HYPOCHOLESTEROLEMIC EFFECT OF ALISOL A-24-MONO- ACETATE AND ITS RELATED COMPOUNDS IN RATS

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Received for publication November 8, 1969

The rhizoma of *Alisma Plantago-aquatica* L. var. *orientale* Samuels used as a component of various preparations of Chinese medicine has long been accepted to have a diuretic effect. The availability of this plant as a pulver in the preparations is a good contrast concerning the presence of practically water insoluble triterpenes to many of the raw plants usually used as a decoctum. Several years ago the extract of this plant with organic solvents was demonstrated to exert a lipotropic effect, which was somewhat different in mode of action from those of other lipotropic agents (1). Further chemical studies on this extract succeeded to isolate the active triterpenes including alisol A-24-monoacetate (ALMA) and its related compounds (2, 3). The present paper describes mainly the hypocholesterolemic activity in the rats maintained on an atherogenic diet.

METHODS

The chemical structure of the representative triterpenes isolated from the *Alisma* plant and a compound derived chemically from alisol A is shown in Fig. 1. Some compounds were chemically derived from the natural triterpenes for the assay (see the footnote of Table 3). The details of the chemical procedures for isolation or modification and the chemical properties of the triterpenes were reported elsewhere (2-4). The test compounds previously dissolved into ethanol or diethylether were given to the rats by mixing in the diet.

Male Sprague-Dawley-JCL rats 5 weeks in age and weighing about 100 g were divided into groups consisted of 5 in each. They were housed individually and fed a commercial diet (CLEA, CE-2) or an atherogenic diet consisting of 67.1% sucrose, 15% milk casein, 10% hydrogenated coconut oil, 4% salt mixture, 2% cellulose flour, 0.5% vitamin mixture in glucose, 1% cholesterol, 0.2% sodium cholate and 0.2% choline chloride. The mixing ratios of ALMA in the diet were 0.0125, 0.025, 0.05, 0.1 and 0.2%. The maintenance of the control two groups on the CE-2 diet or the atherogenic diet and the treated five groups on the atherogenic diet, supplemented with the concentrations of ALMA, for

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A part of this study was presented by Y. Aramaki at the 4th International Congress on Pharmacology (Basel, July, 1969).

FIG. 1. Structures of alisol A-24-monoacetate (ALMA) and its related compounds.

10 succeeding days was followed by the sacrifice of the animals by decapitation. The same experimental design was employed for the comparison of the hypocholesterolemic activity of various alisol-triterpenes. Liver in weight of 2 g was homogenized in 10 volumes of chloroform-methanol mixture (2: 1, v/v) and the crude lipid was extracted according to the method of Folch et al. (5). Cholesterol, phospholipids and triglycerides were assayed following the methods of Abell et al. (6), King (7) and van Handel and Zilversmit (8), respectively. The lowering effect of the test compounds on the elevated levels of plasma cholesterol, plasma cholesterol/phospholipid ratio, liver crude fat and liver cholesterol was evaluated using the following equation: $(X_e - X_i) / (X_e - X_n) \times 100$ in which X_e = mean levels in the group fed the atherogenic diet, X_i = mean levels in the group fed the atherogenic diet supplemented with ALMA, X_n = mean levels in the group fed the CE-2 diet.

In another series of experiment, groups of 5 male rats maintained on the CE-2 diet were fasted previously for 17 hours. Two, 4, 8 and 12 hours after the oral administration of 100 mg/kg of nicotinic acid and 425 mg/kg of ALMA the blood was obtained by decapitation for the estimation of plasma free fatty acids following the method of Dole (9).

RESULTS

The supplement of the atherogenic diet with the concentrations of ALMA lowered the levels of cholesterol in the plasma and liver. The effect was roughly dose-dependent (Table 1). The levels of cholesterol in the plasma and liver in the rats maintained on the atherogenic diet for 10 days were 266 ± 36 mg/100 ml and 2.37 ± 0.07 g/100 g, while those in the animals maintained on the same diet supplemented with 0.0125% ALMA were 187 ± 16 mg/100 ml and 1.86 ± 0.13 g/100 g, respectively. The difference in the levels between both animals was significant. The daily treatment of the animals with ALMA increased considerably the liver weight without affecting the daily food intake. The increased level of the plasma phospholipids by feeding the atherogenic diet was further elevated by

TABLE 1. Dose-response relationship of ALMA in the hypocholesterolemic activity in the male rats fed the atherogenic diet.

