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Abstract

Neocarzinostatin (NCS), an antibiotic with a high molecular weight, showed an inhibitory effect on Rauscher mouse leukemia. In normal mice, no significant changes were found in peripheral blood pictures except a tendency of lymphocytopenia, when 0.05mg/kg/day and 0.50mg/kg/day of NCS were injected intraperitoneally to two groups of mice for three days. On the other hand, peripheral nucleated cells of Rauscher leukemic mice decreased after intraperitoneal administration of NCS in a dose over 0.25mg/kg/day for three days. The cells affected by NCS were mainly erythroblasts and smudged cells. Spleens of Rauscher leukemic mice treated with NCS have been reduced in weight, and histological examinations of livers showed a significant decrease of infiltrating cells. In three groups treated with 0.25mg/kg/day of NCS for seven days, 0.25mg/kg/day for three days and 0.50mg/kg/day for three days, the 50% survival time was longer than in the control group. Particularly, the 50% survival time in Rauscher leukemic mice treated with 0.50 mg/kg/day for three days was over twice that of the control group.

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ANTITUMOR ACTIVITY OF NEOCARZINOSTATIN, EFFECT ON RAUSCHER LEUKEMIA IN MICE

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Abstract: Neocarzinostatin (NCS), an antibiotic with a high molecular weight, showed an inhibitory effect on Rauscher mouse leukemia. In normal mice, no significant changes were found in peripheral blood pictures except a tendency of lymphocytopenia, when 0.05mg/kg/day and 0.50mg/kg/day of NCS were injected intraperitoneally to two groups of mice for three days. On the other hand, peripheral nucleated cells of Rauscher leukemic mice decreased after intraperitoneal administration of NCS in a dose over 0.25mg/kg/day for three days. The cells affected by NCS were mainly erythroblasts and smudged cells. Spleens of Rauscher leukemic mice treated with NCS have been reduced in weight, and histological examinations of livers showed a significant decrease of infiltrating cells. In three groups treated with 0.25mg/kg/day of NCS for seven days, 0.25mg/kg/day for three days and 0.50mg/kg/day for three days, the 50% survival time was longer than in the control group. Particularly, the 50% survival time in Rauscher leukemic mice treated with 0.50mg/kg/day for three days was over twice that of the control group.

Neocarzinostatin (NCS), a new antitumor substance with a high molecular weight, was first isolated from culture filtrates of *Streptomyces carzinostaticus* (1). Studies on the effect of NCS in cultures of *Sarcina lutea* and HeLa cells revealed specific inhibition of the synthesis of DNA followed by DNA degradation (2). Up to now, the antitumor effect of NCS has been recognized in various tumors in mice; sarcoma 180, Ehrlich carcinoma, hepatoma 134 and L-1210 (3, 4). However, the effect on virus-induced leukemic mice has never been reported. The present paper describes a study on the effect of NCS on Rauscher leukemic mice.

MATERIALS AND METHODS

Mice used were 1.5-2.0-month old BALB/c male mice weighing 18-20g. Leukemic changes were confirmed through changes in peripheral blood and splenomegaly at the fourth week after the inoculation of 0.2 ml spleen homogenates which were obtained from maintaining leukemic mice and suspended in 20% physiological saline solution. NCS (Lot. 730559 for clinical trial) was

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kindly supplied by the Kayaku Antibiotics Research Laboratory (Tokyo). Fresh solution of NCS was prepared for each experiment and 0.2 ml of the solution was injected once daily for three or seven days intraperitoneally. Dosage of injected NCS was as follows; 0.05mg/kg/day, 0.25mg/kg/day, 0.50mg/kg/day and 1.00mg/kg/day. Hematological examinations included nucleate cell, red blood cell and thrombocyte counts in peripheral blood. Changes in the differentials of peripheral nucleate cells, spleen weights and histological findings of livers were examined in Rauscher leukemic mice treated with 0.50mg/kg/day of NCS for three days. The effect of NCS on survival time was studied in two groups; one group was treated with each dose of NCS for three days and another group for seven days.

