The effects of functional foods, such as Agaricus, *Pleurotus cornucopiae* (Paulet) Rolland var. *citrinopileatus* (Sing.) Ohira., *Hericium erinaceum*, Arabinoxylan, Shark cartilage and Shark extracted lipid on growth of implanted Mouse LM8 Dunn osteosarcoma.

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Abstract

For the test groups, feed with supplement was started three weeks before LM8 highly lung metastasizing cell line from mouse osteosarcoma was implanted into dorsal subcutaneous tissue and the mice were kept for six to seven weeks following the tumor injection On the other hand, the control group was given feed without the supplement. In experiment 1, Agaricus, *Pieurotus cornucopiae* (Paulet) Rolland var. *citrinopileatus* (Sing.) Ohira., *Hericium erinaceum* and Arabinoxylan did not show obvious effects on the growth of the implanted tumor. In experiment 2, Shark cartilage and Shark extracted lipid showed inhibition of growth in both the implanted and metastasized tumor. The effect of Shark extracted lipid was slightly stronger than that of Shark cartilage. In experiment 3, Shark extracted lipid decreased the number of blood vessels significantly. In this experiment, Shark extracted lipid showed a higher inhibition of tumor growth than experiment 2.

Thus, Shark extracted lipid as a functional food which has anti-tumor activity is worth further investigation.

Introduction

Recently attention has been focused on alternative medicine for cancer treatment. It is able to combine alternative medicine and other treatments. Therefore the number of the users has increased dramatically. Functional foods with biological activity have been studied. Particularly functional foods such as Agaricus and Shark cartilage are remarkable.

However these functional foods are not used actively in clinical practice as the effect of them is not clear. Recently the use of functional foods for animal tumors has increased and the effects have been reported. However there are not many studies which have proved their effectiveness scientifically. Therefore proving the various effects and mechanisms of action of functional foods on tumors is an important issue in animal medicine.

In this study the inhibiting effect of functional foods on the growth of the implanted tumors was evaluated. In experiment.1, Agaricus, Pleurotus cornucopiae var. citrinopileatus, Hericium erinaceum, and Arabinoxylan which are appeared to have the ability of immune

activation were tested. In experiment.2, Shark cartilage and Shark extracted lipid which have an anti-angiogenic activity were tested. In experiment.3, Shark extracted lipid which has already shown its efficacy was evaluated for anti-angiogenic activity.

Materials and Methods

(Skipped)

Results

Experiment.1 (n=6)

Agaricus, *Pieurotus cornucopiae* (Paulet) Rolland var. *citrinopileatus* (Sing.) Ohira. , *Hericium erinaceum* and Arabinoxylan did not show a significant effect on the growth of the implanted tumors.

