Antidiabetic Action of Low Molecular Weight Chitosan in Genetically Obese Diabetic KK-A\(^{\gamma}\) Mice

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Recently, we reported that low molecular weight (LMW) chitosan (chitosan lactate, average MW: 20000) prevents the progression of low dose (100 mg/kg, i.p.) streptozotocin-induced slowly progressive diabetes mellitus in male ICR mice. The present study was designed to clarify the effects of LMW chitosan on hyperglycemia, hyperinsulinemia and hypertriglyceridemia in genetically obese diabetic male KK-A\(^{\gamma}\) mice. LMW chitosan (0.05%, 0.2% or 0.8% water solution) was given daily as drinking water to male KK-A\(^{\gamma}\) mice for 11 weeks, from 5 weeks of age. The non-fasting serum glucose levels of control mice continued to increase slowly throughout the experimental period. LMW chitosan lowered the serum glucose levels in a dose-dependent manner. In these diabetic mice, hyperinsulinemia and hypertriglyceridemia were observed, and LMW chitosan was dose-dependently effective in improving both serum biochemical parameters. LMW chitosan at three doses improved overdrinking and polyuria observed in these diabetic mice. It is concluded from these results that LMW chitosan may be useful for the treatment of obesity-related type 2 diabetes mellitus.

Key words chitosan; insulin resistance; antidiabetic action; KK-A\(^{\gamma}\) mouse

Diabetes mellitus is classified into two types, type 1 (insulin-dependent) and type 2 (non-insulin-dependent). Type 2 diabetes mellitus is divided into two categories, obese type with hyperinsulinemia and non-obese type with hypoinsulinemia. It is established that obesity causes peripheral insulin resistance which leads to hyperinsulinemia. Obesity-related type 2 diabetes mellitus is also characterized by hypertriglyceridemia as well as hyperinsulinemia.\(^1\) It is clear from a number of clinical and experimental studies that triglyceride production is increased in hyperinsulinemia.\(^2,3\) Man et al.\(^5\) have demonstrated that hypertriglyceridemia in non-insulin-dependent diabetes mellitus (NIDDM)-prone OLETF rats results in a high triglyceride content in islets.\(^5\) They suggest that fat droplets in islets may play an important role in hastening the development of NIDDM in this model by the impairment of pancreatic \(\beta\)-cell function. Therefore, the above findings strongly suggest that improving the abnormality of lipid metabolism as well as glucose metabolism may be useful in preventing the development or progression of obesity-related type 2 diabetes mellitus.

Chitosan is chemically a polymeric \(\alpha\)-D-glucosamine, a basic polysaccharide, and is produced by deacetylating chitin, a polymeric \(N\)-acetyl-\(\beta\)-D-glucosamine, with 40—45% NaOH at 120 °C (Fig. 1).\(^3\) Chitin is insoluble in water, acid or alkaline solution. However, chitosan is easily solubilized in acid solution, because it has amino groups in its chemical structure. It has been reported that chitosan has many pharmacological actions such as immunopotentiating,\(^6,7\) anti-hypertensive,\(^8\) serum cholesterol-lowering\(^9—11\) anti-bacterial,\(^12,13\) and wound healing-promoting actions.\(^14—16\)

In our previous study, we reported that chitosan (MW: 25000—50000) has potent gastric cytoprotective and ulcer-healing promoting actions in rats.\(^7\) Miura et al.\(^18\) first showed that chitosan given as a 5% food mixture produces consistent blood glucose- and lipid-lowering effects in normal mice and neonatal streptozotocin (STZ)-induced diabetic mice, one of the animal models of non-obese type NIDDM, but this compound is ineffective in improving these biochemical parameters in KK-A\(^{\gamma}\) mice, one of the animal models of genetically obese type NIDDM with hyperinsulinemia. Recently, we succeeded in creating a new mouse model of slowly progressive NIDDM by only a single i.p. injection of a subdiabetogenic low dose (100 mg/kg) of STZ to 8-week-old male ICR mice.\(^19,20\) We reported that low molecular weight (LMW) chitosan (chitosan lactate, average MW: 20000) prevented the progression of low dose STZ-induced slowly progressive NIDDM.\(^21\) Therefore, in the present study, we examined the effect of the long-term administration of LMW chitosan, given as drinking water, on hyperglycemia, hyperinsulinemia and hypertriglyceridemia of diabetes mellitus in male KK-A\(^{\gamma}\) mice, an animal model of genetically obese-type NIDDM.

