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Antineoplastic Natural Products and the Analogues V.

Antitumor Activity of Skullcapflavon II.

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Abstract ☐ The effect of skullcapflavon II, 5,2′-dihydroxy-6, 7, 8, 6′-tetramethoxyflavone, on the growth of transplantable L1210 and sarcoma 180 tumors in mice was studied. Intraperitoneal treatment of skullcapflavon II caused a significant(T/C=166%) and a moderate (T/C=122%) prolongations of the life spans of ICR and BDF_1 mice respectively, which had been intraperitoneally inoculated with sarcoma 180 and L1210 cells. Peritumoral injection of skullcapflavon II on the solid form of sarcoma 180 in mice inhibited the tumor growth strongly (Inhibition rate=71%).

Keyword ☐ Skullcapflavon II, Antineoplastic, Sarcoma 180, L1210.

In studies aimed at the developemnt of possible antitumor agents from Korean traditional medicines, the extract of Whanggum, the dried roots of *Scutellaria baicalensis*, was found to inhibit the growth of mouse leukemia L1210 cell[1]. It also showed antitumor activity on sarcoma 180 in mice[2]. The cytotoxic principle against L1210 cell was isolated and identified as 5,2′-dihydroxy-6, 7,8,6′-tetramethoxyflavone[3], which had been known as skullcapflavon II[4]. The activity was confirmed by the total synthesis of skullcapflavon II[5].

To investigate the antitumor activity of skullcapflavon II *in vivo*, antitumor tests on L1210 and sarcoma 180 in mice were carried out.

EXPERIMENTAL METHODS

Animals and Tumors

Experimental mice, BDF_1, DBA/2(male), C57 BL/6(female), and ICR, were purchased from Seoul National University. These mice were 6~7 weeks old and weighed 20~25g. They were housed in plastic cages in air conditioned room and supplied with foods and water without limitation. The cell line of L1210 was a gift from Perman's laboratory, University of Wisconsin, Madison, USA. Sarcoma 180 was obtained from the Microbiology laboratory of Seoul National University, Seoul, Korea. L1210 cells were maintained in the peritoneal cavities of BDF_1 mice by transplantation per 5 days. Sarcoma 180 cells were maintained in the peritoneal cavities of ICR mice by weekly transplantation. The tumor cells were harvested from the peritoneal cavities and adjusted to the required concentrations in sterile physiological saline. The numbor of the tumor cells were counted with a hemocytometer(American Optical, Buffalo, N.Y.).

Materials

Skullcapflavon II was synthesized by the methods described previously[5]. It was dissolved in 50% PEG 400 in saline for the injection into mice.

Antitumor Tests in Animals

L1210 system: L1210 cells harvested were suspended in saline (1×10^6 cells/ml) and inoculated into the peritoneal cavities of BDF_1 mice (0.1 ml/mouse). The mortality of mice was recorded as days after the tumor inoculation. 0.1 ml of skullcapflavon II in 50% PEG 400 was administered intraperitoneally once a day for 9 days. The survival rate was calculated by means of the following formula.

$$\text{Survival rate (T/C, \%)} = \frac{\text{mean survival days of treated mice}}{\text{mean survival days of control mice}} \times 100$$

which was referred to NCI protocol[6].

Sarcoma 180 system: For ascitic forms of the tumor, 1×10^6 of sarcoma cells were inoculated intraperitoneally per ICR mouse and the test substance was administered intraperitoneally once a day for 7 days. The mortality of mice was recorded for 60 days and the survival rate was calculated by the above formula. For solid forms of the tumor, tumor cells were inoculated at the left groin of mouse, and then the test substance was administered from 1st to 7th day peritumorally. The mice were killed and the tumor weight was assessed 28 days after the tumor inoculation. The inhibition rate for tumor growth was calculated by means of the following formula[7],

$$\text{Inhibition rate (\%)} = \left(1-\frac{\text{mean tumor weight of the treated mice}}{\text{mean tumor weight of the control mice}}\right) \times 100$$

RESULTS

Effect of Skullcapflavon II on the Life Span of BDF_1 Mice Inoculated with L1210 Cells

BDF_1 mice were inoculated with L1210 cells. The leukemia bearing mice were treated with skullcapflavon II and its effect on the life span was observed. As shown in Table I, treatment with 40mg skullcapflavon II per kg of animal weight showed a survival rate of 122% which is very close to the NCI standard of 125% over which a drug can be considered as a promising antitumor agent.

