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Antidiabetic Activity and Mechanisms of Acarbose in KKA^y Mice

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To elucidate antidiabetic effect and mechanism(s) of acarbose in a polygenic spontaneous hyperglycemic and hyperinsulinemic diabetic animal model, KKA^y mice, acarbose was administered orally for 4 weeks and effects on body weight, plasma glucose and insulin levels, genetic expressions of intestinal sucrase-isomaltase (SI), sodium-glucose cotransporter (sGLT1) and glucose transporter in quadriceps muscle (GLUT4) were examined in this study. Although no differences in body weight were detected between control and acarbose-treated groups, plasma glucose level in acarbose-treated group was markedly reduced as compared to the control. In the mechanism study, acarbose downregulated the SI and SGLT1 gene expressions, and upregulated the GLUT4 mRNA and protein expressions when compared to the control group. In conclusion, the data obtained strongly implicate that acarbose can prevent the hyperglycemia in KKA^y mice possibly through blocking intestinal glucose absorption by downregulations of SI and sGLT1 mRNA expressions, and upregulation of skeletal muscle GLUT4 mRNA and protein expressions.

Key Words: Acarbose, KKA^y mouse, Sucrase-isomaltase (SI), sGLT1, GLUT4

INTRODUCTION

Acarbose is a competitive inhibitor of intestinal glucosidase, and is efficient in reducing postprandial plasma glucose and insulin levels in diabetic subjects (Reaven et al, 1990) and in non-diabetic and diabetic rats fed with carbohydrate diets containing acarbose (Gray & Olefsky, 1982; Lee et al, 1983). Acarbose is also efficient in treating obesity as it decreases plasma insulin levels, and since metabolic abnormalities can be partly ascribed to hyperinsulinemia (Jeanrenaud, 1990). In genetically obese animals (*fa/fa* rats or *db/db* mice), acarbose treatment prevented hyperglycemia and decreased insulin levels. Hyperinsulinemia plays a key role not only in the development of the obese syndrome, since it promotes adipose and liver lipogenesis, but also in the establishment and maintenance of insulin resistance (Jeanrenaud, 1979).

Although quite a few reports have been made on the antidiabetic activity of the acarbose, there is no experiment to elucidate antidiabetic effect and mechanism(s) of acarbose in a polygenic spontaneous hyperglycemic and hyperinsulinemic diabetic animal model, KKA^y mice. Effects of acarbose on body weight, plasma glucose and insulin levels, genetic expressions of intestinal sucrase-isomaltase (SI), sodium-glucose cotransporter (sGLT1) and glucose transporter in quadriceps muscle (GLUT4) were examined in this study.

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METHODS

Animals

Male KKA^y mice (Clea, Tokyo, Japan) weighing 20~25 g were used. The mice were kept for 1 week in a temperature (25±2°C) and moisture (50%) controlled chamber, and fed *ad libitum* a regular laboratory chow (SamYang-Sa, KangWon-Do, Korea) with ready access to water. Animals were divided into two groups containing eight mice each as follows: Group 1, diabetic mice drinking a tap water; Group 2, diabetic mice administered with 50 mg/kg of acarbose. During 28 days of treatment, body weight

and blood glucose level were determined once in a week. Glycated hemoglobin and insulin levels in blood were examined at 28th day. On the last day of treatment, intestine and quadriceps muscle were removed and kept in a deep freezer until SI and sGLT1 mRNA expressions and GLUT4 mRNA and protein expressions were determined, respectively.

Determination of plasma glucose and insulin

Blood samples were obtained from the orbital venous plexus using capillary glass tubes without anesthesia. Plasma glucose was measured once in a week by the glucose-oxidation method (Bergmeyer & Bernt, 1974), and insulin levels were determined at 28th day of treatment by a radioimmunoassay kit (Coat-A-Count Insulin, DPC, USA) using a double-antibody technique to separate free and bound insulin.

Determination of blood glycated hemoglobin (HbA_{1c})

HbA_{1c} was determined using the Glycated Hemoglobin Kit (Sigma, procedure No. 441). HbA_{1c} was separated using a cation exchange resin based on their charge and its absorbance measured at 415 nm (Boil et al, 1980).