	No. of rats	F.B. intake wt. ^{*1} (g)	Food intake (g/rat/day)	Liver wt. (% of B. wt.)	Plasma lipids (mg/100 ml)			Liver lipids (g/100 g)			
					T. chol.	P.L.	c/p	T.G.	Fat	T. chol.	P.L.
Normal	(5)	204	20.1	5.67±0.11 ^{*2}	89±4	126±3	0.71±0.03	50±4	3.94±0.04 (456) ^{*3}	0.22±0.01 (25) ^{*3}	2.85±0.05 (330) ^{*3}
Control	(5)	137	11.8	6.63±0.21	266±36	144±11	1.84±0.22	47±4	9.41±0.14 (855)	2.37±0.07 (215)	2.18±0.09 (198)
ALMA 0.0125% (12.2 mg/kg/day)	(5)	139	11.9	7.17±0.21	187±16	166±12	1.13±0.04 [*]	66±5	7.90±0.37 ^{**} (787)	1.86±0.13 ^{**} (185)	2.09±0.04 (208)
ALMA 0.025% (24.0 mg/kg/day)	(5)	140	11.8	7.17±0.33	182±13	165±7	1.11±0.09 [*]	69±7	6.52±0.22 ^{***} (655)	1.39±0.07 ^{***} (14)	2.10±0.11 (211)
ALMA 0.05% (49.2 mg/kg/day)	(5)	136	11.9	7.13±0.25	171±11 [*]	187±12 [*]	0.92±0.06 ^{**}	72±14	6.53±0.15 ^{***} (633)	1.10±0.15 ^{***} (107)	2.31±0.09 (224)
ALMA 0.1% (97.5 mg/kg/day)	(5)	132	11.6	7.62±0.19 ^{**}	155±4 [*]	158±5	0.98±0.01 ^{**}	71±13	5.76±0.58 ^{***} (580)	0.75±0.16 ^{***} (76)	2.28±0.09 (229)
ALMA 0.2% (196.7 mg/kg/day)	(5)	132	11.7	7.35±0.05	144±6 ^{**}	159±7	0.91±0.07 ^{**}	57±4	5.92±0.38 ^{***} (575)	0.82±0.12 ^{***} (79)	2.51±0.10 (243)

*1: Initial body weight was 105 g.

*2: Mean±standard error

*3: Mg per total liver.

F.B. wt.: Final body weight.

T. chol.: Total cholesterol.

P.L.: Phospholipids.

T.G.: Triglycerides.

c/p: Cholesterol/Phospholipids ratio.

Statistically significant at p=0.05 (*), 0.01 (**) and 0.001 (***).

TABLE 2. Hypocholesterolemic activity of ALMA in the female rats fed the atherogenic diet.

	No. of rats	F.B. wt.* ¹ (g)	Food intake (g/rat/day)	Liver wt. (% of B. wt.)	Plasma T. chol. (mg/100 ml)	Plasma c/p	Liver lipids (g/100 g)	
							Crude fat	T. chol.
Normal	(5)	193	—	5.01±0.18	93±4	0.62±0.04	4.29±0.18	0.26±0.01
Control	(5)	165	13.4	5.64±0.13	252±31	1.85±0.23	10.18±0.54	2.34±0.15
ALMA 0.1%	(5)	168	13.1	7.62±0.25***	154±5**	0.82±0.06**	7.38±0.20**	0.96±0.04***

*1 : Initial body weight was 158 g.

the supplement of the atherogenic diet with ALMA. On the other hand, the level of phospholipids in the liver was rather lowered by the maintenance of the animal on the atherogenic diet with or without the supplement of ALMA. The levels of triglycerides in the plasma was somewhat elevated by the treatment of ALMA, while that in the liver was lowered by the feeding ALMA. No apparent sexual difference in the hypocholesterolemic effect of ALMA was found (Table 2).

The Alisma plant was shown to contain the steroidal compounds such as alisol A, alisol B, alisol A-24-monoacetate, alisol B-23-monoacetate and alisol C-23-monoacetate. The acetolysis of alisol B or acid hydrolysis of alisol B-23-monoacetate yielded alisol A-24-monoacetate (ALMA). For the comparison of the effects on the levels of plasma and liver cholesterol the triterpenes were supplemented to the atherogenic diet in concentration of 0.1%. The results are shown in Table 3. The most effective compound in lowering the elevated levels of plasma and liver cholesterol was alisol A-23, 24-diacetate, which was derived chemically from alisol A. ALMA, alisol C-23-monoacetate, alisol A, alisol B-23-

TABLE 3. Hypocholesterolemic activity of alisol-triterpenes in the male rats fed the atherogenic diet.