MATERIALS AND METHODS

Animals Five-week-old male KK-A\(^{\gamma}\) (Clea, Tokyo, Japan) and male ICR mice (Nippon SLC, Shizuoka, Japan) were used in the experiment. They were housed in an isolator caging system in an air-conditioned animal room at 23±1 °C.

Compound The compound employed was LMW chitosan (Yaizu Suisankagaku Ind., Co., Ltd., Shizuoka, Japan). LMW chitosan was used as water solution.

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Experimental Procedure  LMW chitosan (0.05%, 0.2% or 0.8%) was given to KK-A'Y mice as drinking water for 11 weeks from 5 weeks of age. Control KK-A'Y mice were given distilled water instead of chitosan solution. ICR mice of the same age were used as the normal control, and were given distilled water. Blood samples were withdrawn from the cavaus sinus through a capillary under ether anesthesia at 5, 7, 9, 11, 13 and 16 weeks of age to determine non-fasting serum glucose, insulin, total cholesterol and triglyceride levels. The body weight of each mouse was measured immediately before blood collection. After the collection of blood samples, these animals were kept in individual metabolic cages for 24 h, and drinking water (distilled water instead of LMW chitosan solution only when the 24-h urine was collected) and food consumption per 24 h was measured. Serum glucose and insulin were determined using commercial agents: Glucose CII-test Wako (Wako Pure Chemical Industries, Tokyo, Japan) and ELISA Insulin kit (Seikagaku Industries, Tokyo, Japan). Serum total cholesterol and triglyceride were determined using an Automated Chemistry Analyzer (AU400, Olympus, Tokyo, Japan).

Statistical Analysis  Results obtained were expressed as the mean±S.E. The data were analyzed by one-way analysis and Duncan’s multiple range test or non-parametric statistics. In all cases, p<0.05 was considered significant.

RESULTS

Effects of LMW Chitosan on Non-fasting Serum Glucose and Insulin Levels  Figure 2 shows the effect of LMW chitosan given as drinking water to KK-A'Y mice on non-fasting serum glucose levels for 11 weeks from 5 weeks of age. The non-fasting serum glucose levels of ICR mice of the normal control were 167±195 mg/dl throughout an 11-week observation period from 5 weeks of age. The serum glucose levels of KK-A'Y mice at 5 weeks of age were significantly higher than those of ICR mice at the same age (ICR: 167±10 mg/dl vs. KK-A'Y: 301±17 mg/dl, p<0.01). Thereafter, the glucose levels of the KK-A'Y mice continued to increase gradually as the day went on until 16 weeks of age, when the experiment was terminated (16 weeks of age: 443±45 mg/dl). LMW chitosan markedly lowered the serum glucose levels of KK-A'Y diabetic mice, from about 1 week of age when the experiment was terminated (6 weeks of age: control, 434±21 mg/dl vs. 0.05% LMW chitosan, 288±27 mg/dl; 0.2% LMW chitosan, 318±28 mg/dl; 0.8% LMW chitosan, 257±15 mg/dl, p<0.05; 6-week treatment: control, 431±49 mg/dl vs. 0.05% LMW chitosan, 260±15 mg/dl, p<0.01; 0.2% LMW chitosan, 242±16 mg/dl, p<0.01; 0.8% LMW chitosan, 249±19 mg/dl, p<0.01; 11-week treatment: control, 443±45 mg/dl vs. 0.05% LMW chitosan, 379±23 mg/dl; 0.2% LMW chitosan, 324±28 mg/dl, p<0.05; 0.8% chitosan, 229±20 mg/dl, p<0.01).

The non-fasting serum insulin levels of KK-A'Y mice were markedly higher than those of ICR mice at 6, 10 and 14 weeks of age (6 weeks of age: ICR, 585±53 pg/ml vs. KK-A'Y mice, 8650±1016 pg/ml, p<0.01; 14 weeks of age: ICR mice, 536±87 pg/ml vs. KK-A'Y, 14172±430 pg/ml, p<0.001) (Fig. 3). LMW chitosan dose-dependently lowered the insulin levels of KK-A'Y mice at 5 and 9 weeks after the start of treatment (9-week treatment: control, 14172±430 pg/ml vs. 0.05% LMW chitosan, 11112±1230 pg/ml; 0.2% LMW chitosan, 8671±1185 pg/ml, p<0.01; 0.8% LMW chitosan, 7168±1279 pg/ml, p<0.01).

