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Suppression of Hypertriglyceridemia of Ehrlich Carcinoma-bearing Mice by an Antibiotic, Ascofuranone[1]

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ABSTRACT

Ehrlich ascites carcinoma-bearing mice exhibit hypertriglyceridemia. An antitumor antibiotic, ascofuranone, suppressed tumor-induced hypertriglyceridemia when administered i.p. even when no evident antitumor activity was observed without affecting the levels of free fatty acids, phospholipids, cholesterol, glucose, and total protein in plasma. Ascofuranone did not reduce plasma triglycerides of normal mice. Insulin and clofibrate, known modifiers of lipid metabolism, showed no significant suppression. Ascofuranone is also effective on solid tumor-induced hypertriglyceridemia. Another notable change of metabolism affected by tumor-bearing in the early stage where hypertriglyceridemia has not yet fully progressed is hypoglycemia. Although ascofuranone did not affect hypoglycemia, the suppressive effect on hypertriglyceridemia was more evident when ascofuranone was administered in the early stage than in the later stage. These results suggest that ascofuranone suppresses hypertriglyceridemia by specifically affecting the changes of host metabolism which is induced in the early stage of tumor bearing.

INTRODUCTION

Abnormalities of host metabolism in tumor-bearing animals have been studied in various experimental models (1–3). However, the mechanism of cancer cachexia development in humans and experimental animals is not fully understood. It is reported that Ehrlich ascites carcinoma-bearing mice exhibit hypertriglyceridemia in the course of tumor development (4, 5). Although fasting causes a marked reduction of plasma triglycerides in tumor-bearing mice, depletion of dietary fat never overcomes the hypertriglyceridemia, suggesting that triglycerides are provided endogenously. However, the hepatic secretion rate of triglycerides does not increase prior to or during the development of hypertriglyceridemia in tumorous mice and it is not significantly different from those of control mice (6).

Ascofuranone is isolated from a phytopathogenic fungus, *Ascochyta viciae*, as a hypolipidemic substance having prenylphenolic structure (7). When administered p.o., it reduces the serum lipid level of experimental animals (8, 9). It also modulates lipid metabolism of leukemia L5178Y cells *in vitro* and alters the membrane properties (10).

Recently, we found an antitumor effect of the antibiotic on several experimental tumors, including Ehrlich's carcinoma, Sarcoma 180, and L1210 (11). A characteristic of the antitumor activity is that it is effective with pretreatment as well as posttreatment, suggesting that the antitumor activity is host mediated. Since ascofuranone modulates lipid metabolism, it is interesting to study the relationship between the antitumor activity and modulation of lipid metabolism.

In our present work, we investigated the effect of ascofuranone on tumor-induced hypertriglyceridemia and found that it suppressed the hypertriglyceridemia. We expect ascofuranone to be a useful tool for investigating the mechanism of development of tumor-induced cachexia.

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MATERIALS AND METHODS

Mice. Six-week-old male ddY mice were purchased from Shizuoka Experimental Farm (Hamamatsu, Japan). Commercial pellet diet (CE-2; Clea, Japan, Ltd., Tokyo, Japan) and tap water were fed *ad libitum*.

Chemicals. Purified ascofuranone was supplied by Chugai Pharmaceutical Co. (Tokyo, Japan). For administration, ascofuranone was suspended in 0.5% Tween 80 dissolved in phosphate buffered saline (0.8% NaCl, pH 7.4) with the aid of a Teflon homogenizer. Insulin and clofibrate were purchased from Sigma Chemical Co. (St. Louis, MO) and also were suspended in the same vehicle.

Development of Tumors. Ehrlich carcinoma cells, freshly isolated from the peritoneal cavity, were suspended in phosphate buffered saline and the cell number was adjusted just before implantation. The cell suspension was injected i.p. or s.c. for the formation of ascites or solid tumor, respectively.

Estimation of Plasma Components. Blood was removed from the heart of a mouse, anesthetized with ether, using a heparinized syringe. After a 1-h incubation at room temperature, plasma was obtained by centrifugation. Plasma lipids were determined by the following methods: triglycerides by the Van Handel method (12); phospholipids by the Zilversmit method (13); free fatty acids by the Itaya-Ui method (14); and total cholesterol by the Zurkowski method (15). Plasma glucose and total protein were determined by a blood analyzing system (Chugai Pharmaceutical Co.) based upon an enzymatic method and the biuret reaction, respectively.