RNA extraction and RT-PCR

Total RNA from quadriceps muscle or small intestine was prepared using easy-BLUE (Intron Co., Seoul, Korea) according to the manufacturer's instruction after homogenization with motor-driven pellet pestle (Chomczynski & Sacchi, 1987). The concentration of RNA was determined by absorbance at 260 nm. One microgram of total RNA was reverse transcribed into cDNA using Moloney murine leukemia virus reverse transcriptase with oligo-(dT)12-18 primers. Each base pairs of the coding region of SI. sGLT1 and GLUT4 gene was amplified by polymerase chain reaction (PCR) with total RNA as the template (SI: upstream primer 5'-TAT TCC AGA CAC CCC TAC CA-3', downstream primer 5'-AGG AGT TGG CTG GAA TGT AA-3'; sGLT1 upstream primer 5'-TCA TGC CTA ATG GAC TGC AA-3', downstream primer 5'-AGG TCG ATT CGC TCT TCC TT-3'; GLUT4 upstream primers 5'-ACA GAA GGT GAT TGA ACA GAC-3', downstream primer 5'-ACC CGT CCA AGA ATG AGT ATC-3'). A mouse GAPDH probe was used to monitor the amount of total RNA in each sample. The PCR product of SI, sGLT1 and GLUT4 was 394, 601 and 285 base pairs, respectively. Each cDNA was amplified according to following conditions: initial template denaturation was performed at 94°C for 5 min followed by 30 cycle consisting of denaturation - 30s at 94°C, annealing - GLUT4 and sGLT1; 30s at 57°C, SI; 30s at 55°C, extension - 30s at 72°C. Then, post-incubation was performed at 75°C for 5 min. PCR products were electrophoresised on 1.5% agarose gel and quantitated by a densitometry.

Western blot analysis for GLUT4

For determination of GLUT4 protein, quadriceps muscle was homogenized in a 1 : 10 dilution (w/v) of homogenizing buffer containing 250 mM sucrose, 2 mM EDTA, 10 mM Tris (pH 7.4) and centrifuged at 3,700 g for 25 min at 4°C. KCl was added to the supernatant to 0.8 M, mixed for 30 min at 4°C and followed by centrifugation at 150,000 g for 1h at 4°C. The pellet (total membranes) was resuspended in homogenizing buffer with 25-gauge needle, added same volume of RIPA buffer and centrifuged at 100,000 g for 1h at 4°C. Protein concentration of the supernatant was determined, and membrane proteins were used for Western-blot analysis of GLUT4.

Total protein samples (10 μg) were subjected to SDS-polyacrylamide on a 10% resolving gel. After completion of electrophoresis, proteins were transferred onto nitrocellulose membrane (Hybond N, Amersham Pharmacia Biotech, England) in transfer buffer (50 mM Tris-HCl, pH 7.0, 380 mM glycine, and 20% methanol). The blot was blocked in 5% nonfat dry milk dissolved in phosphate-buffered saline with 0.1% Tween 20, followed by incubation for 2 h with polyclonal goat anti-GLUT4 antibody (Santacruz, California, USA), followed by 1 h incubation with anti-goat IgG conjugated to horseradish peroxidase (Bio-rad, Hercules, USA). The luminescence detection of peroxidase was performed with the enhanced chemiluminescence system (Pierce, Rockford, USA) according to the manufacturer's instructions. The relative amount of positive immunoreactive proteins was quantified with densitometric analysis using a GS-700 imaging densitometer.

Statistical analysis

All the data were expressed as mean$\pm$S.E. and

Student's t-test was used for the statistical analysis. A p value less than 0.05 was considered to be significant.

RESULTS

Body weight and plasma glucose level of KKA^y mice at 28th day after oral administration of acarbose are shown in Table 1. Although no differences in body weight were detected between control and acarbose-treated groups, plasma glucose level in acarbose-treated group was markedly reduced as compared to the control.

Fig. 1 shows a plasma glucose level during four week period. Acarbose-treated group started to show lower the glucose level after one week and maintained lower glucose levels than those of the control group throughout an entire period.

Fig. 2 and 3 illustrate the effects of acarbose on individual HbA_{1c} and insulin levels in KKA^y mice, respectively. Although HbA_{1c} levels between groups are not significantly different, the horizontal bar representing the mean HbA_{1c} value in acarbose-treated group has been lowered than that in control group (5.4 vs 4.9), suggesting that acarbose persistently improved the hyperglycemic abnormality. Acarbose as a starch blocker is known to inhibit an intestinal glucosidase and blunt a postprandial hyperglycemia. As a result, acarbose tends to lower serum insulin level in hyperinsulinemic diabetic model, as shown in Fig. 3 (21.0 vs 12.0). Improvement in hyperinsulinemia might be clinically beneficial since insulin resistance syndrome could be resolved.

