The Hypoglycemic Effects of Acarviosine-Glucose Modulate Hepatic and Intestinal Glucose Transporters In vivo

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Abstract Acarviosine-glucose (AcGl) is an α-glucosidase inhibitor and has similar inhibitory activity to acarbose in vitro. We synthesized AcGl by treating acarbose with Bacillus stearothermophilus maltogenic amylase and fed C57BL/6J and db/db mice with diets containing purified AcGl and acarbose for 1 week. AcGl (50 and 100 mg/100 g diet) significantly reduced plasma glucose and triglyceride levels in db/db mice by 42 and 51%, respectively (p<0.0001). The hypoglycemic and hypotriglyceremic effects of AcGl were slightly, but significantly, greater than those seen with acarbose treatment (p<0.01). The expression of two novel glucose transporters (GLUTs), GLUT10 and GLUT12, were examined by Western blot analysis. GLUT10 was markedly increased in the db/db livers. After AcGl treatment, the expression of hepatic GLUT10 was decreased whereas intestinal GLUT12 was significantly increased in both strains of mice. Our results show that AcGl improves plasma lipid and glucose metabolism slightly more than acarbose. Regulation of hepatic GLUT10 and intestinal GLUT12 may be important in controlling blood glucose levels.

Keywords: α-glucosidase inhibitor, acarviosine-glucose, diabetes mellitus, in vivo, glucose transporter-10, glucose transporter-12

Introduction

Alpha-glucosidase inhibitors block brush border enzymes such as maltase, sucrase, and α-amylase, thus reducing the production of glucose from starch-based foods and improving hyperglycemia in diabetic patients (1, 2). Acarbose, one of the α-glucosidase inhibitor drugs, is efficient for both the treatment and prevention of hyperglycemia (3, 4), and could lower the risk of coronary heart disease among diabetic patients (5, 6). Some adverse effects of acarbose include flatulence and abdominal distension associated with the fermentation of unabsorbed carbohydrates or delayed carbohydrate digestion.

Acarviosine-glucose (AcGl) shows similar levels of α-glucosidase inhibitor activity in vitro (7-9) compared with acarbose and is relatively resistant to intestinal fermentation due to the lack of a fermentable glucose moiety from the non-reducing end of acarbose (10). This characteristic of AcGl may help diminish the adverse effects associated with conventional α-glucosidase inhibitor treatments. AcGl can be produced from acarbose following its incubation with Bacillus stearothermophilus maltogenic amylase (BSMA) (11-13).

Controlling hyperglycemia by α-glucosidase inhibitor treatment could beneficially affect cellular glucose uptake mediated by glucose transporters (GLUTs). The GLUT family is subdivided into three different classes (Class I, II, and III) according to sequence homology. GLUTs mediate facilitative and energy independent transport of glucose across the cell membranes in various tissues. Some GLUTs are insulin sensitive and are thought to be associated with diabetes due to the chromosomal locations of the corresponding genes. GLUT4 is a well-characterized insulin sensitive GLUT that is abundant in muscle. The translocation of GLUT4 from intracellular stores to the plasma membrane in response to insulin is enhanced by exercise and improved hyperglycemia. Two Class II GLUTs, GLUT10 and 12, are highly expressed in the liver and the small intestine, respectively, and both are presumed to be associated with diabetes. This is because the GLUT 10 gene is located in the Type II diabetes-linked region of human chromosome 20, and GLUT 12 is specifically expressed in insulin-sensitive tissues and can be translocated from the cytosol to the cell membrane in response to insulin, as is GLUT4. However, the expression of GLUT10 and GLUT12 as a consequence of improved hyperglycemia is not well understood.

In this report we studied the hypoglycemic effects of AcGl in a mouse model of monogenic diabetes (db/db) and in C57BL/6J control mice. After a 1-week treatment, AcGl compounds effectively improved diabetic hyperglycemia. The expression of hepatic GLUT10 and intestinal GLUT12 was markedly elevated in db/db mice compared with C57BL/6J mice. After AcGl treatment, hepatic GLUT10 was significantly reduced whereas intestinal GLUT12 expression was increased. Our results indicate that short-term treatment with AcGl improves diabetic hyperglycemia in mice and modulates GLUT expression in the liver and small intestine.