Statistics. The results are represented as the mean ± SE. Statistical significance between groups was calculated using Student's *t* test.

RESULTS

Plasma triglycerides of Ehrlich ascites carcinoma-bearing mice were elevated 10-fold higher, compared with normal mice, 8 days after tumor implantation. Ascofuranone, when administered i.p. for 5 consecutive days starting 24 h after tumor implantation, suppressed hypertriglyceridemia in a dose-dependent manner. Suppression by 200 or 400 mg/kg was statistically significant. Although a high dose of ascofuranone also suppressed body weight gain of cancerous animals which resulted from tumor growth (Table 1), it did not prolong the survival time of tumor bearing animals when 4 million tumor cells were implanted. As reported previously, the antitumor activity of ascofuranone is dependent on the number of tumor cells implanted (10), and it shows no significant antitumor activity when more than 1 million tumor cells are implanted. The triglyceride concentration of tumor extracellular fluid was less than that of normal mice. Ascofuranone also reduced the triglyceride levels of tumor extracellular fluid and tumor cells (data not shown).

Tumor-induced elevation of lipid levels was specific for triglycerides while other major lipids in plasma, namely, free fatty acids, phospholipids, and cholesterol were all reduced compared with normal mice (Table 2). Glucose and total protein in the plasma were also reduced. The effect of ascofuranone (200 mg/kg) was also specific for triglycerides and it had only a negligible

Table 1 *Dose-dependent suppression of hypertriglyceridemia by ascofuranone*

Four million tumor cells were implanted in the peritoneal cavity of ddY mice (8 mice/group). Mice were sacrificed 8 days later and triglycerides in the plasma were determined. Ascofuranone was administered i.p. once a day for 5 consecutive days, starting 24 h after tumor implantation.

Ascofuranone[a] (mg/kg)	Body wt (g)	Triglycerides (mg/dl)	P[a]
0	40.0 ± 1.0[b]	401.1 ± 73.8	
100	38.0 ± 1.0	258.2 ± 53.2 (64)[c]	Not significant
200	36.7 ± 0.7	185.1 ± 26.2 (46)	<0.02
400	31.3 ± 1.1	99.1 ± 18.3 (25)	<0.002
Normal mice	30.0 ± 0.5	47.7 ± 3.5 (12)	<0.001

[a] *P* compared to tumor-bearing control group.
[b] Mean ± SE.
[c] Numbers in parentheses, percentage of tumor-bearing control.

Table 2 *Effect of ascofuranone on plasma components*

Four million tumor cells were implanted in the peritoneal cavity (8 mice/group). Ascofuranone (200 mg/kg) was administered i.p. once a day for 5 consecutive days starting 24 h after tumor implantation. Eight days after tumor implantation, concentrations of indicated components in plasma were determined.

	Plasma concentration (mg/dl)		
	Tumor-bearing control	Ascofuranone treated	Normal
Glucose	177 ± 25[a]	245 ± 27 (138)[b]	248 ± 24 (140)
Total protein	48.4 ± 1.4	51.4 ± 2.2 (106)	69.4 ± 2.2[c] (143)
Free fatty acids	17.7 ± 2.2	16.7 ± 2.6 (94)	23.9 ± 3.0 (135)
Phospholipids	5.93 ± 0.67	5.48 ± 0.47 (92)	7.41 ± 0.42 (125)
Cholesterol	28.1 ± 3.7	24.2 ± 3.0 (86)	45.5 ± 3.5[c] (162)

[a] Mean ± SE.
[b] Numbers in parentheses, percentage of tumor-bearing control.
[c] Statistically significant compared to tumor-bearing control ($P < 0.01$).