Next, we examined whether acarbose as an α-glycosidase inhibitor affects the gene expressions of intestinal sucrase-isomaltase (SI) complex enzyme,

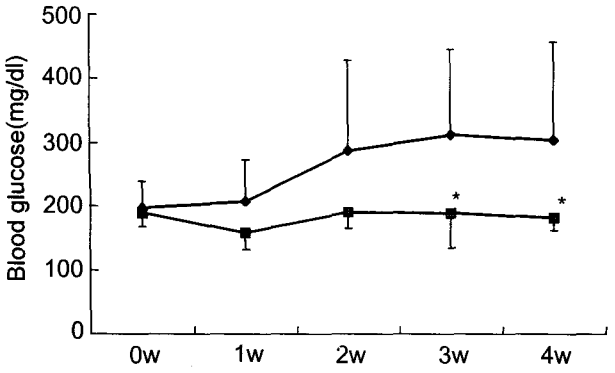

Fig. 1. Effect of acarbose on the blood glucose level in KKA^y mice. Each point represents the mean of 8 mice. -○-, control; -●- , acarbose 50 mg/kg. *P<0.05 compared to the control group.

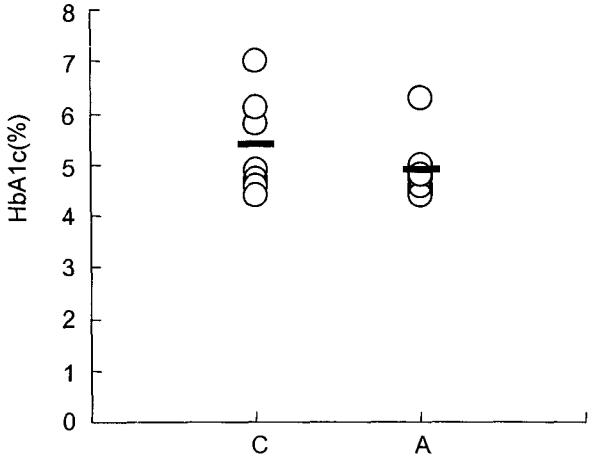

Fig. 2. Effect of acarbose on individual glycosylated hemoglobin level of KKA^y mice. Mice were sampled after 28 days of treatment without (C) or with acarbose (A). Each circle represents an individual glycosylated hemoglobin level and the horizontal bar represents the mean value in each group.

Table 1. Effects of acarbose on body weight and plasma glucose level in KKA^y mice

	Body weight (g)	Body length (cm)	BMI (g/cm^2)	Blood glucose (mg/dl)
Control	40.9±1.5	10.0±0.5	40.9±3.1	304±53
Acarbose	38.3±2.3	9.9±0.6	39.6±5.4	182±19*

BMI, Body Mass Index. Each value represents the mean±SE of 8 mice.
*P<0.05 compared to the control group

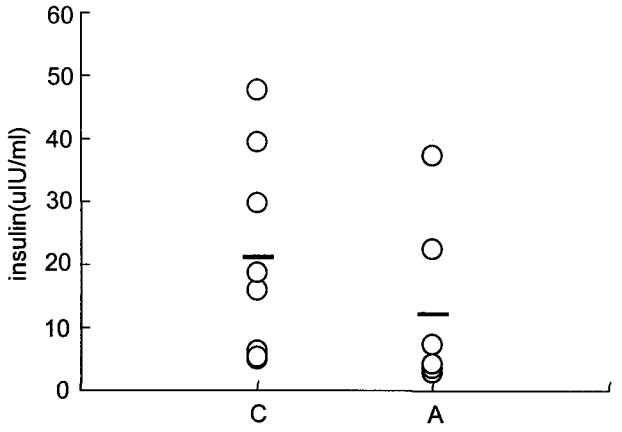

Fig. 3. Effect of acarbose on individual immunoreactive insulin (IRI) level of KKA^y mice. Mice were sampled after 28 days of treatment without (C) or with acarbose (A). Each circle represents an individual immunoreactive insulin level and the horizontal bar represents the mean value in each group.

