Note

Effect of Sesamin on Cholesterol Synthesis and on the Distribution of Incorporated Linoleic Acid in Lipid Subfractions in Cultured Rat Cells

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Received June 17, 1994

Since sesamin influences the metabolism of essential fatty acids, its effects on cholesterol metabolism and on the incorporation of linoleic acid were studied by using cultured rat artery smooth muscle cells (SMCs) and primary cultured rat hepatocytes. Cholesterol synthesis from acetate was inhibited by sesamin in SMCs, and the distribution of incorporated linoleic acid in the lipid and phospholipid subfractions was altered by sesamin in rat hepatocytes.

Sesamin is a lignan in sesame seed oil, and there are some reports on its various biological functions. Sesamin participates in the biological antioxidative reaction,1,2 an anticancer activity,3 and it has been recently reported that sesamin had ability to reduce liver damage caused by alcohol.4 Particularly, numerous studies about the influence of sesamin on lipid metabolism have been conducted. Concerning essential fatty acid metabolism, sesamin inhibited Δ5 desaturation in the pathway of n-6 series fatty acids, and the amount of arachidonic acid production decreased in Mortierella alpina.5 However when we investigated the effect of sesamin on Δ5 desaturation in the pathway of n-3 series fatty acids in primary cultured rat hepatocytes, no inhibition of Δ5 desaturation that was observed in the n-6 series was found.8 We also studied the effect of sesamin on chain elongation in the pathway of both series of fatty acids.9 Additionally, since sesamin influenced cholesterol synthesis,6 we noted its functional importance. In this study, we investigated 1) the effect of sesamin on cholesterol synthesis in cultured rat artery smooth muscle cells, and 2) the effect of the distribution of incorporated linoleic acid in the lipid subfractions and phospholipids in primary cultured rat hepatocytes.

Sesamin (sesamin:episesamin = 1:1) prepared by the method of Fukuda et al.7 was kindly donated by Idemitsu Petrochemical Co.

SMCse were prepared from male rats (200 g, Wistar strain) by the method of Morisaki et al.,8 while hepatocytes were prepared by the method of Nakamura et al.9 SMCs and hepatocytes were seeded at 1 × 10^5 cells and 1 × 10^5 cells per dish, respectively, in Dulbecco's modified Eagle medium (Nissui Pharmaceutical Co.) containing 10% fetal bovine serum (Gibco) and gentamicin (Scherling-Plough Co.).3 SMCs were used for the experiments after 5 days, and hepatocytes after 17 h of incubation.

Sesamin was dissolved in ethanol and added to the culture medium at a concentration of up to 100 μg/ml, and [1-14C]acetate (56 mCi/mmol, ICN Biomedicals) was added to the culture medium at a final concentration of 0.1 μCi/ml. [1-14C]Linoleic acid (53.9 mCi/mmol, Amersham Japan Co.) was added at a final concentration of 1 μCi/ml.

SMCs were incubated for 24 h with sesamin and [1-14C]acetate for measurements on cholesterol synthesis. Hepatocytes were incubated for 24 h with sesamin and [1-14C]linoleic acid to investigate the linoleic acid distribution in neutral lipids and phospholipids. Cellular lipids were extracted by the method of Folch et al.10 The free cholesterol fraction and each fraction in the neutral lipids were separated by TLC by the method of Skipski et al.11 The phospholipids were also separated into subfractions by the method of Skipski et al.12 and protein was measured by the method of Lowry et al.13

To investigate effect of sesamin on cholesterol synthesis from acetate, SMCs were incubated with 0.1 μCi/1.8 nmol/ml of [1-14C]acetate and with 0, 0.05, 0.075, or 0.1 mg/ml (final concentration in the medium) of sesamin for 24 h. The amount of free cholesterol (FC) was significantly decreased when more than 0.075 mg/ml of sesamin was present (Fig.). In this experiment, although sesamin was added to the medium by dissolving ethanol, and the medium included less than 6% ethanol, this concentration of ethanol had no adverse effect on cholesterol synthesis (data not shown).

The effect of sesamin on the distribution of linoleic acid in neutral lipids was investigated. When sesamin was added with 1 μCi/19 nmol/ml of [1-14C]linoleic acid, the concentrations of linoleic acid in the triacylglycerol (TG) and free fatty acid (FFA) fractions were significantly increased. In the other hand, linoleic acid in the cholesterol ester (CE) fraction was significantly decreased according to the amount of sesamin in the medium. In the phospholipid (PL) fraction, sesamin had no effect. The total incorporated linoleic acid concentration was significantly increased (Table 1).

Next, the effect of sesamin on the distribution of linoleic acid in the PL subfractions was investigated. The concentrations of linoleic acid in phosphatidylethanolamine and in phosphatidylinositol (PS+PI) were decreased by adding sesamin. Expressing the distribution of linoleic acid in each PL as a percentage, linoleic acid in the PC fraction was increased and, in the phosphatidylethanolamine fraction, decreased (Table 1).

Fig. Effect of Sesamin on Cholesterol Synthesis.

Confluent smooth muscle cells were incubated for 24 h in a culture medium containing [1-14C]acetate (0.1 μCi/1.8 nmol/ml) and sesamin (0-0.1 mg/ml). The free cholesterol fraction was separated by TLC and its radioactivity counted. Bars with an asterisk are significantly different from the no-sesamin control (*p < 0.05).
Sesamin, particularly, affects the metabolism of linoleic acid in PL. It is considered that the alteration of FC/PC ratio in blood induces variation in the blood vessel surface and participates in the progression of arteriosclerosis. Therefore, it is interesting that sesamin affects the cholesterol synthesis and the distribution of fatty acid in PL. Although we used in this study linoleic acid, in the future, we need to investigate the effect of sesamin on the metabolism of the other important essential fatty acids: linolenic acid, arachidonic acid, eicosapentenoic acid, and docosahexaenoic acid.

References