RESULTS AND DISCUSSION

Effects of NCS on normal mice are shown in Fig 1. No significant

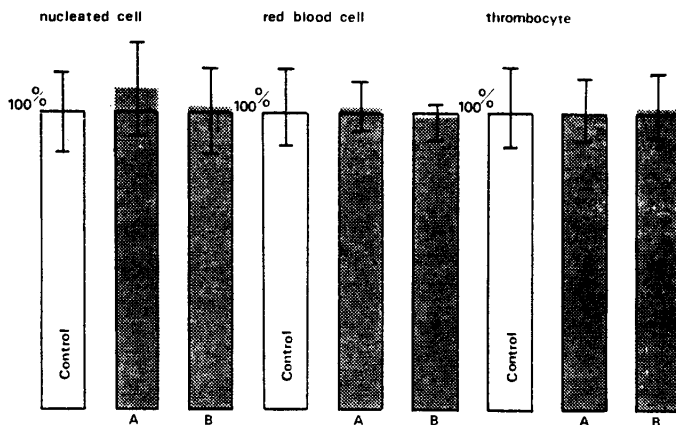


Fig. 1. Effect of Neocarzinostatin on peripheral blood in normal BALB/c mice

A: 0.05 mg/kg of NCS was injected for 3 days, i. p, B: 0.50 mg/kg of NCS was injected for 3 days, i. p, Cell Count (C. C) after injection of NCS/C. C before injection $\times 100$

changes in peripheral nucleated cell, red blood cell and thrombocyte counts were found in normal mice injected with 0.05mg/kg/day and 0.50mg/kg/day of NCS for three days. Neither any significant changes in the differentials of peripheral nucleated cells were recognized, except for a tendency of lymphocytopenia in mice injected with 0.50mg/kg/day of NCS. Effects of NCS on peripheral nucleate cell counts (NCC) in Rauscher leukemic mice are shown in Fig. 2. In non-treated Rauscher leukemic mice, NCC increased up to $128.3 \pm 27.7\%$ for three days. On the other hand, they decreased significantly after injection of NCS in a dose more than 0.25mg/kg/day for three

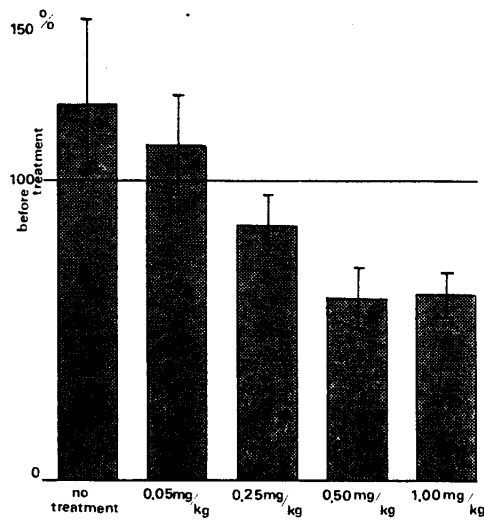


Fig. 2. Effect of Neocarzinostatin on peripheral nucleated cell count in Rauscher leukemic mice

NCS, injected intraperitoneally for 3 days, NCC after treatment/NCC before treatment × 100%

days. In Rauscher leukemic mice treated with 0.05 mg/kg/day of NCS for three days, NCC were increased slightly although the ratio of the increase tended to be lower than in the non-treated group. Changes in the differentials of peripheral nucleated cells are demonstrated in Figs. 3 and 4. It is

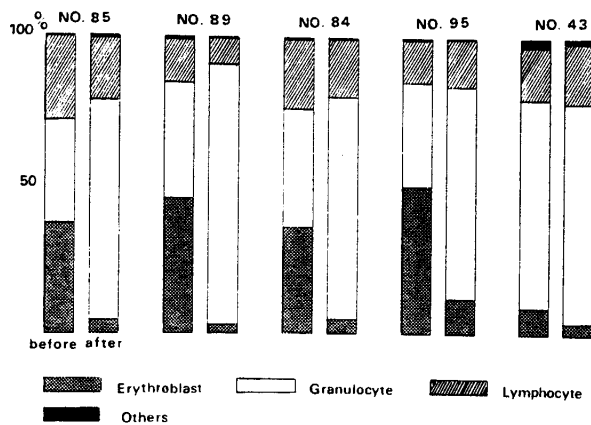


Fig. 3. Effect of Neocarzinostatin on differentials of peripheral blood in Rauscher leukemic mice NCS, 0.50 mg/kg/day × 3 days, i. p