Triterpens	Dose (% in diet)	Lowering effect (%)			
		Plasma T. chol.	Plasma c/p	Liver fat	Liver T. chol.
Alisol A-23, 24-diacetate*	0.1	72	68	68	69
Alisol A-24-monoacetate (ALMA)	0.1	61	64	65	61
Alisol C-23-monoacetate	0.1	55	46	31	35
Alisol A	0.1	54	45	45	30
Alisol B-23-monoacetate	0.1	51	46	49	26
3-Dihydroalisol A*	0.1	47	56	6	0
23-O-Methylalisol A*	0.1	43	48	34	30
25-Anhydroalisol A*	0.1	39	51	40	25
Epi-alisol A	0.1	22	32	26	14
Alisol A-11, 24-diacetate*	0.1	0	0	19	0
Alisol A-11, 23, 24-triacetate*	0.12	0	0	25	10
Alisol B	0.1	0	34	47	20
Alisol A-tetranoraldehyde*	0.1	0	7	10	0
β-Sitosterol	0.25	5	0	29	27
Cholestane-3β, 5α, 6β-triol (CT)	(0.25) (0.5)	(17) (38)	(6) (34)	(41) (89)	(39) (51)

* Chemically derived from the natural triterpenes.

monoacetate, 3-dihydroalisol A, 23-O-methylalisol A, 25-anhydroalisol A and epi-alisol A were found also to be effective in order of description. Other derivatives including alisol B produced no hypocholesterolemic activity. The supplement of the atherogenic diet with 0.25% β -sitosterol exerted a weak hypocholesterolemic effect. The supplement of the same diet with 0.25 and 0.5% cholestane-3 β , 5 α , 6 β -triol (CT), which was shown to exert the hypocholesterolemic and anti-atherogenic effects in rats, chickens and rabbits (10), was also found to be less effective.

Groups of the rats were maintained for 10 days on the CE-2 diet supplemented with 0.1% ALMA, 0.25% CT or 0.1% ethyl p-chlorophenoxyisobutyrate (CPIB) (Table 4). The daily measurement of the body weight and food intake was followed by the sacrifice of the animal on the 11th day for weighing of the liver and estimation of the levels of plasma cholesterol and phospholipids and liver crude fat, cholesterol and phospholipids. The supplement of the diet with ALMA did not affect significantly the parameters, while the

TABLE 4. Effect of ALMA in the male rats fed the stock diet.

	No. of rats	F.B. wt.* (g)	Food intake (g/rat/day)	Liver wt. (% of B. wt.)	Plasma lipids (mg/100 ml)		Liver lipids (g/100 g)		
					T. chol.	P.L.	Crude fat	T. chol.	P.L.
Control (Normal)	(5)	199	21.2	5.05 $\pm$ 0.08	75 $\pm$ 3	123 $\pm$ 4	4.33 $\pm$ 0.10	0.21 $\pm$ 0.01	3.19 $\pm$ 0.11
ALMA 0.1%	(5)	200	21.2	5.57 $\pm$ 0.08**	71 $\pm$ 7	111 $\pm$ 8	4.70 $\pm$ 0.06*	0.21 $\pm$ 0.00	3.65 $\pm$ 0.07**
CT 0.25%	(5)	169	15.2	5.35 $\pm$ 0.21	61 $\pm$ 2**	97 $\pm$ 2***	4.34 $\pm$ 0.08	0.21 $\pm$ 0.01	3.53 $\pm$ 0.12
CPIB 0.1%	(5)	199	22.0	6.54 $\pm$ 0.18***	51 $\pm$ 2***	91 $\pm$ 2***	5.40 $\pm$ 0.08***	0.19 $\pm$ 0.00	3.94 $\pm$ 0.10**

*1: Initial body weight was 103 g.

FIG. 2. Plasma free fatty acid changes induced by single oral administration of ALMA or nicotinic acid in fasted rats. Drugs were given in rats previously fasted for 17 hours. Each point represents the mean (or $\pm$ S.E.) of 5 male rats weighing about 180 g. Significant at $p=0.05$ (*) and 0.001 (***).

phospholipid content in the liver was elevated considerably. On the other hand, CPIX or CT revealed a lowering of the plasma level of cholesterol as well as phospholipids.

