Effects of Pantetheine on Cholesteryl Ester Synthesis in the Arterial Wall of Rats on High Cholesterol Diet

Kohji Shirai, Nobuo Matsuoka, Yasushi Saito, Akira Kumagai and Hiromichi Okuda*

The Second Department of Internal Medicine, School of Medicine, Chiba University, Chiba 280, and *the Second Department of Medical Biochemistry, School of Medicine, Ehime University, Ehime 791-02

SHIRAI, K., MATSUOKA, N., SAITO, Y., KUMAGAI, A. and OKUDA, H. Effects of Pantetheine on Cholesteryl Ester Synthesis in the Arterial Wall of Rats on High Cholesterol Diet. Tohoku J. exp. Med., 1979, 128 (4), 355-358 — Increase of acyl-CoA synthesis was observed when extracts of rat arterial wall were incubated with pantetheine [D-bis-(N-pantothenyl-β-aminoethyl)-disulfide]. Cholesteryl ester synthesis from palmitate in the arterial wall extract in vitro was higher with arteries from rats on high cholesterol diet than with those from rats on normal diet, but the synthesis was reduced in the arteries of rats on high cholesterol diet with pantetheine. Triglyceride synthesis was higher with arterial wall extracts of rats on high cholesterol diet than with preparations from rats on normal diet and was not reduced with those of rats on high cholesterol diet plus pantetheine. The value of the effects of pantetheine on lipid metabolism in the prevention of atherosclerosis is pointed out. —— arterial wall; pantetheine; acyl-CoA synthesis; lipid synthesis; high cholesterol diet

Development of atherosclerosis is associated with various changes in metabolism of the arterial wall. One characteristic of atherosclerosis is the accumulation of lipids due to their infiltration from the blood or to synthesis in the arterial wall (Day and Wahlqvist 1968; Zilversmit 1968; Chobanian and Manzur 1972; Ross and Flomset 1973). It is reported that in rat liver pantetheine is incorporated into thiolase A (CoA) (Hoagland and Novelli 1954; Levintow and Novelli 1954), which is a cofactor in many enzyme reactions (Jaenicke and Lynen 1960). This paper reports the effects of pantetheine [D-bis-(N-pantothenyl-β-aminoethyl)-disulfide] on lipid metabolism in the arterial wall and the results are discussed in relation to prevention of atherosclerosis.

MATERIALS AND METHODS

Animals and diets. Groups of 15 male Wistar-King strain rats, weighing 150 g, were fed on the following diets for 4 weeks: i) normal diet, ii) normal diet plus pantetheine.
iii) high cholesterol diet, iv) high cholesterol diet plus pantetheine. The high cholesterol diet contained 1% cholesterol and 0.5% cholic acid (W/W), and the diet with pantetheine contained 1% of the substance. These diets were obtained from Oriental East Co. Rats were given 20 g of diet per day for 4 weeks.

Preparation of enzyme source. Rats were killed by decapitation and the arterial wall from the aortic arch to the common iliac artery was quickly removed and washed with ice cold 0.9% NaCl. The adventitia was carefully removed away. The arterial wall was cut up with scissors and homogenized in 50 mM Tris HCl buffer (pH 7.5), in Hiscotron (Nichion Ltd., Japan) for three 1-min periods to give a 10% (W/W) homogenate. The mixture was then centrifuged at 800 x g for 10 min and the supernatant was used as the enzyme solution.

Chemicals. Cholesterol was obtained from Wako Pure Chemicals Co. ATP, CoA and thin layer chromatography plates were obtained from Sigma Chemical Co. and palmitic acid 1-14C was supplied by Japan Isotopic Company. Pantetheine was kindly supplied by Daiichi Seiyaku Co., Ltd.

Estimation of acyl-CoA synthesis. Acyl-CoA synthesis was estimated by the method of Bar-Tana et al. (1971) with slight modifications. The incubation mixture consisted of 50 mM Tris-HCl buffer, pH 7.4, 6.6 mM ATP, 6.6 mM MgCl2, 2.25 mM reduced glutathione, 0.4 mM potassium palmitate (1 μCi/4 μmoles), pantetheine (1 mg/ml) and enzyme solution in a final volume of 0.25 ml. Reactions were made to proceed for 10 min periods at 37°C and terminated by adding 1 ml of Dole’s extraction mixture (Dole 1965). The test tube was shaken vigorously and centrifuged and the heptane layer was removed. The lower phase was washed five times with 0.6 ml volumes of heptane, the heptane extracts were discarded and 0.4 ml of the lower phase was used to measure radioactivity.

