Antidiabetic Effects of Ajoene in Genetically Diabetic KK-Ay Mice

Atsuhiko HATTORI, Northiko YAMADA, Tomoaki NISHIKAWA*, Hiroyuki FUKUDA, and Tsuchiyoshi FUJINO
Biodevelopment Division, Central Institute, Nagoya Seiraku Co. Ltd., 310 Nakasuna-cho, Tempaku-ku, Nagoya, Aichi 468-0065, Japan
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Summary Antidiabetic effects of ajoene, derived from garlic, were investigated in genetically diabetic KK-Ay mice. Four-week-old male KK-Ay mice were kept on a laboratory diet containing 0.02 or 0.05% of ajoene for 8 wk. The elevation of water intake was suppressed depending on ajoene intake. The levels of plasma glucose in the 0.05% ajoene-containing diet group was significantly suppressed to 73.8% compared with the control group at the 8th wk. Similarly, the plasma triglyceride level was significantly suppressed. It is suggested that hyperglycemia and hypertriglyceridemia are suppressed by ajoene treatment.

Key Words hypoglycemic effect, ajoene, garlic, KK-Ay mice, diabetes mellitus

Garlic (Allium sativum L.) has been used worldwide as a medicinal plant since ancient times, and its beneficial properties against cardiovascular diseases, cancer and infections are well known (1). Garlic products and compounds derived from garlic have also been reported to have anti-diabetic effects. Mathew and Augusti reported allicin (diallyl thiosulfinate) produced hypoglycemic action comparable to tolbutamide in alloxan-induced diabetic rabbits (2). Aged garlic extract is also effective to prevent hyperglycemia in hyperglycemic mice induced by immobilization stress (3).

Oil-maceration is one method for processing garlic, and this type of garlic product is a common health food in Europe (4). Ajoene [(E, Z)-4, 5,9-trithiadodeca-1, 6,11-triene-9-oxide], one of the derivatives of allicin, has been found to be a major sulfur-containing compound in oil-macerated garlic products and is known as an inhibitor of platelet aggregation (Fig. 1) (5). Ajoene has also been reported to exhibit antibiotic (6), hepatoprotective (7) and antitumor (8) effects. However, nothing is known about its antidiabetic effects in a type 2 (non-insulin dependent) diabetes mellitus model using KK-Ay mice. The KK-Ay mice which were developed by transferring the yellow obese gene, A, into KK mice were improved as a type 2 (non-insulin dependent) diabetes mellitus model animal (9). As the result, KK-Ay mice show earlier and severer obesity, hyperglycemia and hyperlipidemia than KK mice. In this paper, we report antidiabetic effects of ajoene in genetically diabetic mice, KK-Ay.

Z-Ajoene, which had a purity of more than 98%, was used in the series of experiments. The preparation and purity check of Z-ajoene were carried out according to the methods described by Block et al. (5) and Lawson et al., (4) respectively.

Four-week-old male KK-Ay mice were obtained from CLEA Japan, Inc. (Tokyo, Japan). The mice were kept individually in plastic cages in a room maintained at 24±1°C and 50% humidity with a 12h light/dark cycle. The mice were allowed free access to a standard laboratory diet (CE-2, CLEA Inc.) and drinking water for one week to accustom themselves to the surroundings. In the antidiabetic test, the mice were kept on a CE-2 powdered diet containing 0.02 or 0.05% of ajoene for 8 wk. The diet containing ajoene was prepared as follows. Ajoene of required quantity was suspended in 0.5% carboxymethyl cellulose sodium salt solution. The suspension was added to CE-2, and they were mixed well.

Blood samples were taken from the tail vein at 1:00 p.m, after removing the mice from their diets at 8:00 a.m. and plasma was separated every week. The concentrations of plasma glucose and plasma triglyceride were measured with their corresponding assay kits (Glucose C-II and Triglyceride E-, Wako Pure Chemical Industries, Ltd., Osaka, Japan). This experimental design was approved by the Committee on Animal Research at Nagoya Seiraku and the mice were cared for in line with the Guidelines for Care and Use of Laboratory Animals.

Each value is presented as the mean±SD. All data were analyzed by one-way ANOVA, and then the statistical significances among means were evaluated by Duncan’s multiple range test (10), being considered significant at p<0.05.

Water intake in this experiment is shown in Fig. 2A. Body weight and food intake were almost the same among all groups (data not shown). But water intake was inclined to be less in 0.05% ajoene-containing diet groups than in the control group. The decrease in water intake depended on ajoene intake. After the beginning of this experiment, the intakes of water in the control and 0.02% ajoene containing diet groups were immediately increased. Water intake in the 0.05% ajoene-containing diet group was kept at a constant level till the
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Fig. 1. Structures of E- and Z-ajoenes. Z-Ajoene was used in this series of experiments.

Fig. 2. Effects of ajoene on water intake, plasma glucose and triglyceride levels in KK-Ay mice. Four-week-old KK-N mice were kept on a CE-2 powdered diet containing ajoene at a level of 0 (○), 0.02 (●) or 0.05 (△)% respectively, for 8 wk. The points and bars represent the means±SD for 5 mice. Differences in water intake among the three groups at each period were analyzed by Duncan’s multiple range test; points with out common superscript letters differed significantly (p<0.01 versus water intake, p<0.05 versus plasma glucose as well as triglyceride).

The levels of plasma glucose and triglyceride are shown in Fig. 2B, C. The level of plasma glucose in the 0.05% ajoene-containing diet group was suppressed to 73.8% compared with the control group, but the level in the 0.02% ajoene containing diet group was not significantly different from that in the control group at the 8th wk. The increase in water consumption was proportional to the elevation of plasma glucose content and is also a characteristic of KK-AY mice, as generally observed in diabetes. The level of plasma triglyceride in the control group was gradually elevated. In the 0.02% ajoene-containing diet group, the level of plasma triglyceride was elevated from the second week and it reached the same as in the control group at the 6th wk. The gradual increase of plasma triglyceride was shown in the control group but that of the 0.05% ajoene-containing diet group was suppressed to 74.5% at 8 wk.

Ajoene suppressed the rise of the blood glucose and triglyceride levels in this experiment, but the mechanism was not clear. Similarly, although the effect of garlic on diabetes has been reported, the mechanism has not been clarified. Ajoene was not effective in improving the glucose tolerance test in normal mice (data not shown). A proposed mechanism is due to spare insulin from the sulphydryl group. Insulin is inactivated by the sulphydryl group. According to Mathew and Augusti (2), allicin can effectively combine with compounds like cysteine and enhance serum insulin. It is possible that ajoene functions in a similar manner. Garlic was also effective in reducing serum cholesterol and triglyceride (11). Yeh and Liu (12) reported garlic depressed several hepatic activities of lipogenic enzymes. The preventive effect of ajoene against hypertriglyceridemia may be primarily due to inhibition of these enzymes.

In conclusion, ajoene derived from garlic reduced the levels of plasma glucose and triglyceride in non-insulin dependent diabetic mice, though the mechanism of action remains to be further investigated.

REFERENCES

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