The oral administration of 100 mg/kg of nicotinic acid to the previously fasted rats resulted in the significant decrease in the level of the plasma free fatty acids two hours after the administration and the succeeding elevation above the previous level at least for several hours. The levels of the plasma free fatty acids in the animal treated with the oral administration of 425 mg/kg of ALMA tended to be elevated considerably (Fig. 2).

DISCUSSION

Many triterpenes were found in the plant *Alisma Plantago-aquatica* L. var. *orientale* Samuels. The previous assay of the triterpenes on the hypocholesterolemic effect in the rats fed the atherogenic diet containing 1% cholesterol and 0.2% sodium cholate for 10 days revealed that several triterpenes were effective. The existence of the most effective alisol A-23, 24-diacetate in the plant was not yet confirmed. The other effective triterpenes were alisol A-24-monoacetate (ALMA), alisol C-23-monoacetate, alisol A, 25-anhydroalisol A, alisol B-23-monoacetate, 3-dihydroalisol A, 23-O-methylalisol A and epi-alisol A. The structure-action relationship indicates that the glycerol moiety at the side chain of the triterpenes is essential for the hypocholesterolemic effect, because alisol A-tetranoraldehyde, in which the glycerol moiety is lost, proved to be inert. The acetylation of the hydroxyl group at 23 and/or 24 position of the side chain was found to enhance the activity, whereas the same procedure at 11 position abolished the activity.

ALMA, which was present in considerable amount in the plant and easily derived from alisol B or alisol B-23-monoacetate, was next effective to alisol A-23, 24-diacetate. Therefore, the hypocholesterolemic effect was mainly studied on ALMA. The elevated levels of the plasma and liver cholesterol in the rats maintained for 10 days on the atherogenic diet were dose-dependently lowered by the supplement of the diet with ALMA. The elevated levels of the plasma and liver cholesterol in the rats were slightly lowered by the supplement of the atherogenic diet with 0.25% β -sitosterol. On the other hand, the supplement of the same diet even with 0.0125% ALMA produced a significant lowering of the cholesterol level in the plasma and liver. In this respect, ALMA proved to be much more effective than cholestane-3 β , 5 α , 6 β -triol (CT) (10). Since the absorption of cholesterol in the rat with a thoracic duct-fistula was markedly depressed by the oral administration of ALMA (11), the presence of ALMA in the intestinal tract seemed to interfere with the absorptive mechanism of cholesterol.

Differing the effect of nicotinic acid (12-14) the single oral administration of ALMA did not decrease the plasma level of free fatty acids in the fasted rats. The supplement of the stock diet with ALMA in the rats did not also affect the levels of plasma and liver cholesterol, while the same procedure of ethyl p-chlorophenoxyisobutyrate, which was reported to inhibit the cholesterol biosynthesis (15-17), lowered both levels. Therefore, the inhibition of cholesterol synthesis by ALMA was unlikely occur. It has been demonstrated that 40% or more of orally administered ALMA-³H is absorbed via portal vein but

not via lymphatic route, and that the absorbed ALMA and its metabolites are largely excreted via bile duct into the feces (18). The variable changes of the plasma and liver levels of phospholipids in the rats maintained on the stock or atherogenic diet supplemented both with ALMA seemed to be indicative for some role in the liver. The lack of the behavioral abnormality in the animals fed the diet supplemented with ALMA reflected the finding that the oral administration of ALMA interfered only with the cholesterol absorption but not with the absorption of fatty acids in the intestine (11).

SUMMARY

Hypocholesterolemic effect of alisol A-24-monoacetate (ALMA) and its related compounds was investigated in the normal and hypercholesterolemic rats. Among the compounds easily available ALMA was found to have a potent activity in the rats fed a diet containing 1% cholesterol and 0.2% sodium cholate, though alisol A-23, 24-diacetate was the most effective. Even the supplement of 0.05% ALMA in the diet showed a statistically significant lowering of the plasma and liver cholesterol levels. ALMA had no influences on the cholesterol level of the plasma and liver in the rats fed the stock diet. Unlike nicotinic acid, ALMA did not inhibit the elevation of the plasma free fatty acids in the fasted rats.

Acknowledgements: We thank Dr. S. Tatsuoka, General Manager of Research and Development Division, and Dr. K. Shimamoto, Research Manager of Biological Research Laboratories, for their encouragement and helpful advice. Thanks are also due to Dr. M. Miyamoto and Mr. T. Murata for the supply of the triterpenes used.

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