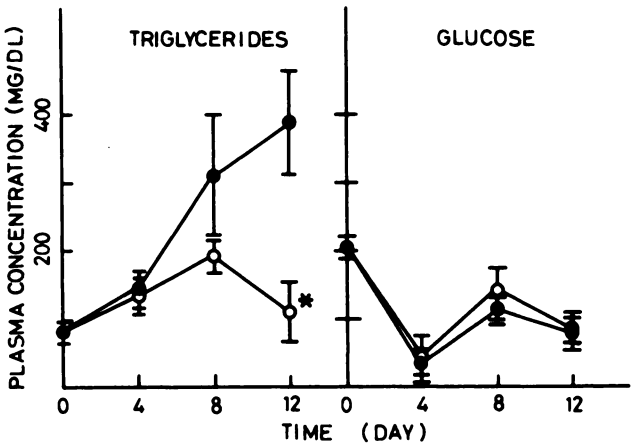

Fig. 1. Time course study of the effect of ascofuranone on tumor-induced hypertriglyceridemia. Four million tumor cells were implanted in the peritoneal cavity and ascofuranone (400 mg/kg) was administered i.p. once a day for 5 consecutive days starting 24 h after tumor implantation. Plasma triglycerides and glucose were determined 4, 8, and 12 days after the implantation. ●, tumor-bearing control mice; ○, ascofuranone-treated mice. *Bars*, SE based upon 8 mice. *, statistically significant compared to tumor-bearing control ($P < 0.05$).

effect on the changes of other plasma components. Although glucose was reduced by ascofuranone, the effect was not statistically significant. In fact, another experiment (Fig. 1) showed that ascofuranone did not affect tumor-induced hypoglycemia.

Since only triglycerides of plasma lipids were elevated in tumor-bearing mice and the effect of ascofuranone is specific for triglycerides, it is likely that ascofuranone specifically modulates the lipid metabolism which is changed in tumor-bearing mice. This prompted us to examine the effect of ascofuranone on the triglycerides of normal mice and we found no significant reduction. On the contrary, a high dose of ascofuranone (400 mg/kg) elevated triglycerides significantly (Table 3). Ascofuranone also suppressed solid tumor-induced hypertriglyceridemia

Table 3 *Effect of ascofuranone on plasma triglycerides of normal mice*

Ascofuranone was administered i.p. once a day for 5 consecutive days (8 mice/group) and plasma triglycerides were determined 4 days after the last administration.

Ascofuranone (mg/kg)	Body wt (g)	Triglycerides (mg/dl)	P[a]
0	31.0 ± 0.5[b]	69.7 ± 2.6	
100	31.4 ± 0.6	79.7 ± 9.6 (114)[c]	Not significant
200	29.9 ± 0.5	73.4 ± 3.4 (105)	Not significant
400	28.9 ± 1.0	102.8 ± 10.6 (147)	<0.02

[a] *P* compared to control (0 mg/kg).
[b] Mean ± SE.
[c] Numbers in parentheses, percentage of control.

Table 4 *Suppression of solid tumor-induced hypertriglyceridemia by ascofuranone*

Four million tumor cells were implanted s.c. and ascofuranone was administered i.p. once a day for 5 consecutive days starting 24 h after tumor implantation (10 mice/group). Plasma triglycerides were determined 15 days after tumor implantation.

Ascofuranone (mg/kg)	Triglycerides (mg/dl)	P[a]
0	170.0 ± 22.1[b]	
100	111.0 ± 11.4 (65)[c]	<0.05
200	116.0 ± 15.8 (68)	Not significant
400	139.0 ± 14.2 (82)	Not significant
Normal mice	94.3 ± 15.1 (55)	<0.05

[a] *P* compared to tumor-bearing control (0 mg/kg).
[b] Mean ± SE.
[c] Numbers in parentheses, percentage of tumor-bearing control.

Table 5 *Effect of clofibrate and insulin on tumor-induced hypertriglyceridemia*

In experiment 1, 4 million tumor cells were implanted in the peritoneal cavity and clofibrate (200 mg/kg) was administered i.p. once a day for 5 consecutive days starting 24 h after tumor implantation (5 mice/group). Plasma triglycerides were determined 8 days after the implantation. In experiment 2, 10 million tumor cells were implanted in the peritoneal cavity and insulin (5 units/kg) was administered once a day for 4 consecutive days after tumor implantation (5 mice/group). Plasma triglycerides were determined 9 days after the implantation.

