ANTI-LEUKEMIC ACTIVITY OF 15-DEOXYSPERGUALIN AGAINST *N*-BUTYL-*N*-NITROSOUREA-INDUCED AUTOCHTHONOUS RAT LEUKEMIA

JUNPEI ITO, TAKUMI YAMASHITA, KATSUTOSHI TAKAHASHI, HIROO HORINISHI, TERUYA NAKAMURA†, TOMIO TAKEUCHI†† and HAMAO UMEZAWA††

Research Laboratories, Pharmaceuticals Group, Nippon Kayaku Co., Ltd., 3-31-12 Shimo, Kita-ku, Tokyo 115, Japan
†Central Research Laboratory, Takara Shuzo Co., Ltd., 3-4-1 Seta, Ohtsu-shi, Shiga-ken 520-21, Japan
††Institute of Microbial Chemistry, 3-14-23 Kamiosaki, Shinagawa-ku, Tokyo 141, Japan

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Spergualin is a new anticancer antibiotic isolated from culture filtrates of *Bacillus laterosporus* BMG162-aF2.[1] After determination of chemical structure[2] and the total synthesis,[3] a number of analogs have been synthesized and evaluated.[4,5] Among these analogs, 15-deoxyspergualin(DSG) had the strongest anticancer activity against mouse L1210 and P388 leukemias.[6,7] In this paper, we report on its anti-leukemic activity against *N*-butyl-*N*-nitrosourea(BNU)-induced autochthonous leukemia of rats, which seems to be most relevant to human leukemia compared with other experimental transplantable leukemias.

Female 11 week-old Donryu rats were purchased from Nippon Rat Co. (Tokyo, Japan). BNU was purchased from Nakarai Chemicals (Kyoto, Japan). DSG was prepared by Takara Shuzo Co., Ltd. (Kyoto, Japan). Rat leukemia was induced by BNU according to ODASHIMA *et al.*[8] Briefly, rats were raised with drinking water containing 0.04% BNU for 125 days from 11 weeks of age, because it had already been shown that the 125 days administration of BNU were sufficient to induce leukemia with an incidence of 100%.[8] The rats were hematologically examined on the 107th and 113th day after the initial BNU administration. All surviving rats, in which anemia, atypical cell and a number of nucleated cells were observed in peripheral blood, were randomly allocated to two groups; a DSG administration group and a control group. To 15 rats in the DSG group, DSG was administered intraperitoneally from the next day after the completion of BNU administration. The administration of DSG was scheduled as 4 mg/kg/day once daily for 5 consecutive days per week for 11 weeks. However, due to the appearance of body weight loss and inanition, the dose was reduced to 2 mg/kg/day from the 5th week and the administration was omitted at the 4th and 9th week (see Fig. 1). The 20 rats in the control group were administered saline ip. During the observation period of 110 days, moribund-sacrificed or dead rats were subjected to macroscopic and microscopic examinations. The major organs were fixed in 10% buffered formalin solution, embedded in paraffin, sectioned and stained with hematoxylin and eosin. For the histological classification of leukemias, peripheral blood smears and stamps of the bone marrow, spleen, lymph node and liver were stained with May-Gruenwald Giemsa and periodic acid Shiff. The smears and stamps were also stained for peroxidase, alkaline phosphatase and nonspecific esterase activities.

Fig. 1 shows the survival pattern of rats in the DSG administration and control groups. In the control group, the rats died in the early part of study and the survival had decreased to 15% at the end of observation period. In the DSG group, the rats did not die in the early period but they died from about 40 days after the initial administration and the survival had decreased to 13% at the end of the observation period. Median survival days in the DSG and control groups were 62.5 and 35.5, respectively, and the T/C(%) was 176.

The anti-leukemic activity of DSG was further examined histopathologically. Fatal lesions of all moribund-sacrificed or dead rats in both groups were classified in five groups; erythroleukemia, lymphoblastic leukemia, thymic lymphoma, squamous cell carcinoma and infectious pneumonitis. The survival time and the fatal lesion of each rat are shown in Fig. 2. Two and three rats survived during the observation period in the DSG and control groups, respectively. The surviving two rats in the DSG group had no leukemia on other malignancies, while the two surviving rats in the control group had myelocytic leukemia and the other had squamous cell

Fig. 1. Survival pattern of rats in DSG and control groups after initiation of the DSG treatment.

Statistical analysis was performed by the generalized WILCOXON.[15] The difference in survival rate between both groups was not significant at the end of observation period but was significant up to 55 and 60 days with $P<0.01$ and $P<0.05$, respectively.

