Anti-tumor Effects of Orally Administered Soft-Shelled Turtle Powder in Mice

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The effects of oral administration of cryomilled powder of the soft-shelled turtle (SST powder) on solid and ascites tumor-bearing C3H/He mice were studied. The SST powder was administered orally at a dose of 100 or 500 mg/kg.d for two or four weeks, and then MH134 hepatoma cells were inoculated; oral administration was continued after inoculation. Treatment with the SST powder attenuated the increase in diameter and weight of the tumor and prolonged the survival of mice with solid tumors. However, the same treatment did not show any significant effect in mice with ascites tumor, indicating that the SST powder has no direct killing action on tumor cells. These results suggest that oral administration of the SST powder decreases the growth of solid tumors possibly by activating the host immune system.

Key words soft-shelled turtle; anti-cancer effect; chronic effect

Soft-shelled turtle (SST) has been traditionally and clinically considered as an effective crude drug to treat tumors in China. There are many different prescriptions available and numerous accounts describing the tumor-suppressive effects of soft-shell turtle. 1) However, there have been few pharmacological studies substantiating these effects and the mechanism of the anti-tumor activity of soft-shelled turtle and its pharmacological effectiveness still require proper confirmation.

In previous papers we have reported the anti-hypertensive and anti-hepatitic effects of chronic oral administration of soft-shelled turtle in the rat. 2) In the present study, the anti-tumor effects of the SST powder against solid and ascites tumors were investigated in mice.

MATERIALS AND METHODS

Animals and the SST Powder  Commercially available 4-week-old male C3H/He mice were purchased. After 1 week of acclimatization, the mice were subjected to the experiments. The cryomilled SST powder (Iwatani International Co., Tokyo, Japan) was suspended in saline containing 0.5% carboxymethyl cellulose sodium (CMC-saline). The suspension for oral administration was freshly prepared each day.

Solid Tumor Experiments  The mice were divided into six groups consisting of 10 mice per group. The first group was taken as a control and received oral CMC-saline daily. The SST powder suspension, at a dose of 100 or 500 mg/kg, p.o., was administered daily to groups 2 and 3 for 4 weeks and groups 4 and 5 for 2 weeks. In the solid tumor model, these four mouse groups were then injected endemically in their abdomen with 1 × 10^5 MH134 hepatoma cells per animal. Administration of CMC-saline or SST powder suspension was continued after inoculation until the end of the experiment. The sixth group mice were injected with mitomycin C (MMC) at a dose of 30 mg/mouse three times every other day starting from the day after inoculation. A micrometer was used to measure the maximum (d max) and minimum diameter (d min) of each tumor every three days, the square root of d min × d max, an index of the size of tumor, was calculated. On the 35 day, the mice were killed and the tumors dissected and weighed.

Ascites Tumor Experiments  The same tumor cells were injected into the abdominal cavity at a ratio of 0.5 × 10^7 cells/mouse to produce the ascites tumor. As an index of the growth and increase in tumor cells, the weight of the mice was measured and the survival period recorded. Administration of the SST powder and Mitomycin C and division into six groups were the same as in the solid tumor experiment.

RESULTS

Effects on Solid Tumor  As shown in Fig. 1, after the tumor cells were inoculated, the tumor diameter of control mice increased daily. The tumor diameter in the group injected with Mitomycin C was significantly reduced after the 18th day. The tumor diameter of the groups which received 100 or 500 mg/kg of SST powder daily was smaller than that of the control group. A statistically significant difference compared with the control group was detected in the group which received 500 mg/kg for 4 weeks before inoculation. In the case of the 500 mg/kg group, the longer the duration of treatment, the smaller the diameter. However, the decrease in tumor diameter produced by prophylactic treatment of 100 mg/kg for 4 weeks was no greater than for 2 weeks.

Table 1 shows the tumor weight 30 d after inoculation. Treatment with the SST powder tended to reduce the tumor weight, but a statistically significant difference was detected only in the group receiving 500 mg/kg for 4 weeks. Mitomycin C showed strong anti-tumor effects, and both the diameter and weight of the tumor were significantly reduced.

Effects on Ascites Tumor  After the tumor cells were inoculated, the weight of the mice in the control group continued to increase daily, while in the group injected with Mitomycin C, the increase was significantly attenuated (Table 2). The survival period in the control group was 16.9 ± 0.77 d, but Mitomycin C prolonged

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survival to $31.5 \pm 2.54$ d. However, the significant decrease in weight was not seen in groups receiving the SST powder. In addition, survival was not significantly prolonged by treatment with SST powder.

**DISCUSSION**

We have previously shown that semi-chronic administration of SST powder attenuates the increase in blood pressure of hypertensive animals (Feng et al., 1990) and has a beneficial effect on carbon tetrachloride-induced liver injury (Feng et al., 1991). The effects were manifested very slowly and treatment for several weeks was necessary. In the present study, we found another action of SST powder, namely its anti-tumor effect. However, this does not mean that the anti-tumor effect is different from the previously reported effect on liver injury. Restoration of cellular function is also important in the anti-tumor effect. Traditionally, SST has been used for the treatment of various types of tumor in China. The present experiments clearly showed for the first time that SST powder has an antitumor effect.

The experimental method involving ascites tumor is mainly used for investigation of the anti-tumor effects of agents which can kill tumor cells directly, while solid tumor is used to detect the effect on the host immune system. The present results show that administration of SST powder is not effective on ascites tumor, but works on solid tumor. This suggests that the suppressive effect of SST powder on the solid tumor does not involve killing the tumor cells directly. Improvement of the host immune system is one of the possible mechanisms of such an indirect anti-tumor effect. Further experiments to elucidate the effective component(s) in the SST powder are necessary.

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**REFERENCES**