Experiment	Group	Triglycerides (mg/dl)	P[a]
1	Tumor bearing		
	Control	72.6 ± 13.3[b]	
	Clofibrate	173.4 ± 43.3 (238)[c]	Not significant
	Normal	51.9 ± 6.7 (71)	Not significant
2	Tumor bearing		
	Control	279.0 ± 30.4	
	Insulin	193.0 ± 32.5 (69)	Not significant
	Normal	62.0 ± 3.5 (22)	<0.001

[a] *P* compared to tumor-bearing control.
[b] Mean ± SE.
[c] Numbers in parentheses, percentage of tumor-bearing control.

significantly (Table 4). Body weight of solid tumor-bearing mice was not significantly changed when compared with normal mice. Administration of 200 or 400 mg/kg of ascofuranone reduced body weight significantly, without affecting tumor weight (data not shown). These results suggest that hypertriglyceridemia of ascites tumor-bearing mice was not produced by the secretion of ascites fluid or direct destruction of organs in the peritoneal cavity, and that ascofuranone did function on the changes of lipid metabolism induced by tumors.

Insulin and clofibrate are known modulators of lipid metabolism. However, neither of them showed any statistically significant effect on tumor-induced hypertriglyceridemia (Table 5), although insulin and clofibrate had a tendency to suppress and enhance hypertriglyceridemia, respectively. Compared with Table 1, tumor-bearing nontreated mice in Experiment 1 did not show the usual degree of hypertriglyceridemia, presumably because of the difference in the batches of mice between the two experiments.

The level of plasma triglycerides in tumor-bearing mice in-

Table 6 *Dose schedule-dependence of suppression of tumor-induced hypertriglyceridemia by ascofuranone*

Ten million tumor cells were implanted in the peritoneal cavity on day 0, and ascofuranone (200 mg/kg) was administered i.p. once a day on the indicated days (5 mice/group). Plasma triglycerides were determined on day 9. The difference between ascofuranone-treated groups was also statistically significant ($P < 0.005$).

Group	Triglycerides (mg/dl)	Tumor wt[a] (g)	P[b]
Tumor-bearing control	223.0 ± 35.7[c]	1.71 ± 0.11	
Ascofuranone treated			
Days 1–4	77.3 ± 13.9 (35)[d]	1.94 ± 0.23 (113)	<0.005
Days 5–8	175.3 ± 35.2 (79)	1.69 ± 0.27 (99)	Not significant
Normal mice	82.6 ± 8.2 (37)		<0.005

[a] Wet weight of cells in the peritoneal cavity packed by centrifugation.
[b] *P* of triglycerides compared to tumor-bearing control.
[c] Mean ± SE.
[d] Numbers in parentheses, percentage of tumor-bearing control.

creased as the tumor cells grew and a significant elevation was observed 8 or 12 days after tumor implantation, whereas only a slight increase was observed 4 days after the implantation. The suppression of hypertriglyceridemia by ascofuranone was evident 12 days after tumor implantation although it was administered only during the early stage of tumor bearing, when hypertriglyceridemia has not yet fully progressed (Fig. 1). This suggests that ascofuranone modifies the change of metabolism which is induced in an early stage of tumor bearing and triggers the hypertriglyceridemia in the later stage. It should be noted that plasma glucose was drastically reduced in the early stage and was improved in the later stage of tumor growth (Fig. 1). Ascofuranone showed no effect on hypoglycemia.

The results of Fig. 1 were further confirmed in Table 6, in which the suppressive effect of early stage administration and that of later stage administration were compared. Although both schedules suppressed hypertriglyceridemia, early stage administration gave complete and significant suppression, whereas later stage administration gave only partial suppression and it was not statistically significant. Administration of ascofuranone by either schedule did not affect tumor growth, when determined by tumor wet weight. These results strongly suggest that ascofuranone suppresses hypertriglyceridemia indirectly by influencing the effect of the tumor on the host's metabolism at early stages of tumor growth, rather than by directly influencing the rates of triglyceride secretion or utilization, the two processes that determine plasma triglyceride levels.

