EFFECT OF ASCOFRANONE ON SERUM LIPIDS OF RATS FED A CHEOLESTEROL RICH DIET

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Abstract—Ascofuranone, a fungal metabolite, significantly reduced serum lipid levels, when orally administered to male Wistar rats fed a cholesterol rich diet. The treatment also resulted in a marked reduction of hepatic and cardiac cholesterol contents without affecting the body weight gain. The serum albumin/globulin ratio increased significantly in the treated rats. This increase is presumably due to the decrease of β-lipoprotein. The mode of action differentiates from clofibrate in so far as the former effectively prevents hepatic and cardiac cholesterol deposits in the cholesterol feeding rats.

Abnormal lipid metabolism plays a major role in the development of atherosclerosis and coronary heart disease in man (1-3). Ascofuranone (Af, Fig 1), a metabolite of a plant pathogenic fungus, Ascochyta viiae Libert, was isolated in our screening for hypolipidemic agents (4-6). Oral administration of Af resulted in significant reduction of serum lipid levels in Wistar strain rats fed a normal diet. The purpose of the present study was to establish the hypolipidemic property of Af in the rat on an atherogenic regimen.

MATERIALS AND METHODS

Thirty male Wistar rats, weighing 260-30 g, were divided equally into three groups. The rats were housed in an air-conditioned room at 24°C and 40%, relative humidity under 12 hr photocycle. A hyperlipidemia inducing diet had been prepared according to Fujiwara et al. (7), but it was hardly touched by the rats so that commercial diet was admixed, as shown in Table 1. The diet and water were given ad libitum. Af (20 mg/rat/day) and a positive control agent, clofibrate (30 mg/rat/day) were orally given as gum arabic suspensions once a day for 10 consecutive days. The treated groups as well as the untreated
control were sacrificed under chloroform 6 hr after the final administration, since for each agent the lowest peak of serum lipid levels is attained 6-10 hr after administration. The blood was obtained by a cardiac puncture using a plastic syringe and after centrifugation in a plastic tube, serum lipids were estimated as reported in a previous paper (6). The organs were carefully removed, weighed and homogenized with phosphate buffer saline (pH 7.0) followed by extraction with a mixture of chloroform-methanol (2:1) (8). Total cholesterol was estimated by Zurkowski's method (9) with slight modification. Tissue phospholipid, triglyceride and free fatty acid were determined as reported previously (6).

RESULTS

The positive control agent, clofibrate, can be regarded as an effective and safe drug for lowering elevated serum lipid in humans. It is remarkably free from side effects, since slight gastrointestinal disorders and transient rise of serum GOT and GPT have rarely been reported as side effects (10). Thorp and Waring (11) reported that clofibrate lowered serum

| Table 1. Cholesterol and saturated triglyceride rich diet administered to Wistar rats |
|-----------------------------------------------|
| Cholesterol | 10 (g) |
| Hydrogenated coconut oil | 75 |
| Cholic acid | 2 |
| Casein | 150 |
| Sucrose | 671 |
| Commercial diet (Nihon CLEA, CE-2) | 1000 |

| Table 2. Change in serum lipids and proteins of Af treated rats |
|-----------------------------------------------|
| Serum lipids | Untreated control | Ascofuranone | Clofibrate |
| Cholesterol (mg/dl) | 97.4 ± 4.5 | 69.0 ± 6.3** | 101.8 ± 11.2 |
| (mg/dl) | (29.2) | (44.4) | (50.0) |
| Triglyceride (mg/dl) | 72.0 ± 13.2 | 40.4 ± 7.7 | 36.2 ± 4.2* |
| Phospholipid (mg/dl) | 427 ± 27 | 410 ± 16 | 427 ± 13 |
| Free fatty acid (μeq/dl) | 82.0 ± 2.7 | 69.9 ± 2.9** | 68.9 ± 5.4** |
| Total protein (g/dl) | 6.80 ± 0.14 | 6.53 ± 0.15 | 6.89 ± 0.09 |
| Albumin (g/dl) | 3.00 ± 0.07 | 3.12 ± 0.23 | 3.42 ± 0.09 |
| A-G ratio | 0.79 | 0.91 | 0.99 |
| GOT (karnem unit) | 195 ± 10.0 | 139 ± 6.0 | 156 ± 17.5 |
| GPT (karnem unit) | 61 ± 6.1 | 38 ± 2.5 | 43 ± 7.0 |
| Body weight (g/rat) | Initial | 267 | 265 |
| Final | 320 | 318 | 322 |

Each value is expressed as mean ± SE. Figures in parenthesis represent serum lipid reduction rates in per cent.

* 0.05 > P > 0.01  ** P < 0.01
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lipid levels in normolipidemic rats at a daily dosage of 100-200 mg/kg by either oral or
subcutaneous administration. In the present study, no interference with growth of the
clofibrate treated rats occurred at this dose level. Af treated rats also showed the same
body weight gain as the untreated control and no sign of abnormality was noted at the
time of post mortem examination (Table 2).