Effects of LMW Chitosan on Non-fasting Serum Total Cholesterol and Triglyceride Levels  The non-fasting serum total cholesterol levels of KK-A'Y mice were not significantly different from those of ICR mice throughout the observation period of 5 to 16 weeks of age (16 weeks of age: ICR, 139±5 mg/dl vs. KK-A'Y, 147±7 mg/dl). The total cholesterol levels of KK-A'Y mice were not affected by long-term treatment with LMW chitosan.

Figure 4 shows the effects of LMW chitosan given as drinking water on non-fasting serum triglyceride levels. The non-fasting serum triglyceride levels of KK-A'Y mice had already been markedly higher than those of ICR mice at 5 weeks of age. Thereafter, the triglyceride levels of KK-A'Y mice gradually increased throughout the observation period (16 weeks of age: ICR, 85±5 mg/dl vs. KK-A'Y, 430±67 mg/dl, p<0.01). LMW chitosan was markedly effective in lowering the triglyceride levels of KK-A'Y diabetic mice from 1 week after the start of treatment (1-week treatment: control, 216±13 mg/dl vs. 0.05% LMW chitosan, 142±14 mg/dl, p<0.01; 0.2% LMW chitosan, 150±10 mg/dl, p<0.01; 0.8% LMW chitosan, 133±6 mg/dl, p<0.01; 11-week treatment: control, 430±67 mg/dl vs. 0.05% LMW chitosan, 199±15 mg/dl, p<0.05; 0.2% LMW chitosan, 208±21, p<0.01; 0.8% LMW chitosan, 133±15 mg/dl, p<0.01).
KK-Ay control and LMW chitosan-treated KK-Ay mice, from 5 to 16 weeks of age. At the onset of the experiment (at 5 weeks of age), the average body weight of ICR and KK-Ay mice was 24.8 and 26.5 g, respectively. The ICR and KK-Ay control mice gained 19.8 and 18.7 g, respectively, by 16 weeks of age when the experiment was terminated. On the other hand, the body weight of 0.05, 0.2 and 0.8% LMW chitosan-treated mice increased by 17.5, 15.8 and 14.7 g, respectively. Thus, body weight gain in chitosan-treated mice was dose-dependently lower than that of the control mice.

**Effects of LMW Chitosan on Drinking Water Consumption and Urine Volume**

Figure 6 shows the effects of LMW chitosan on drinking water consumption (left) and urine volume (right). The drinking water consumption and urine volume per 24 h of KK-Ay mice were significantly higher than those of ICR mice from 9 weeks of age. The increase in drinking water consumption and urine volume of KK-Ay mice from 9 weeks of age was significantly inhibited by the daily administration of 0.05, 0.2 or 0.8% LMW chitosan.

**DISCUSSION**

The present study indicates that daily administration of LMW chitosan as drinking water is effective in improving hyperglycemia, hyperinsulinemia and hypertriglyceridemia of KK-Ay mice with genetically obese type 2 diabetes mellitus. Diabetes of KK-Ay mice closely resembles that of obesity-related NIDDM in humans with hyperinsulinemia and hypertriglyceridemia. In the present experiment, the non-fasting serum glucose and triglyceride levels of the experimental mice at 5 weeks of age were significantly higher than those of normal ICR mice at the same age. Thereafter, both serum biochemical parameters continued to increase until 16 weeks of age, when the experiment was terminated. When 0.05, 0.2 or 0.8% LMW chitosan was given as drinking water, this compound markedly lowered the serum glucose and triglyceride levels of the experimental mice at 5 weeks of age were significantly higher than those of normal ICR mice at the same age. Thereafter, both serum biochemical parameters continued to increase until 16 weeks of age, when the experiment was terminated. When 0.05, 0.2 or 0.8% LMW chitosan was given as drinking water, this compound markedly lowered the serum glucose and triglyceride levels throughout the experimental period until 11 weeks from 1 week of the start of treatment. In this case, the degree of serum glucose-lowering action of LMW chitosan at lower concentrations (0.05, 0.2%) decreased daily from 6 weeks after the start of the treatment.