DISCUSSION

Our present results show that ascofuranone suppressed hypertriglyceridemia of Ehrlich carcinoma-bearing mice almost completely. Since reduction of plasma trigycerides was not observed when ascofuranone was administered to normal mice, it can be assumed that ascofuranone specifically modifies the metabolic change induced by tumors. The suppression was not observed when insulin and clofibrate, known modulators of lipid metabolism, were administered. In addition, the effect of ascofuranone was evident when administered in the early stage of tumor bearing, suggesting that the antibiotic modifies the metabolic change induced in the early stage of tumor bearing.

However, several other explanations for the mechanism of suppression may be possible. First, administration of ascofuranone might suppress tumor growth itself and the resultant retardation of tumor development reduces the elevation of triglycerides. The antitumor effect of ascofuranone is dependent on the inoculum size of the tumors (11). Ascofuranone completely suppressed tumor-induced hypertriglyceridemia when 4 or 10 million tumor cells were implanted, the condition wherein ascofuranone never prolongs the survival time of tumor-bearing mice. When 10 million tumor cells were implanted, ascofuranone (200 mg/kg), which significantly suppressed hypertriglyceridemia did not reduce the tumor packed volume. In addition, ascofuranone reversed the change of plasma triglycerides without affecting changes of other plasma lipids, glucose, and total protein, indicating that tumor cells in mice treated with ascofuranone grow to the same extent as those in non-treated mice. These observations imply that the first explanation is hardly plausible.

The second possible explanation is anorexia. Fasting for 24 h reduces serum triglycerides of tumor-bearing mice to a level less than those of normal mice (4, 5). Administration of ascofuranone suppressed body weight gain, implying that ascofuranone-treated mice exhibited anorexia. However, fasting also decreases serum triglycerides of normal mice, whereas ascofuranone rather increased them. In addition, fasting also reduces the triglyceride level which had already increased, whereas ascofuranone showed no significant effect on the increased triglyceride level in the late stage of tumor bearing. These facts suggest that anorexia is not plausible as the mechanism of suppression.

AS-6, an antibiotic which has a prenylphenolic structure like ascofuranone, is active in both obese hyperinsulinemic (16) and insulin-deficient diabetic mice (17), when given p.o. and suppresses hypertriglyceridemia induced by diabetes. In the obese diabetic mouse, C57BL/KsJ ab/ab, insulin cannot improve the syndrome (16). The adipocytes of genetically obese diabetic animals increase insulin-binding capacity by the administration of AS-6 and respond to insulin. As a result, the uptake of 2-deoxyglucose is stimulated (20). Furthermore, the combined treatment with insulin and AS-6 synergistically decreases serum glucose (16). Thus, the proposed action mechanism of AS-6 is the restoration of the sensitivity of organs to insulin. The plasma glucose of tumor-bearing mice drastically decreased preceding hypertriglyceridemia and reversed in the course of tumor development. Kannan *et al.* (6) reported that hypertriglyceridemia is also spontaneously reversed. These observations imply that critical changes of metabolism, including hormonal regulation, may take place to keep the metabolic homeostasis. Therefore, it is possible that ascofuranone prevents the progress of hypertriglyceridemia by modulating hormonal regulation which is changed in the early stage of tumor bearing.

Our results also indicate that reduction of plasma triglycerides to the normal level by itself has no influence on the survival time of the host, because ascofuranone suppressed hypertriglyceridemia in the condition when it did not prolong the survival time of tumor-bearing mice. Thus, ascofuranone probably presents the host-mediated antitumor property by a mechanism other than the suppression of hypertriglyceridemia. We found that ascofuranone increases splenic natural cytotoxicity *in vivo* (18) and that it activates macrophages *in vitro* (19). Therefore, in the case of antitumor activity by pretreatment, activation of the host immune response is a plausible mechanism. Modulation of lipid metabolism probably has some role in the activation of the immune system. In fact, ascofuranone reduces splenic triglycerides concomitantly with the activation of splenic natural cytotoxicity (18). Thus, restoration of normal lipid metabolism by ascofuranone might contribute in some way to the antitumor activity as well as suppression of hypertriglyceridemia.

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