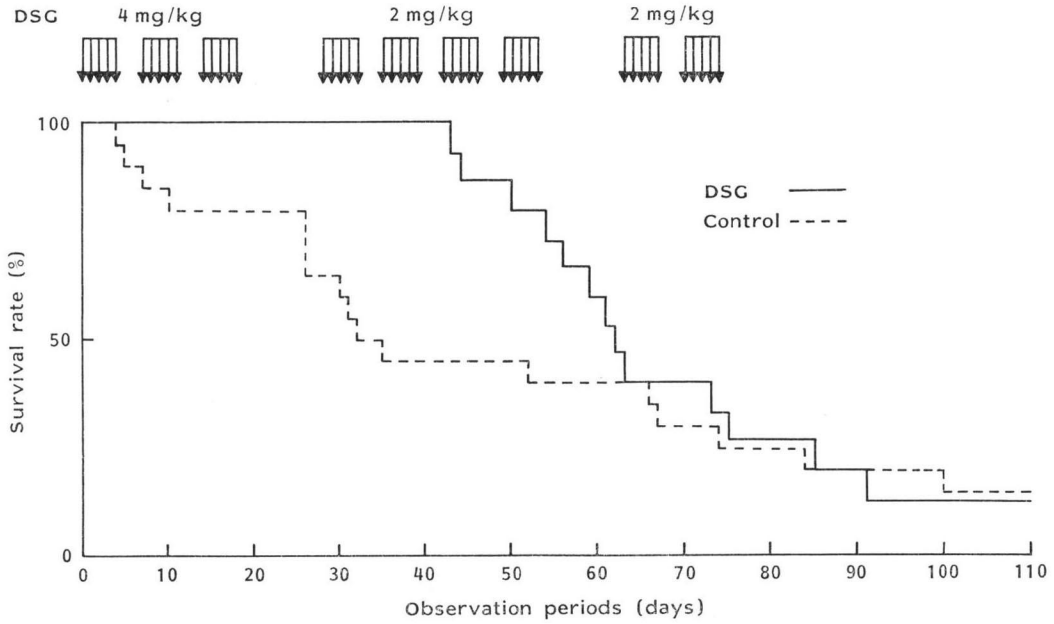
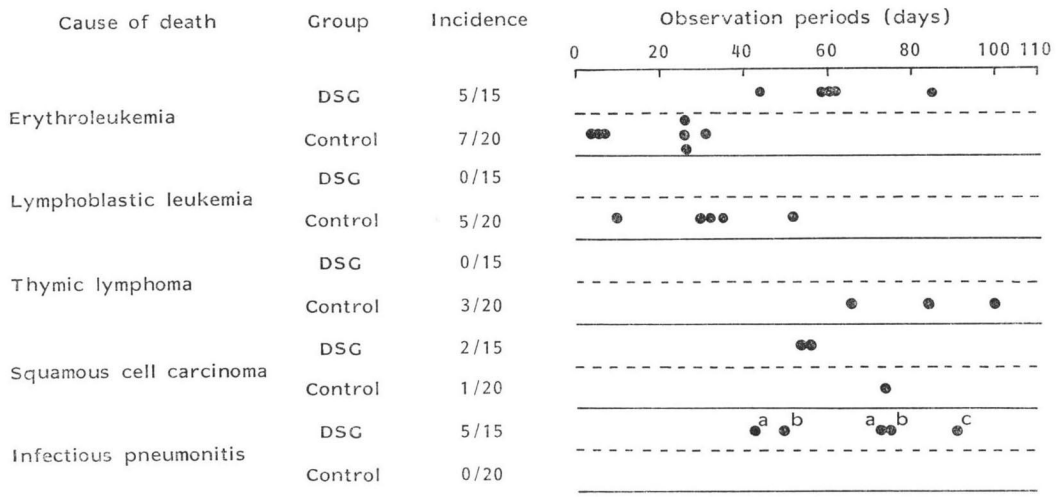


Fig. 2. Survival time of individual rat and its cause of death in DSG and control groups.

In each group, there was one rat with strong post-mortem changes and the histopathological diagnosis was not obtained. Therefore, they were omitted from the figure. Day of death or moribund-sacrifice of each rat is showed with the symbol: (●). The rats, which died of infectious pneumonitis, had a slight lesion of erythroleukemia (a), myelocytic leukemia (b) and lymphoblastic leukemia (c).



carcinoma. The survival time of rats with erythroleukemia was prolonged by DSG markedly, although the incidence of death was almost the same in both groups. In the DSG group, no rat died of lymphoblastic leukemia and thymic lymphoma, while five and three rats in the control group died of these leukemia and lymphoma, respectively. Five rats in the DSG group died

of infectious pneumonitis with bone marrow hypoplasia, while death by infectious pneumonitis was not observed in the control group. Among these five rats, two rats had erythroleukemia, two had myelocytic leukemia and one had lymphoblastic leukemia, but the severity and extent of leukemic lesions were minimal, and were only observed by microscopy.

Autochthonous rat leukemia induced by the nitrosourea is a model of human leukemia,[9] and has been used for the evaluation of anti-leukemic drugs with high clinical predictability.[10,11] As reported recently,[12~14] the anti-leukemic drugs should be selected properly because the drug sensitivity is found to be different in each type of human leukemias. It has been known that several types of leukemia can be induced in rats by BNU.[8,9] Therefore, we studied the activity of DSG against various types of leukemia induced by BNU. This was also examined histopathologically.

DSG exhibited inhibitory activity against several types of leukemia induced by BNU such as erythroleukemia and lymphatic leukemia. The effect against myelocytic leukemia was not clear because of the low incidence. Among DSG responsive leukemias, lymphatic leukemia seemed to be most sensitive to DSG, because no animal treated with DSG died of this leukemia. The myelo-suppressive effect of DSG was observed at the present dosis. The pneumonitic death in the DSG group may be due to this myelo-suppression. This death by myelo-suppression may be controlled by a supplement therapy using antibacterial antibiotics. From the high clinical predictability of this model, DSG is expected to show the efficacy against human leukemia, especially against lymphatic leukemia, although the optimal treatment schedule to avoid the adverse action remains to be further studied.

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