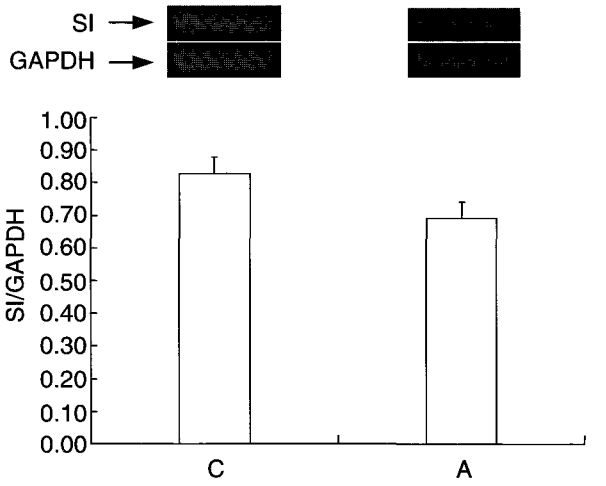

Fig. 4. Expression of SI mRNA in small intestine from control (C) and acarbose (A) treated KKA^y mice. Quantification of mRNA was normalized with GAPDH.

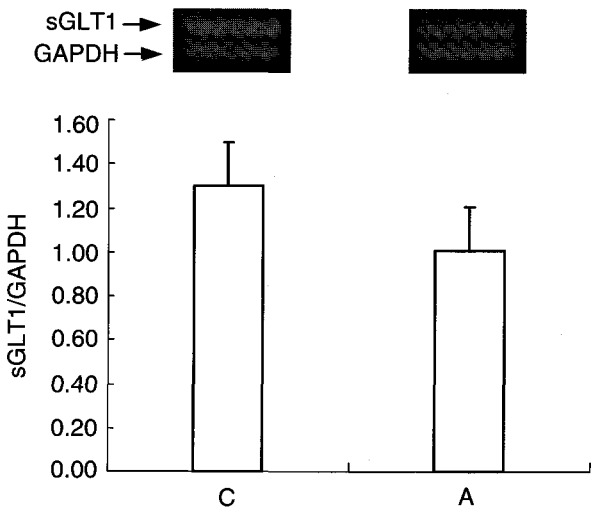

Fig. 5. Expression of sGLT1 mRNA in small intestine from control (C) and acarbose (A) treated KKA^y mice. Quantification of mRNA was normalized with GAPDH.

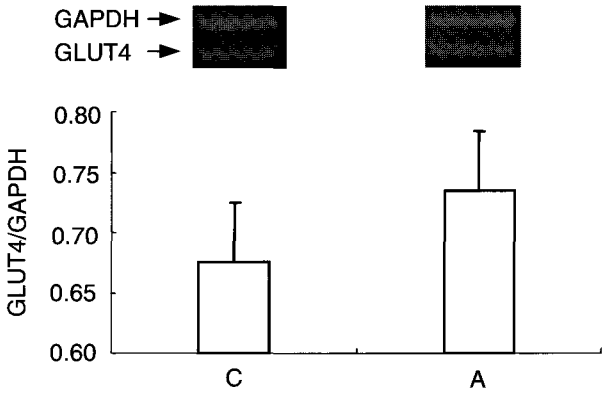

Fig. 6. Expression of GLUT4 mRNA in quadriceps muscle from control (C) and acarbose (A) treated KKA^y mice. Quantification of mRNA was normalized with GAPDH.

which is a major enzyme among glycosidases. We also examined whether acarbose affects intestinal glucose transporter protein or not. Effect of acarbose on mRNA expression of SI gene was examined at 28th day of administration. As shown in Fig. 4, acarbose-treated group (A) revealed a lower mRNA expression of SI gene when compared to control group (C). To determine whether acarbose also affects on monosaccharide absorption through the sodium-glucose cotransporter (sGLT1) or not, we explored the effect of acarbose on mRNA expression of sGLT1 gene. Fig. 5 shows that acarbose-treated group (A) had reduced sGLT1 gene expression as compared with control group (C), probably due to reduction in the amount of free monosaccharides available for absorption.

