HYPOLIPIDEMIC PROPERTY OF ASCOFURANONE

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Ascofuranone significantly reduced serum lipid levels of rats fed with normal diet 6 hours after a single oral administration of 108 mg/kg. When the antibiotic was orally given for consecutive 10 days to normolipidemic rats, the treatment resulted in marked reduction of serum cholesterol, triglycerides, phospholipids and free fatty acids without affecting organ weight gain, serum total protein, albumin/globulin ratio and serum transaminases. Reduction was also noted with cardiac cholesterol content but liver total sterol and fecal sterol excretion were unchanged. Acute toxicity of ascofuranone is weak to mice and rats and the antibiotic did not induce hepatomegaly which is the main side effect of a positive control agent, ethyl-*p*-chlorophenoxyisobutyrate.

Hyperlipidemia as well as high blood pressure and aging is causative of coronary heart disease in man[1]. Recently, ethyl-*p*-chlorophenoxyisobutyrate (Clofibrate; Clf.) succeeded in preventing incidence of coronary heart disease due to pharmacological lipid lowering property[2,3]. However, the agent is still unsatisfactory in its side effect and activity. In our search for a hypolipidemic agent from microorganisms, ascofuranone (Af) has been isolated from the filter cake of the fermented broth of a fungus *Ascochyta viciae*[4]. This paper deals with hypolipidemic property of Af in normolipidemic rats.

Materials and Methods

Af used in this experiment was pure crystalline preparation produced in the pilot plant fermentors of Chugai Pharmaceutical Co., Ltd. Gum arabic suspension was prepared by glass homogenizer and used for administration, since Af is insoluble in water. Clf was selected as a positive control agent because of the world-wide clinical application against atherosclerosis.

Male swiss albino mice strain *ddY*, 5 weeks old, and male rats strain Wistar, weighing 250±30 g were used in this study. The animals were housed in an air-conditioned room at 24°C and 60% relative humidity under 12-hour photocycle. Commercial pellet diet (Nihon Clea Co., grade CE-2) and water were given *ad libitum*. The treated rats as well as the untreated controls were sacrificed by anesthetizing with chloroform 6 hours after the last administration and a blood was removed from the heart.

Rapid blood analyzer system (RaBA, a product of Chugai Pharmaceutical Co., Ltd.) was used for estimation of serum total cholesterol (s-TC), serum triglycerides (s-TG), serum total protein, serum albumin, serum glutamate-oxaloacetate transaminase (s-GOT) and glutamate-pyruvate transaminase (s-GPT). The following assay methods were used; s-TC was determined by *o*-phthalaldehyde method[5], ZACK method[6], FRIED method[7] and Choleskit reagent[8]; s-TG, by VAN HANDEL method[9]; serum phospholipids by ZILVERSMIT-YOSHIDA method[10]; free fatty acids by ITAYA-UI method[11] and serum globulin by GOLDENBERG method[12].

Determination of heart and liver cholesterol: The heart and liver were carefully dissected out and weighed. After rinsing with RINGER solution, they were homogenized in a blender with phosphate buffer (pH 7.0). The lipid was extracted with chloroform-methanol (2: 1)[13]. Cholesterol was precipitated

from the extract as digitonide and determined by RaBA system.

Results and Discussion

Acute toxicity of Af is weak to mice and rats (Table 1). No death was observed by an oral administration of 7 g/kg and the tolerated animals showed normal body weight gain without any sign of toxicity. The LD_{50}s by intraperitoneal route are also high for both animals. Excess administration caused severe diarrhea and the animals died due to gastrointestinal disorder. No other toxic symptoms were found except diarrhea. This low toxicity is noteworthy when compared with a positive control agent, Clf.

Table 1. Acute toxicity of ascofuranone

	LD_{50} (mg/kg)			
	mouse		rat	
	Oral	Intraperitoneal	Oral	Intraperitoneal
Ascofuranone	>3,700 (male) >4,000 (female)	2,220 (1,776~2,775) (male) 2,250 (1,923~2,633) (female)	>5,000 (male)	1,350 (male)
Ethyl *p*-chlorophenoxyisobutyrate	1,700		2,300	

Table 2. Serum cholesterol levels of the rats by a single oral administration of ascofuranone

	Dose (mg/kg)	Serum total cholesterol (mg/dl)				
		RaBA method mean±SE	OPA method mean±SE	ZACK's method mean±SE	FRIED's method mean±SE	Choleskit method mean±SE
Ascofuranone	108	54.2±0.2 (−17.4%)	73.7±2.7 (−18.5%)	50.3±3.3 (− 6.7%)	55 ±2.5 (−24.7%)	41.4±0.8 (−13.2%)
Ethyl *p*-chlorophenoxyisobutyrate	109	58.8±3.8 (−10.3%)	79.7±4.4 (−11.1%)	49.1±3.7 (− 8.1%)	57 ±2.3 (−21.9%)	44.5±3.4 (− 6.7%)
Untreated control	—	65.6±2.6	89.0±6.2	53.9±3.0	73 ±9.0	47.9±3.0

Figures in parenthesis are s-TC reduction rate; 100-(s-TC of the treated/s-TC of the untreated ×100) (%). Five rats were used in each dose.