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Materials and Methods

Preparation of acarbose and AevGlc Acarbose was generously provided by Bayer Korea (Seoul, Korea). The culture and purification of B. steatorrhophilus maltogenic amylase (BSMA) was described previously (11). AevGlc was produced enzymatically from acarbose by incubation with BSMA as described previously (8, 9). Acarbose and AevGlc were analyzed using high-performance ion chromatography (HPIC). Filtered samples were applied to a CarboPac PA1 column (0.45x25 cm, Dionex, Sunnyvale, CA, USA) equipped with an electrochemical detector (ED40; Dionex). The sample was eluted with a linear gradient ranging from 100% eluent A (150 mM NaOH in double distilled water) to 40% eluent B (150 mM NaOH + 600 mM sodium acetate) over 40 min. The flow rate of the mobile phase was maintained at 1.0 mL/min.

Animals and feeding studies The db/db mice (BKS.Cg-mj+/-Leprdb/)) provide a monogonic mouse model of type II diabetes due to leptin receptor deficiency, and C57BL/6J mice serve as the control. The control mice have an identical genetic background to the db/db mice. The mice were purchased from Jackson Laboratory (Bar Harbor, ME, USA) and were maintained in the animal facilities at Hae-Eun Biotech (Seoul, Korea). The animals were kept in a specific-pathogen-free room at 21-25°C with a 12-hr day/night cycle, and water and standard chow were provided ad libitum. All experiments were performed using protocols approved by the Committee on Animal Experimentation of the Hae-Eun Biotech Research Committee. Ten to 12 weeks old mice were fed normal chow or chow containing purified α-glucosidase inhibitors (acarbose or AevGlc) for one week. Two different amounts of the two α-glucosidase inhibitors were used, 50 mg/100 g chow (Acarbose 50 and AevGlc 50) and 100 mg/100 g chow (Acarbose 100 and AevGlc 100). After feeding, the mice were fasted overnight (16-19 hr) and then blood samples were collected retro-orbitally in purple-topped EDTA tubes for further analysis.

Measurements Blood plasma was separated by centrifugation, and then glucose, total cholesterol, total triglyceride, and free fatty acid levels were measured enzymatically (Shin-Yang Chemical, Seoul, Korea). Protein concentration was determined using a Bio-Rad protein kit (Bio-Rad, Hercules, CA, USA) with bovine serum albumin (Sigma Chemical Co., St. Louis, MO, USA) as a standard.

Oral glucose tolerance test (OGTT) Four age-matched male and female mice from each genotype were raised on the AevGlc 100 diet. After one week on the Acarbose 100 diet, mice were fasted for 18 hr, then anesthetized and given glucose (1.0 g/kg of 20% D-glucose, injected orally) for the OGTT. The test was performed based on previously reported methods with minor modifications (14, 15). Blood samples were obtained retro-orbitally at 0, 15, 30, 40, 60, and 120 min after glucose administration. Glucose elimination was quantified as the Kg (i.e., the glucose elimination constant), which is defined as the reduction in circulating glucose between 1 and 20 min after the intravenous administration of glucose and is calculated after the logarithmic transformation of the individual plasma glucose values. Kg is expressed as the percent elimination of glucose per min. For the OGTT, the Kg was calculated based on the glucose elimination rate between 15 and 60 min.

Western blot analysis Liver membrane preparations were made as previously described (16). The anti-mouse low density lipoprotein (LDL) receptor and LDL receptor related protein (LRP) antibodies were gifts from Dr. Allen Cooper (Stanford School of Medicine). Anti-mouse apoE antibody was purchased from Academy Biomedical (Houston, TX, USA). Anti-GLUT5, -GLUT10, and -GLUT12 antibodies were purchased from Alpha Diagnostics International (San Antonio, TX, USA). Horseradish peroxidase (HRP)-conjugated secondary antibody was purchased from Merck-Korea (Seoul, Korea). Western blots were performed by standard procedures and bands were visualized using the ECL method (Amersham-Pharmacia Korea, Seoul, Korea). The density of each band was quantified using Signagel software (Jandel Scientific, San Rafael, CA, USA).

Data analysis The data were expressed as the mean ± SE, unless otherwise indicated. Student's t-test was performed for two-group comparisons. Values were considered statistically significant at p<0.05.

Results and Discussion

Animal feeding studies, baseline glucose, and lipid concentrations The purity of the AevGlc used in the feeding studies was approximately 96% (Fig. 1). One week of feeding with diets containing acarbose or AevGlc reduced the fasting glucose and triglyceride levels in both strains of mice (Table 1). The body weight and food intake of each strain was unchanged during feeding (Table 1). Overall, the hypoglycemic and hypolipidemic effects of AevGlc were similar to those of acarbose. However, in C57BL/6 mice, AevGlc 100 reduced plasma glucose and triglyceride slightly more efficiently than acarbose (Table 1). The reduction in free fatty acids was significant with the AevGlc 100 diet (154±8, baseline; 118±8 μeq, AevGlc100, p<0.01), but not with the Acarbose 100 diet. In db/db mice, the effects of acarbose and AevGlc on blood glucose and triglyceride levels were similar (Table 1).