Incorporation of palmitate into triglyceride, phospholipid and cholesteryl ester. A sample of 50 mg of slices of arterial wall was incubated in Krebs-Ringer bicarbonate buffer (pH 7.4) containing 1 mM potassium palmitate (1.5 μCi/μ mole) in a final volume of 1 ml for 30 min at 37°C. The reaction was terminated by adding chloroform-methanol mixture (2:1, V/V). Lipids were extracted by the method of Folch et al. (1951) and were separated by thin layer chromatography using a two-step development system. The first step was developed with isopropyl ether: acetic acid, 96:4 (V/V) and the second step with petroleum ether: ether: acetic acid, 90:10:1 (V/V). The spots of lipid were scratched off the plates and their radioactivities were determined in scintillation solution (DPO 4 g, POPOP 100 mg, Toluene 1000 ml) in a scintillation counter. Protein was assayed by the method of Lowry et al. (1951).

RESULTS AND DISCUSSION

Pantetheine was found to be converted to CoA in rat liver (Hoagland and Novelli 1954; Levintow and Novelli 1954). Therefore, the effect of pantetheine on acyl-CoA synthesis from FFA in the arterial wall was investigated. Acyl-CoA synthesis in the arterial wall increased when pantetheine was added in vitro (Fig. 1). This result suggests that pantetheine is metabolized to CoA and that it promotes the synthesis of acyl-CoA from FFA in the arterial wall. Pantetheine may also contribute to glucose metabolism, lipid metabolism and protein metabolism (Jaenicke and Lynen 1960).

As shown in Table 1, phospholipid synthesis in the arterial wall was slightly increased in the group on high cholesterol diet, as reported by Zilversmit et al. (1954). It has been suggested that increased phospholipid may be used to remove
accumulated lipid in the arterial wall (Zilversmit et al. 1954). Phospholipid synthesis in the arterial wall has not changed in rats on a high cholesterol diet containing pantetheine compared with those on a high cholesterol diet.

Fig. 1. Effect of pantetheine on acyl-CoA synthesis in rat arterial wall. Acyl-CoA synthesis was measured as described in Materials and Methods. 0.25 mg of pantetheine was added to reaction mixture. Enzyme solution was the 800 g supernatant of the 10% aortic homogenate.

Table 1. Effect of pantetheine on lipid synthesis in rat arterial wall

<table>
<thead>
<tr>
<th></th>
<th>Cholesteryl ester synthesis (cpm/g wet wt.)</th>
<th>Triglyceride synthesis (cpm/g wet wt.)</th>
<th>Phospholipid synthesis (cpm/g wet wt.)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal diet</td>
<td>(n=6) 2930±302</td>
<td>7980±822</td>
<td>8440±586</td>
</tr>
<tr>
<td>Normal diet + pantetheine</td>
<td>1873±228‡</td>
<td>11402±1902‡</td>
<td>10681±1030‡</td>
</tr>
<tr>
<td>High cholesterol diet</td>
<td>(n=6) 5375±567†</td>
<td>1218±1079</td>
<td>10685±1040†</td>
</tr>
<tr>
<td>High cholesterol diet + pantetheine</td>
<td>(n=6) 3831±210†</td>
<td>12816±1336†</td>
<td>10305±999†</td>
</tr>
</tbody>
</table>

Mean±s.e. * p<0.1. † p<0.05. ‡ p<0.01.

It has been found that cholesteryl ester is mainly deposited in atheroma (Insull and Bartsch 1966). The mechanism of the deposition has been reported by many workers (Hata et al. 1974; Peters and De Dube 1974). As shown in Table 1, synthesis of cholesteryl ester in the arterial wall was significantly higher in rats on high cholesterol diet than in those on normal diet. Significant decrease of cholesteryl ester synthesis was observed in rats on high cholesterol diet plus pantetheine. These results suggest that pantetheine may prevent deposition of cholesteryl ester in the arterial wall.

As shown in Table 1, triglyceride synthesis was increased in rats on diet containing pantetheine and on high cholesterol diet. But no significant difference was seen between rats from high cholesterol diet with and without pantetheine.

It was reported that cholesteryl ester hydrolase, but not lipase was low in atheromatous lesion (Patelski et al. 1970; Zemplenyi and Grafnetter 1959). Therefore, in these lesions triglyceride may be hydrolysed and cleared out.

Our work shows that pantetheine has the following merits as an antiatherosclerosis agent. It increases CoA, which in turn activates metabolic paths such as the
tricarboxylic acid cycle, and FFA and cholesterol metabolism (Jaenicke and Lynen 1960). It also reduces cholesteryl ester synthesis, which contributes to protection of the arterial wall from atherosclerosis. Experiments are in progress on the mechanisms of the effects of pantetheine on increasing triglyceride synthesis from FFA, and reducing cholesteryl ester synthesis.

Acknowledgments

We are grateful to Daiichi Seiyaku Co., Ltd. for providing pantetheine used in these studies. This work was supported partially by Chiyoda Seimei Foundation, and by Ministry of Education, Science and Culture, Japan (248187).

References