Glucose transporter in nonepithelial mammalian tissues occurs by facilitated diffusion mediated by a family of glucose transporters. Among six different tarnsporter proteins of the facilitative diffusion type, GLUT4 expression is circumscribed to tissues where glucose transporter is insulin sensitive, i.e., skeletal and cardiac muscles, and brown and white fat. GLUT4 mRNA and protein expressions in KKA^y

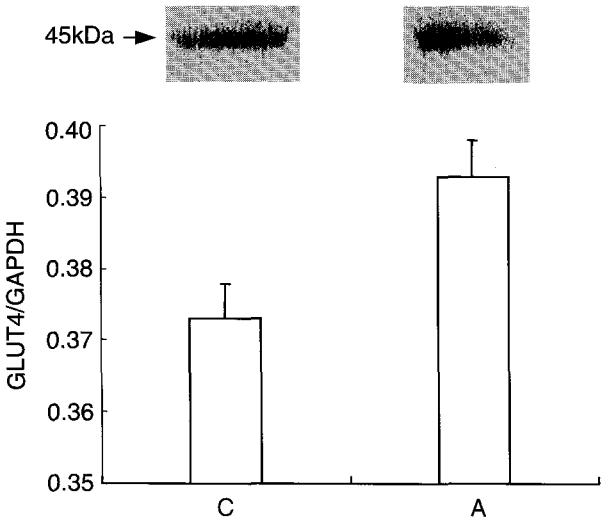

Fig. 7. Western blot analysis of GLUT4 protein in quadriceps muscle membrane from control (C) and acarbose (A) treated KKA^y mice. Skeletal muscles were sampled after 28 days of treatment without or with acarbose. 10 μg of membrane protein was separated by SDS gel electrophoresis and Western blotted.

mice was known to be depressed in fat cells and skeletal muscles. Therefore, we like to know whether acarbose may have any effect on the gene expressions of GLUT4 or not. As shown in Fig. 6 and 7, acarbose significantly increased the GLUT4 mRNA and protein levels as compared to control group.

DISCUSSION

Hyperglycemia is one of the chronic complications that occur in diabetes mellitus. Oral administration of the α-glucosidase inhibitor acarbose has been shown to reduce postprandial blood glucose levels in both experimental animals and humans in a dose-dependent manner. To date, the success of this approach has reinforced the accepted view that α-glucosidase inhibitors reduce glucose absorption by inhibiting disaccharide digestion and thus delay the subsequent monosaccharide absorption (Legler, 1967). To examine the possibility that α-glucosidase inhibitor could also act on monosaccharide absorption directly or indirectly, we determined the gene expressions of sucrase-isomaltase (SI) complex and sGLT1, both of which are on the surface of the intestine brush border membrane.

We propose that there is a linkage between α-glucosidase and sGLT1. As shown in Fig. 4 and 5, mRNA expressions of SI complex and sGLT1 were all reduced when compared to the control group. Reduction of the gene expression of SI complex, which is responsible for degradation of starch and oligosaccharides, would in turn cause a decrease in the absorption of glucose and consequently inhibit the increase in postprandial blood glucose. Decrease in the amount of free monosaccharides available for absorption resulting from administration of acarbose could have an influence on the glucose transporter activity. Recently Hirsh et al reported that α-glucosidase inhibitors can decrease D-glucose absorption in the jejunum by inhibiting the uptake of free glucose across the brush border membrane via sGLT1, although it does not exert its effect directly on the transport protein (Hirsh et al, 1997). Hirsh et al, however, do not know how glucosidase inhibitor act on the entry of free glucose into the enterocyte. Fig. 5 shows that sGLT1 gene expression was reduced in the acarbose-treated group, additionally resulting in correction of postprandial hyperglycemia.

Decreased glucose uptake has been substantiated in different tissues such as skeletal muscle (Le Marchand-Brustel et al, 1978) or adipocytes (Kahn & Cushman, 1985) of obese animals and humans. This alteration is thought to play a major role in the development of hyperglycemia and insulin resistance. This diminution results, at least in part, from the reduction in the number of glucose trasporters. In adipocytes from fasted and refed rats, the number of glucose transporters strongly correlates with the specific mRNA abundance. It is tempting to hypothesize that acarbose is able to prevent both the decrease in transporter mRNA and in glucose transporter activity, which occurs in obese animals. In this study, we measured the mRNA and protein expressions of glucose transporter GLUT4 presented in skeletal muscle. As shown in Fig. 6 and 7, acarbose significantly upregulated the GLUT4 mRNA and protein levels. Clearly, more studies are needed to clarify the mechanism of its action, since we do not know exactly whether this upregulation is due to the direct action of acarbose on the glucose transporter or simply due to the consequence of reduction in insulin resistance.

In conclusion, the data obtained strongly implicate that acarbose can improve the hyperglycemia in KKA^y mice possibly through blocking intestinal glucose absorption by inhibition of α-glucosidase

activity, and upregulation of skeletal muscle GLUT4 mRNA and protein expressions.

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