As shown in Table 2, male Wistar strain rats are resistant to an atherogenic regimen,
since elevation of the serum lipid levels was not so high as expected even in the untreated
control. The positive control agent failed to affect the serum cholesterol level (s-TC),
although the serum triglyceride (s-TG) and free fatty acid (s-FFA) significantly decreased.
Grafnetter (12) observed that clofibrate was ineffective in lowering serum cholesterol in
rats fed a cholesterol rich diet. In contrast with the positive control agent, Af significantly
reduced s-TC together with a considerable reduction in s-TG and s-FFA.

Af slightly reduced serum total protein concentration but had little effect on serum
albumin and transaminase concentrations. Serum albumin/globulin ratio rose significantly
in both treated groups. This rise is attributable to the decrease of β-lipoprotein included
in globulin fraction. Whether or not Af affects a certain specific lipoprotein fraction has
to be clarified.

As pointed out in a previous paper (6), clofibrate induced enlargement of liver; liver/
body weight ratio rose from 4.55% of the untreated control to 5.47% of the clofibrate group
in this study (Table 3). Af treatment also increased the ratio, although the change was
smaller. Heart weights were unaffected by either agent.

Af and clofibrate reduced hepatic cholesterol and free fatty acid, when the changes
were calculated on the basis of the lipid content per gram wet liver. However, total he-

| Table 3. Change in organ lipids in Af treated rats |
|-----------------------------------------------|
|          | Untreated control | Ascofuraneone | Clofibrate |
|-----------------------------------------------|
| Liver | Weight (g rat)  | 14.57±0.46 | 15.24±0.75 | 17.62±0.81** |
|       | Cholesterol (mg/g) | 12.21±1.00 | 9.44±0.44* | 9.53±0.59* |
|       | Triglyceride (mg/g) | 9.10±1.00 | 9.70±0.74 | 8.00±1.00 |
|       | Phospholipid (mg/g) | 20.0±1.3 | 19.4±1.1 | 21.8±9.0 |
|       | Free fatty acid (meq g) | 31.3±1.23 | 19.6±0.89** | 19.1±2.3** |
| Heart | Weight (g rat)  | 0.94±0.29 | 0.91±0.03 | 1.01±0.01 |
|       | Cholesterol (mg/g) | 2.31±0.05 | 1.67±0.04** | 2.27±0.11 |
|       | Triglyceride (mg/g) | 4.29±0.35 | 3.91±0.09 | 4.39±0.22 |
|       | Phospholipid (mg/g) | 52.9±1.2 | 44.0±1.8 | 49.8±1.4 |
|       | Free fatty acid (meq g) | 49.9±5.8 | 32.0±1.9** | 40.9±2.8 |

* 0.05>P>0.01  ** P<0.01

Each value is expressed as mean ± SE. Figures in parenthesis represent per cent
changes of organ lipids.
patic cholesterol of the clofibrate group was approximately the same as that of the un-
treated control, whereas Af reduced total hepatic cholesterol. Af was capable of reduc-
ing cardiac cholesterol deposit in the cholesterol feeding rats.

**DISCUSSION**

Regarding the mode of action of clofibrate, Dalton et al. suggested the agent is
able of inducing hepatic synthesis of mitochondrial membrane, lowering serum lipid
levels in compensation for hepatomegaly through a proliferation of intracellular mem-
branes (13). On the other hand, Af lowered total hepatic cholesterol at a considerable rate,
suggesting that Af either interferes with intestinal cholesterol absorption or stimulates
the excretion as bile acids. In fact, oral administration of Af resulted in an increase of
bile acid excretion as observed in the feces of Wistar strain rats. Moreover, a single oral
administration of Af stimulated bile acid excretion at the rate of 20-30%, increase as com-
pared with the control in the bile-duct cannulized rats. In vitro, Af significantly inhibited
active transport of bile acid and cholesterol in everted gut sacs of the rats. Therefore,
both stimulation of cholesterol catabolism and inhibition of exogenous cholesterol ab-
orption appear to be involved in the mode of action. Details regarding the mechanism
of action will be reported elsewhere.

Although elevation of serum lipid levels was not so high as expected, it has been con-
firmed in the present study that Af alters lipid metabolism of the rat fed on an atherogenic
regimen without affecting body weight gain. These alterations are represented by hypo-
lipidemic activity and inhibition of cholesterol deposit in liver and heart. In this respect,
it is very likely that the mode of action on lipid metabolism differs from that of clofibrate.
It has yet to be proven that inhibition of lipid deposit in the heart and liver decrease the
rate of such deposits in the arterial wall thus preventing both occurrence and progression
of atherosclerosis. In addition to the effect on lipid metabolism, Af in small doses,
shows potent hypotensive activity in Okamoto strain spontaneously hypertensive rats
(personal communication). Therefore, Af is a potential candidate for a clinical agent
against atherosclerosis in view of its activity and safety. Experiments are now in pro-
gress to determine whether or not Af therapy alters lipid deposits in the aorta intima thereby
protecting experimental animals from atherosclerosis.

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