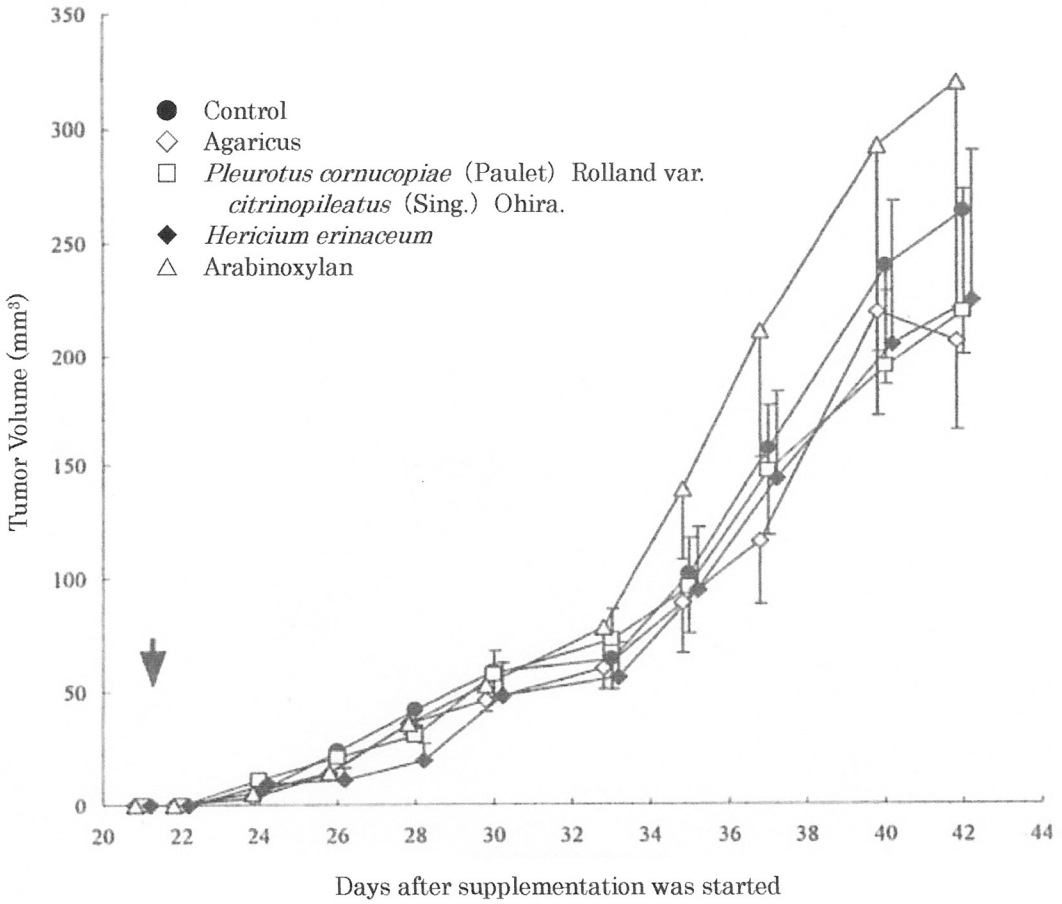

Figure.1 Change in the volume of tumor

- ↓ : Tumor cells (1.0×10^6) were injected after 21 days after supplementation was started.
- Tumor volume was measured every a few days after tumor cell injection.
- Mean±S.E. (n=6)

Expeliment.2 (n=10)

1) The implanted tumor volume of the Shark cartilage group and Shark extracted lipid group was smaller than that of the control group, however, there was no significant difference among the three groups. (Figure.2)

2) The percentage of tumor area of lung metastasis of the Shark cartilage group and Shark extracted lipid group were lower than that of the control group, however, there was no significant difference among the three groups. (Table.2)

Positive lung metastases were seen histologically in every mouse of the control group. On the other hand, the Shark cartilage group and Shark extracted lipid group had two mice and three mice which did not have any metastasis respectively.

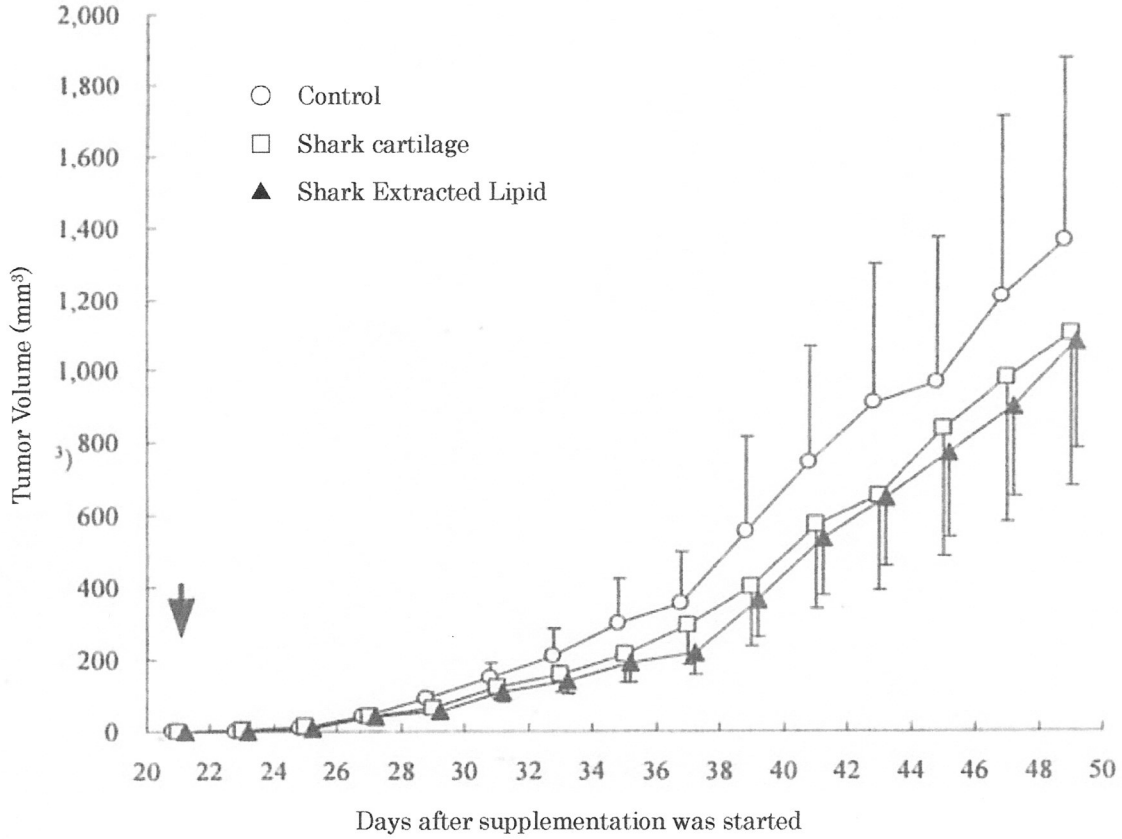

Figure.2 Change in the volume of tumor

・Mean±S.E. (n=10)

