J. Appl. Glycosci., **52**, 367−368 (2005)

Note

Antitumor Effect of Trehalose on Sarcoma 180 in ICR Mice

(Received March 4, 2005 ; Accept June 8, 2005)

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Abstract: An antitumor activity on oral administration of trehalose was investigated by using ICR mice implanted Sarcoma 180. After implanting of Sarcoma 180, five kinds of saline solutions containing trehalose (10 to 250 mg/kg) were administrated to mice daily for 10 days. The antitumor effect of trehalose was evaluated by measuring tumor sizes for 3 weeks and tumor weights on week 3. Approximately 70% inhibition ratio was observed in the tested groups admistered a dose higher than dose of 25 mg/kg.

Key word: trehalose, antitumor activity, Sarcoma 180

Trehalose, a non-reducing disaccharide of α-D-glucopyranosyl α-D-glucopyranoside, distributes widely in bacteria, yeasts, fungi, mushrooms, plants and invertebrates.[1)] The saccharide was mainly extracted from yeast, but a new production system of trehalose has been established recently from starch with enzymatic methods.[2)] An abundant and cheap supply of trehalose became possible to use widely in the food industry. Although many interesting biological functions such as stabilization of proteins,[3)] degradation of unsaturated fatty acid by boiling,[4)] antioxidative activity,[5)] protective efficacy of osteoporosis,[6)] and inhibition of inducing TNFα[7)] have been reported, there is no report about antitumor activity of trehalose.

We found significantly high antitumor activity of crude polysaccharide extracted from a newly cultivated mushroom, Hatakeshimeji (*Lyophyllum decastes* Sing.) on intraperitoneal administration and a (1→3)-β-D-glucan-type polysaccharide showing strong antitumor activity was isolated from crude polysaccharide fractionated hot-water-extract of the edible mushroom.[8)] We found that the extract also showed respectable effect in oral administration.[9)] In this paper, the antitumor activity of trehalose was investigated by oral administration in order to make clear an occurrence of a low-molecular-weight material showing antitumor activity because it was reported that Hatakeshimeji contained considerable trehalose.[10)]

Trehalose was obtained from Hayashibara Co., Ltd (Okayama, Japan). Five-week-old female ICR mice were purchased from Japan SLC Inc. (Shizuoka, Japan). The mice were housed in an animal room with controlled temperature (25±1°C), relative humidity (55±5%) and artificial lighting (8:00−20:00) and fed an experimental food (CE-2pellet diet, Clea, Osaka, Japan). Water was supplied *ad libitum*. The experimental plan was in accordance with the Guidelines for Animal Experimentation of Suzuka University of Medical Science. Sarcoma 180 was initially supplied by the National Cancer Center Research Institute (Tokyo, Japan) in 1969. The established tumor was maintained in mice by weekly intraperitoneal injection (5×10^6 cells), seven-day-old tumor suspension (2×10^6 viable cells) of Sarcoma180 was implanted subcutaneously into the right groin of each mouse.

The mice were implanted subcutaneously with 4×10^5 tumor cells. Starting at 24 h after tumor cell implantation, saline solutions (0.2 mL) containing trehalose corresponding to 0, 10, 25, 50, 100 and 250 mg/kg were administered by using a stomach sonde. The oral administration was conducted daily for 10 days. For each sample, 7 mice were tested and fed for 3 weeks. Tumor size was measured twice a week with a calliper and the tumor volume was calculated using the formula: $4/3\pi a^2b/2$, where a is the smallest and b is the largest diameter (mm).[11)] The antitumor inhibition ratio was calculated using the formula $(1-T/C)\times100(\%)$, where T is the average tumor weight of the treated mice and C is that of the control mice. All values are presented as the mean±SD. The significance of differences was determined by ANOVA using Duncan's multiple-comparison procedure. Probabilities of less than 5% ($p<0.05$) were considered significant.