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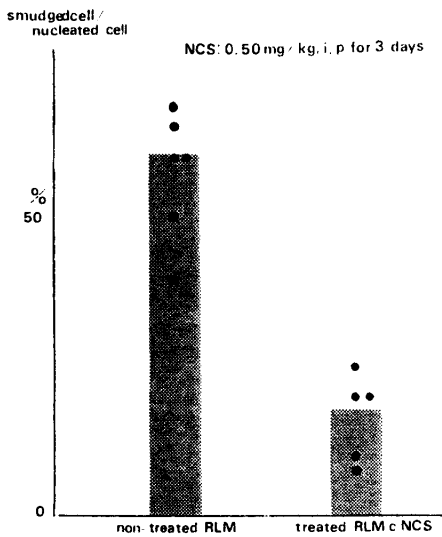


Fig. 4. Smudged cell in peripheral blood

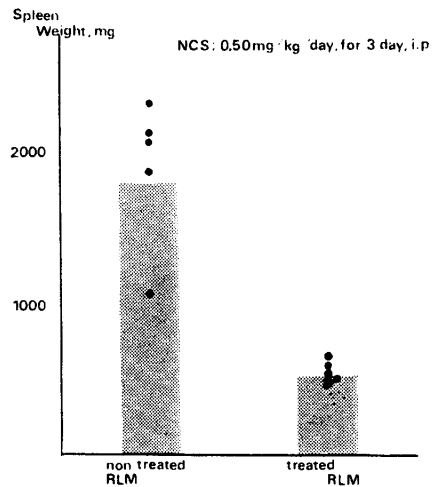


Fig. 5. Effect of Neocarzinostatin on spleen weight in Rauscher leukemic mice

postulated that affected cells with NCS are mainly composed of erythroblasts and smudged cells. We have already reported hematological findings in Rauscher leukemic mice (5). In BALB/c mice inoculated with Rauscher leukemic virus (RLV), erythroblasts begin to appear in the peripheral blood at around the second or third week and erythroblasts and smudged cells are gradually increased in quantity as spleen becomes larger. Histologically, there are marked splenomegaly infiltration, and proliferation of erythroblasts in hematopoietic organs. In Rauscher leukemic mice treated with 0.5mg/kg/day of NCS for three days, the significant decrease in the spleen weight with a decrease in number of peripheral nucleated cells was recognized (Fig. 5). Infiltrating cells were also decreased in the liver of this group (Figs. 6, 7).

Effects of NCS on survival time are shown in Fig. 8. Each group was composed of five Rauscher leukemic mice. When 0.25mg/kg/day, 0.50mg/kg/day and 1.00mg/kg/day of NCS was given to three groups for seven days, a slight prolongation of 50% survival time was recognized only in the group treated with 0.25mg/kg/day of NCS. In the group treated with 1.00mg/kg/day of NCS, Rauscher leukemic mice began to die at day 2 after treatment and all mice have died during treatment. On the other hand, when 0.25 mg/kg/day or 0.50mg/kg/day of NCS was given for three days, the 50%

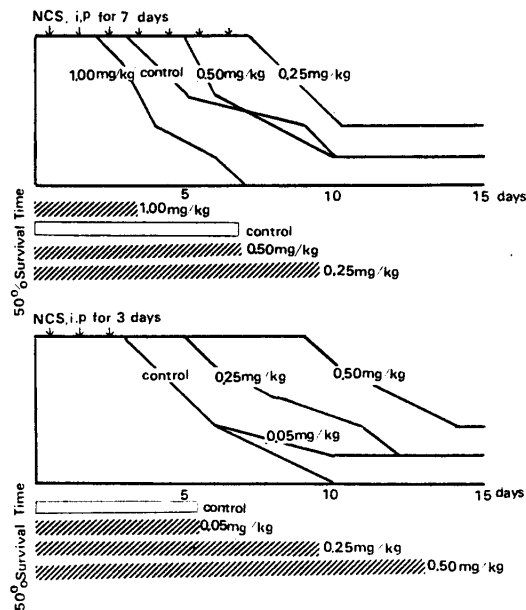


Fig. 8. Effect of Neocarzinostatin on survival time in Rauscher leukemic mice

survival times were longer than in the control. Particularly, the 50% survival time of Rauscher leukemic mice treated with 0.50mg/kg/day of NCS was over twice that of the control. From these results, it can be said that NCS has also an influence on Rauscher leukemic mice. However, the question whether or not NCS acts on RLV remains to be solved. Further studies of antitumor effects of NCS on Rauscher leukemic mice are necessary.