Effect of Skullcapflavon II on the Life Span of ICR Mice Bearing Sarcoma 180 Cells

Skullcapflavon II was tried to treat an ascitic form of tumor instead of the leukemia type. ICR mice were inoculated with sarcoma 180 cells and administered daily with skullcapflavon II from the day after the inoculation until 7th day. The mortality of the mice was recorded for 60 days and the results were summarized in Table II. The optimum dose of skullcapflavon II was 40mg/kg and the survival rate was 166%. Seven out of 24 mice tested could survive longer than 60 days.

Effect of Skullcapflavon II on the Growth of Solid form of Sarcoma 180

Th ICR mice were inoculated with sarcoma 180 cells at the left groin and skullcapflavon II was injected around the inoculation point daily for 7 days. The mice were killed 28 days after the inoculation, the tumors were cut off and the weights were measured. As shown in Table III, the daily injection of skullcapflavon II at the doses of 40 to 60 mg/kg inhibited the growth of tumors by 70%. Furthermore, among the 16 mice tested, six showed complete regressions at the 40 mg/kg dose.

DISCUSSION

Skullcapflavon II showed the significant antitumor activity on both the ascitic and solid forms of sarcoma 180 (Table II and III). Intraperitoneal administration of skullcapflavon II for the solid

forms of sarcoma 180 showed no regression, while direct injection around the transplantation point resulted in the inhibition of tumor growth. These observation led to the suggestion that this cytotoxicity can be attained only through direct contact of the substance with the tumor cells, but not through a systemical route. It might be due to the rapid metabolism of the flavonoids in the animal organs[8].

Skullcapflavon II has a moderate effect on L1210 inoculated BDF_1 mice (Table I). It showed T/C value of 122 % at 40 mg/kg dose which is a comparable value to 125 % of NCI for considering as active agent. In their analysis of antutumor flavonoids, Edwards et al[9] found that no flavone was active against L1210 and only one synthetic flavone, 3, 6-dichloro-2′, 4′, 6′-trimethoxyflavone, was effective on sarcoma 180 among the 140 flavones tested. It is noteworthy that skullcapflavon II, as a naturally occurring flavone, has a considerable antitumor effect on sarcoma 180 and l1210.

Table I. Effect of the intraperitoneal administration of skullcapflavon II on the life span of BDF_1 mice bearing L1210 cells.

Dose (mg/kg)	Mean survival days	30day survivors	T/C (%)
0	12.0(10~15)[a]	0/16	100
20	14.1(11~20)	0/16	116
40	14.6(10~24)	0/16	122
90	10.4 (9~13)	0/16	87

[a] Range

Table II. Effect of the intraperitoneal administration of skullcapflavon II on the ascitic form of sarcoma 180.

Dose (mg/kg)	Mean survival days	60 day survivors	T/C (%)
0	21.3(14~38)[a]	0/36(0)[b]	100
1	23.2(18~51)	0/30(0)	109
10	24.6(15~49)	2/26(8)	115
20	28.3(20~27)	3/24(13)	113
40	35.4(17~34)	7/24(29)	166
60	30.9(20~40)	3/24(13)	145
90	29.6(22~35)	3/24(13)	139

[a] Range for the dead mice
[b] %

Table III. Effect of the peritumoral administration of skullcapflavon II on the solid forms of sarcoma 180.

Dose (mg/kg)	Tumor weight (g, mean±S.D)	Complete regression	Inhibition rate(%)
0	6.96±2.39	0/17(0)[a]	0
1	5.68±1.74	0/16(0)	18
10	3.16±1.94	3/17(18)	55
20	2.65±2.10	4/16(25)	58
40	1.99±2.15	6/16(38)	71
60	2.06±2.04	4/16(25)	70

[a] %

Considering the use of the roots of *S. baicalensis* in the various prescriptions for the treatment of influenzas, fever and hypertension in the practice of the traditional medicines in Korea for hundreds of years, it seemed that the skullcapflavon II, the isolated flavone from the root, might have low toxicity on the host at an effective dose. Furthermore, the fact that the flavonoids in effective doses generally have little deleterious effects, rather a number of beneficial physiological effects on animals[10], provides a basis for further study with skullcapflavon II and other flavonoids for their antitumor actions.

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