This result suggests that treatment with LMW chitosan at concentrations less than 0.2% may be not produce a beneficial effect on obese-type diabetes of KK-Ay mice that has.
dramatically progressed. In this experiment, we also examined the effects of LMW chitosan on drinking water consumption and urine volume per 24 h during the process of the progression of diabetes in KK-A<sup>y</sup> mice. The taste of the water solution of this compound may affect the drinking water consumption. Therefore, we gave distilled water instead of LMW chitosan solution only when each mouse was kept in an individual cage to measure drinking water consumption. As a result, overdrinking and polyuria were not observed in KK-A<sup>y</sup> mice given LMW chitosan solution. As mentioned in the introduction, hypertriglyceridemia may play an important role in the development or progression of diabetes mellitus of KK-A<sup>y</sup>. Therefore, it is suggested that the blood glucose-lowering action of LMW chitosan may be due in part to the triglyceride-lowering action of this compound. In this experiment, blood glucose and triglyceride-lowering actions of LMW chitosan preceded the insulin-lowering action of the compound. This result suggests that the insul-
lowering the action of LMW chitosan may be a secondary action as the result of improvement of abnormal lipid and glucose metabolism in KK-A^y diabetic mice. Miura et al.\(^{18}\) first demonstrated that chitosan given as a 5% food mixture for 4 weeks was significantly effective in lowering blood glucose levels in normal and neonatal STZ-induced diabetic mice, although this compound failed to lower serum glucose and triglyceride levels in KK-A^y mice. We previously reported that LMW chitosan given as drinking water prevented the progression of low dose STZ-induced slowly progressive non-obese NIDDM in ICR mice.\(^{21}\) However, in a previous report, this compound was ineffective in lowering serum glucose levels in normal ICR mice. Thus, our results obtained with the administration of chitosan to KK-A^y diabetic mice and normal mice are not in agreement with their results. The difference in molecular weight as well as the solubility and viscosity in the stomach of chitosan used, may affect the blood glucose- and lipid-lowering activities of this compound.

In the present experiment, no apparent increase in non-fasting serum total cholesterol levels was observed in KK-A^y diabetic mice. Chitosan did not affect the total cholesterol levels. However, we have demonstrated that the daily administration of LMW chitosan given as drinking water markedly prevents an increase in the total cholesterol levels in normal ICR mice fed a cholesterol-rich diet (unpublished data, Ito et al.).

Recently, it has been reported that activation of the hexosamine pathway causes insulin resistance induced by chronic hyperglycemia.\(^{22-24}\) LMW chitosan may further increase insulin resistance of KK-A^y diabetic mice by activating the hexosamine pathway, because it is chemically a polymer of D-glucosamine. However, the daily administration of LMW chitosan showed anti-diabetic action and improved hyperinsulinemia. Therefore, it is unlikely that the anti-diabetic action of LMW chitosan is due to D-glucosamine, a monosaccharide contained in chitosan. The mechanism of the absorption of chitosan from the small intestine has not yet been well defined. It is believed that chitosan may be primarily absorbed after it has been transformed into oligosaccharides by chitosanase secreted from intestinal bacteria, or by lysozyme in intestinal fluid. Consequently, LMW chitosan may exert its anti-diabetic action with the oligosaccharides, but not with the monosaccharide.

In the present experiment, the drinking administration of LMW chitosan showed a slight but dose-dependent anti-obesity action, although food intake was not affected by the administration of this compound. The anti-obesity action of LMW chitosan may be in part related to its blood glucose-lowering effect. As mentioned above, however, LMW chitosan failed to reduce non-fasting serum glucose in normal ICR mice, although this compound revealed a slight anti-obesity action.\(^{21}\) Therefore, it is unlikely that the anti-obesity action of LMW chitosan affects the blood glucose-lowering activity of this compound to a great degree. It has been reported that high molecular weight chitin-chitosan may partly cause the lipid-lowering and anti-obesity actions in mice fed a high fat diet by inhibiting the intestinal absorption of dietary fat.\(^{25}\) However, the exact mechanism by which LMW chitosan improves the hypertriglyceridemia in KK-A^y diabetic mice remains unclear. Further study is needed to clarify this mechanism.

We have reported that the daily administration of LMW chitosan as drinking water prevents the progression of low dose STZ-induced slowly progressive NIDDM in mice.\(^{21}\) We have shown that this progressive diabetic model is non-obese NIDDM, which is characterized by an impaired insulin response to glucose stimulation.\(^{20}\) Therefore, LMW chitosan may be useful for the treatment of both types of non-obese and obese type 2 diabetes mellitus.

REFERENCES