Serum lipid lowering by a single oral administration: Clf has been reported to exert rapid serum lipid lowering activity[14], the lowest peak of which is 6~8 hours after a single oral administration. The s-TC as well as s-TG was estimated by several methods to select an adequate method, because there have been a number of papers that dealt with serum lipid estimation, but there have been few that can estimate serum lipid levels with accuracy, simplicity and reproducibility. Significant s-TC reduction was noted in the Af-treated rats with four methods out of five except that ZACK's method gave a poor reduction rate (Table 2). The positive control agent, Clf, also exerted significant s-TC reduction with three methods out of five. These data indicated that figures obtained by each method represent only relative concentrations of s-TC but not absolute ones. RaBA system was adopted thereafter because of its simplicity and accuracy.

Serum triglycerides were also determined by two methods using the same serum as in Table 2.

Table 3. Serum triglyceride levels of the treated rats

Agents	Dose (mg/kg)	Serum triglyceride (mg/dl)			
		VAN HANDEL method		Turbidimetric method	
		mean±SE	% change	mean±SE	% change
Ascofuranone	108	39±3.7	−35.0	43.8±3.0	−48.7
Ethyl *p*-chlorophenoxyisobutyrate	109	40±3.4	−33.3	76.8±10.9	− 3.4
Untreated control	—	60±2.9	—	79.5±1.5	—

% change = − (1 − s-TG of the treated/s-TG of the untreated) × 100 (%)

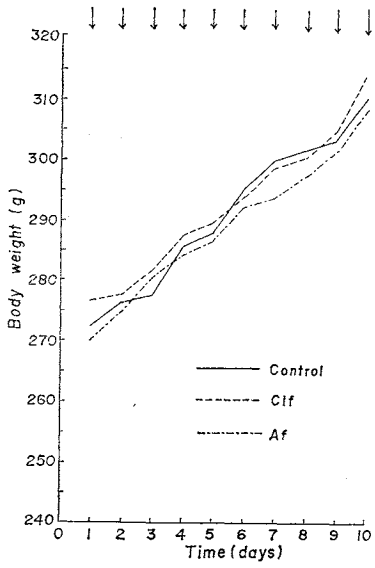
Table 4. Reduction of serum phospholipids and serum free fatty acids by a single oral administration of ascofuranone

Agents	Dose (mg/kg)	Serum phospholipids (mg/dl)		Serum free fatty acids (μeq/dl)	
		mean±SE	% change	mean±SE	% change
Ascofuranone	108	91±3.0	−11.9	44±3.5	−13.8
Ethyl *p*-chlorophenoxyisobutyrate	109	88±10.0	−14.3	55±4.6	+ 7.8
Untreated control	—	105±2.5	—	51±2.9	—

Table 3 shows the results. Marked reduction was observed in the Af-treated rats with both methods but Clf showed an insignificant reduction rate with the turbidimetric method. The latter is based on estimation of serum turbidity which is believed to represent s-TG concentration. However, the data presented here indicated that the serum turbidity does not always reflect s-TG concentration. Therefore, the former was adapted for s-TG determination.

Serum phospholipids (s-PL) and free fatty acids (s-FFA) reduction on a single oral administration are listed in Table 4. Af lowered both lipids but Clf was ineffective on the s-FFA level.

Fig. 1. Body weight gain during the treatment.



GRESHAM claimed that the rat strain Wistar is inadequate for the study of atherosclerosis[15]. In fact, as can be seen in Table 2, s-TC level is as low as one half that of mice and one fourth that of man. Therefore, to detect a small change in s-TC concentration using the rat, it is necessary to completely prevent hemolysis and rapidly separated blood cell from sera. We were able to attain with the use of a plastic disposable apparatus, such as a syringe and test tube.

The data presented above are so promising that the experiment was carried out to establish hypolipidemic effect of Af on long term treatment. Af and Clf were orally given to the rats once a day for 10 consecutive days and body weight gains were compared with that of the untreated control (Fig. 1). Af slightly retarded body weight gain. Body weight gain is not universal index of

Table 5. Effect of consecutive 10-day treatment by ascofuranone on serum lipid levels

Agents	Dose (mg/rat/day)	Serum total cholesterol (mg/dl)		Serum triglycerides (mg/dl)		Serum phospholipids (mg/dl)		Serum free fatty acids (μeq/dl)	
		mean±SE	% change	mean±SE	% change	mean±SE	% change	mean±SE	% change
Asofuranone	20	56.4±3.5	−16.5	24.5±3.1	−44.3	104±9.1	−33.8	72.6±3.1	−20.2
Ethyl *p*-chlorophenoxyisobutyrate	30	51.4±0.9	−23.9	22.7±3.5	−48.4	92±4.1	−40.8	68.9±7.5	−24.3
Untreated control	—	67.6±3.0	—	44.0±3.6	—	158±18.2	—	91.0±9.6	—

Ten rats were used in each group.