Table.2 The percentage of tumor area of lung metastasis (%)

	The percentage of tumor area of lung metastasis (%)
Control	34.9±5.5
Shark Cartilage	31.6±7.8
Shark extracted lipid	30.3±7.6

・The percentage of tumor area of lung metastasis was assessed by image analysis software.

・The percentage of tumor area of lung metastasis (%)

= Total area of lung metastasis (mm^2) / Total area of lung tissue (mm^2) × 100

・Mean±S.E. (n=10)

Expeiment.3 (n=7)

1) The implanted tumor volume of the Shark extracted lipid group was smaller than that of the control group, however, there was no significant difference between the two groups. (Figure.3)

2) The percentage of tumor area of lung metastasis of the Shark extracted lipid group was lower than that of the control group, however, there was no significant difference between the two groups. (Table.3)

The control group and Shark extracted lipid group had one mouse and two mice which did not have any histological lung metastasis respectively.

3) The number of blood vessels in the tumor of the Shark extracted lipid was less than that of the control group and there was a significant difference between those two groups.

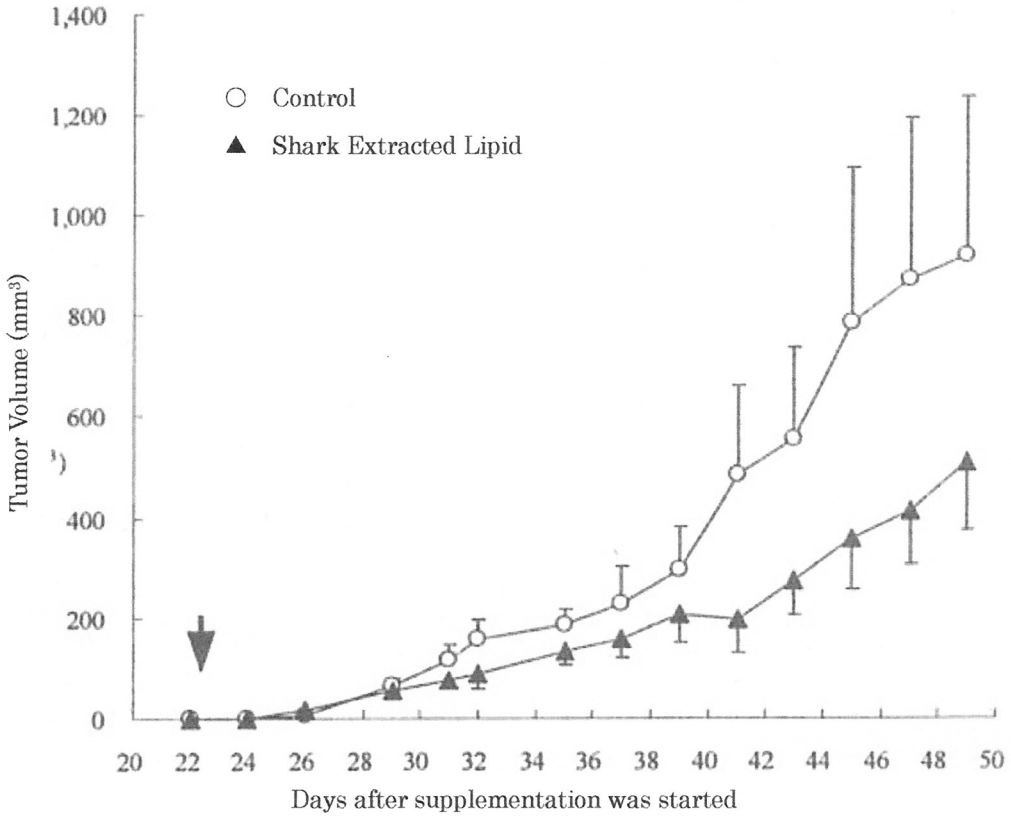

Figure.3 Change in the volume of tumor

・Mean±S.E. (Control : n=6, Shark extracted lipid : n=7)

Table.3 The percentage of tumor area of lung metastasis (%)

	The percentage of tumor area of lung metastasis (%)
Control	32.2±12.3
Shark extracted lipid	17.3±4.5

・Mean±S.E. (Control : n=6, Shark extracted lipid : n=7)

Table.4 The number of blood vessels in the implanted tumor

	The number of blood vessels in the implanted tumor
Control	87.5±4.4
Shark extracted lipid	55.4±8.6*

・The tissue specimen was assessed by immunostaining using antibodies to FactorⅧ which is a vascular endothelial cell specific antigen.