As shown in Fig. 1, it seemed that efficacy was seen in all groups tested according to the order of dosage amount, however, the tumor size of the dose group of 10 mg/kg increased considerably after the end of oral administration. It could be evaluated that the four groups tested with a higher dose than 25 mg/kg maintained an antitumor effect during the test period. From data of tumor weights on week 3 shown in Table 1, it was found that the four groups with dosages of 25, 50, 100 and 250 mg/kg showed around 70% inhibition ratio, suggesting that trehalose has an ability of antitumor activity on oral administration. Although antitumor activity of trehalose on intraperitoneal administration was also conducted and effective data was obtained, the degree of effectiveness was not so different from that of oral administration. It was re-

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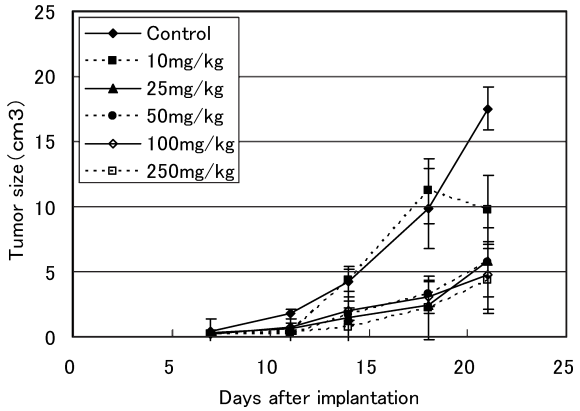

Fig. 1. Changes of tumor size of mice administrated trehalose orally against sarcoma 180.

The mice were implanted subcutaneously with 4×10^5 tumor cells. A saline solution (0.2 mL) containing trehalose (10–250 mg/kg) for antitumor activity analysis was administered orally to the mice implanted with tumor cells 24 h previously and dosage was administered daily for 10 days. Each value is the mean±SD of 7 mice per group. Tumor size was measured twice a week with a caliper and the tumor volume was calculated.

Table 1. Antitumor activity of trehalose administrated orally against sarcoma 180.

Dose (mg/kg)	Tumor weight (g)	Inhibition ratio (%)
Control	10.9 ± 2.3^a	—
10	5.71 ± 3.93^b	47.7
25	3.19 ± 4.51^b	70.8
50	3.31 ± 3.90^b	69.7
100	2.94 ± 4.64^b	73.1
250	3.41 ± 3.54^b	68.7

The mice were implanted subcutaneously with 4×10^5 tumor cells. A saline solution (0.2 mL) containing trehalose (10–250 mg/kg) for antitumor activity analysis was administered orally to mice implanted with tumor cells 24 h previously and dosage was administered daily for 10 days. Each value is the mean±SD of 7 mice per group. Superscript letters a and b are assigned when the mean values differ from each other significantly at $p<0.05$.

ported that oral administration of trehalose in mice caused the total number of the Peyer's patches lymphocytes (PPL), which are essential for mucosal immune responses, of the small intestine.[12] Moreover, *in vitro* cytokine production, such as interleukin-6 or interferon-γ, from PPL was influenced by the administration of trehalose. These results suggested the possibility that trehalose ingestion might modify the intestinal immune environment. The possible modification of the intestinal immune environment might be related to the antitumor effect of trehalose. The trehalose content was 34.1 g /100 g dry matter in Hatakeshimeji.[10] The inhibition ratio of crude polysaccharide (10 mg/kg) from Hatakeshimeji was 89.2% on intraperitoneal administration.[13] The edible mushroom of Hatakeshimeji contains both polysaccharide and trehalose and an investigation of the cooperative biological effects against cancer will be conducted in the future.

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トレハロースの ICR マウス Sarcoma180 固形ガンに対する抗腫瘍活性

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ICR マウス Sarcoma180 固形ガンに対するトレハロースの抗腫瘍活性について研究を行った．Sarcoma180 を接種したマウスに 5 種類の濃度のトレハロースを 10 日間投与した．トレハロースの抗腫瘍活性は 3 週間までの腫瘍サイズ，ならびに 3 週間後の腫瘍重量を測定した．25 mg/kg 以上の投与量のグループでおよそ 70% の抗腫瘍活性が認められた．