Table 6. Effect of consecutive 10-day treatment by ascofuranone on serum proteins and serum transaminases.

Agents	Dose (mg/rat/day)	Total serum protein (g/dl) mean±SE	Serum albumin (g/dl) mean±SE	A/G ratio	Serum γ-globulin (g/dl) mean±SE	s-GOT Karmen unit mean±SE	s-GPT Karmen unit mean±SE
Ascofuranone	20	6.32±0.12	2.36±0.17	0.60	2.82±0.13	164±12	38±2
Ethyl *p*-chlorophenoxyisobutyrate	30	6.05±0.05	2.20±0.05	0.57	2.90±0.09	147±5	42±1
Untreated control	—	6.25±0.03	2.35±0.34	0.60	3.01±0.09	151±7	40±3

toxicity, because we have experienced that mortality was 25~30% in the Clf-treated mice at a dose of 600 mg/kg/day for 7 consecutive days, although the body weight gain was comparable to that of the untreated control. In this experiment, no side effect was observed and the dose was comparatively well tolerated.

In comparison with the untreated control, Af and Clf reduced serum lipid levels on long term treatment (Table 5). Special emphasis should be laid on s-PL and s-TG reduction. Af as well as Clf lowered s-PL, s-TG and s-FFA in the normolipidemic rats according to s-TC reduction. This fact indicates that s-TC is an index of serum lipid change.

Effect of Af and Clf on serum proteins and transaminases were examined using the same serum as in Table 6. Both agents had little effect on serum protein concentrations and the A/G ratio. Liver function is normal in both treated groups as exemplified by normal levels of serum GOT and GPT levels.

Change in organ weights were examined using same rats as in Table 6. Hepatomegaly was clearly seen in the rats treated with Clf accompanied with atrophy in spleen and heart. On the contrary, Af treatment slightly reduced liver/body weight ratio, although the hepatic function was normal. It is unclear why such change in organ weights occurred in the rats treated with both hypolipidemic agents (Table 7).

Af-treatment caused a small increase of liver cholesterol and triglycerides on the basis of mg/g of wet liver, but the total liver cholesterol and triglycerides were nearly the same as those of the untreated control (Table 8). Clf reduced liver cholesterol and triglycerides on the basis of mg/g of wet liver, as a number of papers have reported, although total liver cholesterol and triglycerides were unchanged due

Table 7. Effect of ascofuranone on organ weights

Agents	Dose (mg/rat/day)	Liver (g/rat)		Spleen (mg/rat)		Heart (mg/rat)	
		mean±SE	liver wt./body wt. (%)	mean±SE	spleen wt./body wt. (%)	mean±SE	heart wt./body wt. (%)
Ascofuranone	20	11.41±0.41	3.82	701±35	0.23	960±29	3.21
Ethyl *p*-chlorophenoxyliso-butyrate	30	14.41±0.69	4.60	696±16	0.22	900±35	2.88
Untreated control	—	12.93±0.32	4.17	748±37	0.24	980±24	3.16

The agents were given once a day for consecutive 10 days and 6 hours after the last administration the organs were carefully dissected out and weighed. Ten rats were included in each group.

Table 8. Liver cholesterol and triglycerides change in the rat treated with ascofuranone

Agents	Dose (mg/rat/day)	Cholesterol (mg/g liver)		Triglycerides (mg/g liver)	
		mean±SE	% change	mean±SE	% change
Ascofuranone	20	3.19±0.19	+ 6.7	6.5±0.58	+ 4.8
Ethyl *p*-chlorophenoxyisobutyrate	30	2.69±0.11	−10.7	4.9±0.44	−21.0
Untreated control	—	2.99±0.14	—	6.2±0.79	—

The liver cholesterol and triglycerides were determined with the use of the same livers as in Table 7.

to the hepatomegalic effect. Heart cholesterol content was also determined by the same procedure as hepatic cholesterol. Af lowered total heart cholesterol at a rate of 13.7% ($P<0.01$), although Clf was ineffective on heart cholesterol.

Thus, it is evident that Af has a considerable hypolipidemic activity which is comparable to Clf. In addition, Af differentiates from Clf in the fact that it induces no hepatomegaly and effectively prevents accumulation of cholesterol in heart.

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