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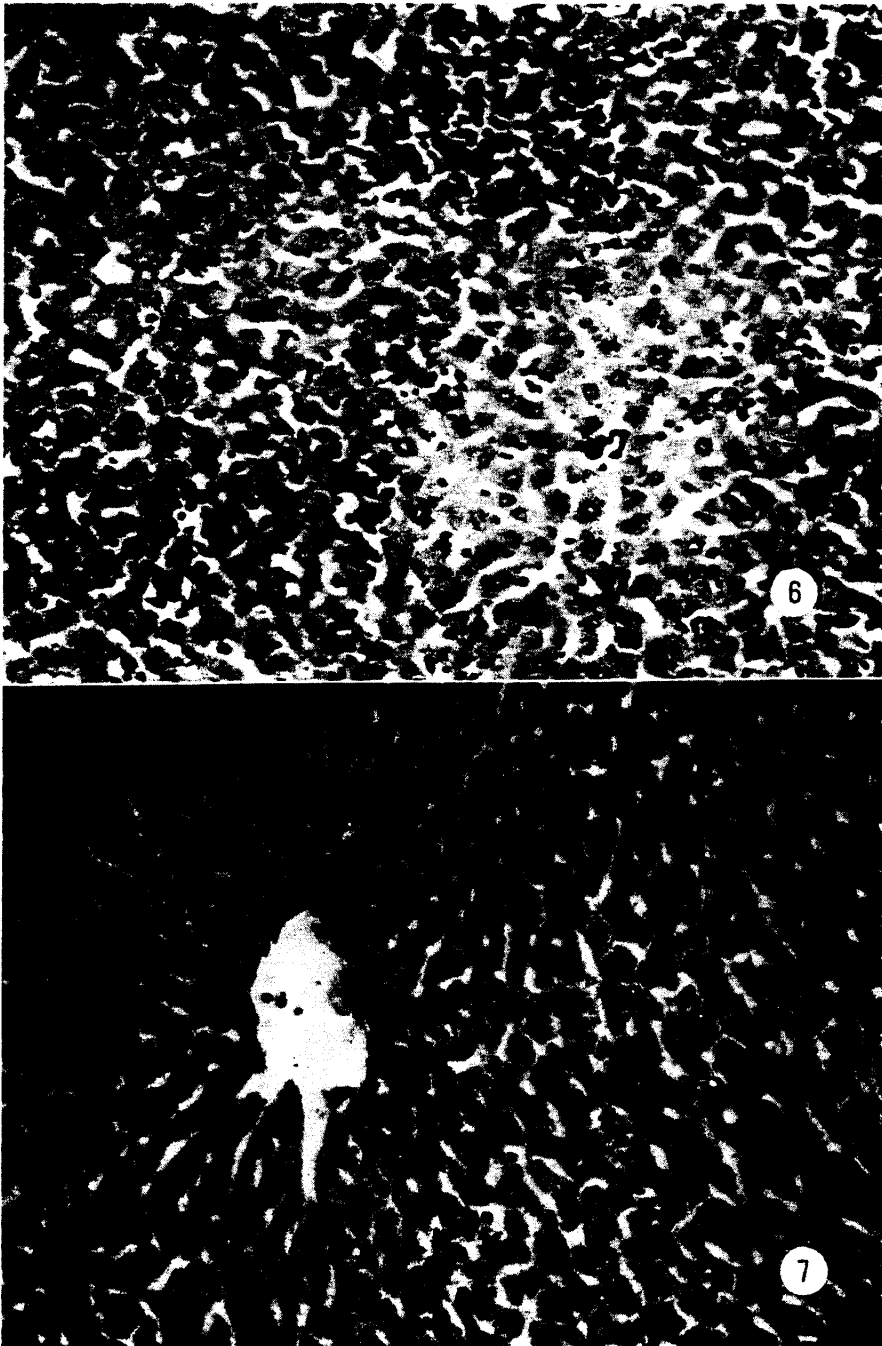


Fig. 6. Liver of Rauscher leukemic mouse

Ffg. 7. Liver of Rauscher leukemic mouse treated with 0.50mg/kg/day of Neocarcinostatin for 3 days (i. p)