of KB-53 on NK cell activity at 3 d after the administration was dose-dependent in a dose range of 125—500 mg/kg.

In a previous paper, we reported that KB-53 augmented the mouse cytotoxic-T-lymphocyte (CTL) activity. From these results, it is suggested that KB-53 augmented not only the CTL activity which shows specific cytotoxicity but also NK cell activity which shows nonspecific cytotoxicity.

Therefore, it is considered that KB-53 assists in the elimination of the hepatitis B virus from the liver in patients with chronic active hepatitis by the activation of CTL and NK cell activities.

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REFERENCES

2) F. Dinola: (+)-Cyanidanol-3 in acute viral hepatitis, Lancet, 1, 1379—1380 (1980).