・The tissues which the tumor was observed in were selected randomly from non-necrotic tissue at 100-fold magnification and the number of blood vessels was totaled in ten tumor tissue sections selected randomly at 400-fold magnification.

・The same operations were performed 3 times and the mean value was calculated.

・Mean±S.E. (Control : n=6, Shark extracted lipid : n=7)

・$p<0.05$ Significantly difference (* : $p<0.05$)

Discussion

In experiment 1, there were two mice in the control group whose tumor grew once and then disappeared, and the mean value of tumor volume was lower. As a result, the growth of the tumors in the control group was inhibited. The implanted tumor volume and weight in the Arabinoxylan group were clearly larger than in the other groups as the implanted tumor of one mouse in the Arabinoxylan group grew remarkably. However the reason was unknown. Consequently, it was not concluded that the growth of implanted tumor of Agaricus, *Pleurotus cornucopiae* (Paulet) Rolland var. *citrinopileatus* (Sing.) Ohira. or *Hericium erinaceum* was stimulated .

In experiment 2, Shark cartilage and Shark extracted lipid showed an inhibiting effect on the growth of the implanted tumor. Shark extracted lipid showed an obviously stronger inhibiting effect on the growth of the implanted and metastasized tumors in experiment 3 than that in experiment 2. It resulted from Shark cartilage and Shark extracted lipid having anti-angiogenic activity. As a result, an inhibiting effects on the growth of implanted and lung metastasized tumors was observed in the Shark cartilage group and Shark extracted lipid group. (Figure.2, Figure.3, Table.2, Table.3) The number of blood vessels in the implanted tumors in the Shark extracted lipid group was significantly less than that of the control group. (Figure.4) Therefore it appears that the Shark extracted lipid group showed a stronger

inhibiting effect on the growth of implanted and lung metastasized tumors than control the group. (Figure.3, Table.2, Table.3)

There is a possibility that Shark cartilage and Shark extracted lipid inhibit the growth of a tumor at the stage when the number of tumor cell in a mouse is small.

This was able to be evaluated by the histological finding of lung metastasis in experiment 3. The growth of an implanted or metastasized tumor was inhibited in the initial stage by anti-angiogenesis activity and the effect of the immune activation of Shark cartilage and Shark extracted lipid. There were fewer mice with lung metastasis in the groups which were fed Shark supplements. It appeared that the number of tumor cells running to the lung from the implanted tumor through blood vessels in the groups which were fed Shark supplements was lower than that of the control group due to the effect of anti-angiogenesis. It seems that this effect of Shark extracted lipid was stronger than that of Shark cartilage.

Each supplement has not been regarded as a medicine though, they are foods with some functionality. In this study, there were two conflicting factors; one was that the effect of supplements is not strong and does not appear immediately, and the other was that the cell line of the tumor implanted was of a high grade tumor. Therefore the obvious inhibiting effect on the growth of the tumor was not able to be shown statistically. This should have been considered in the suitability of the design of this experiment especially with mushrooms as the implanted tumor was a high grade tumor. LM8 Dunn highly lung metastasizing cell line from osteosarcoma was an isograft to the C3H/HeN mouse and there is a possibility that the effect of immune activation with regarded to the real effect of mushrooms on tumors was not observed.

In this study, in order to avoid these problems, the amount of supplement was increased to 5% of total feed weight and the period of supplementation was extended further.

It has shown that functional foods have a dose-dependent inhibiting effect on the growth of tumors. On the other hand, it has been reported that functional foods had no side effects even though the percentage of the functional food of total feed weight was 30%. The mice received feed ad lib, therefore, it was necessary to mix more supplement with feed than the quantity required per day.

They took all the feed in this feeding method but the consumption of each mouse was unknown. It appeared that there was no difference of consumption among mice from the change of weight of each mouse although this was not shown in the result. As far as the period of supplementation is concerned, it has been shown that the case in which supplementation is started before tumor injection has stronger inhibiting effect on the growth of an implanted tumor than the case in which supplementation is started after tumor injection. By being aware of this, the feeding with supplement was started three weeks before tumor injection. However there was no obvious effect of this.

In conclusion, Shark extracted lipid has been shown to have an inhibiting effect on the growth of implanted and lung metastasized tumors and an anti-angiogenesis activity even

though the tumor cell line was that of a high grade tumor.
Therefore Shark extracted lipid appears to have an efficacy as a functional food.