Oral glucose tolerance test Glucose tolerance was examined after 1 week on the AevGlc 100 diet (Fig. 2A and 2B). The administration of AevGlc improved glucose tolerance significantly in both hyperglycemic db/db and C57BL/6J mice (p<0.0001). In C57BL/6J, the plasma glucose level reached a maximum at 30 min after glucose challenge. Thereafter, glucose elimination persisted until 120 min when the original plasma glucose level was restored. AevGlc treatment lowered the maximum glucose level at 30 min to the fasting glucose concentration (Fig. 2B). By contrast, there was little glucose elimination between 15 and 60 min in db/db mice, which suggests severe glucose intolerance (Fig. 2A). In both animals, the
Acarbose-Glucose Reduces Intestinal Glucose Transporters

(A) 

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\begin{array}{l}
\text{Acarbose} \quad \xrightarrow{\text{BSMA}} \quad \text{Acarviosine-glucose (AcvGlc)} + \text{D-glucose}
\end{array}
\]

(B) 

![Diagram of retention time vs. voltage for Acarbose and AcvGlc]

Fig. 1. A. Enzymatic production of acarviosine-glucose from acarbose by Bacillus steareothermophilus maltogenic amylase (BSMA). B. HPEC analyses of acarbose and purified AcvGlc. (a) Acarbose before treatment with BSMA, (b) AcvGlc purified after treatment with BSMA. The purities of acarbose and AcvGlc are 92 and 96%, respectively.

Table 1. Plasma concentrations of glucose, triglycerides (TG), and cholesterol in control (C57BL/6J) and diabetic (db/db) mice

<table>
<thead>
<tr>
<th></th>
<th>Body weight (g)</th>
<th>Food intake (g/day)</th>
<th>Glucose (mg/dL)</th>
<th>TG (mg/dL)</th>
<th>Cholesterol (mg/dL)</th>
</tr>
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<tbody>
<tr>
<td>C57</td>
<td></td>
<td></td>
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<tr>
<td>Baseline</td>
<td>21.1±0.6</td>
<td>2.8±0.2</td>
<td>130.0±18.0</td>
<td>79.9±19.4</td>
<td>94.4±14.7</td>
</tr>
<tr>
<td>Acarbose 50</td>
<td>21.2±0.7</td>
<td>2.7±0.1</td>
<td>123.0±13.0</td>
<td>78.3±14.6</td>
<td>98.3±12.6</td>
</tr>
<tr>
<td>Acarbose 100</td>
<td>21.1±0.5</td>
<td>2.7±0.2</td>
<td>106.9±8.5&lt;sup&gt;3&lt;/sup&gt;</td>
<td>72.9±11.3</td>
<td>83.8±20.0</td>
</tr>
<tr>
<td>AcvGlc 50</td>
<td>20.5±0.4</td>
<td>2.8±0.1</td>
<td>119.4±19.3</td>
<td>55.9±3.3&lt;sup&gt;3&lt;/sup&gt;</td>
<td>91.2±6.5</td>
</tr>
<tr>
<td>AcvGlc 100</td>
<td>21.0±0.5</td>
<td>2.8±0.3</td>
<td>59.0±4.8&lt;sup&gt;2,4&lt;/sup&gt;</td>
<td>55.6±4.7&lt;sup&gt;4&lt;/sup&gt;</td>
<td>85.1±13.3</td>
</tr>
<tr>
<td>DB</td>
<td></td>
<td></td>
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</tr>
<tr>
<td>Baseline</td>
<td>41.5±1.7</td>
<td>7.3±0.2</td>
<td>479.9±12.8</td>
<td>125.3±7.4</td>
<td>113.5±5.1</td>
</tr>
<tr>
<td>Acarbose 50</td>
<td>40.9±1.6</td>
<td>7.3±0.1</td>
<td>365.6±24.0&lt;sup&gt;2&lt;/sup&gt;</td>
<td>107.9±8.6</td>
<td>112.5±7.5</td>
</tr>
<tr>
<td>Acarbose 100</td>
<td>41.1±2.0</td>
<td>7.4±0.2</td>
<td>281.4±18.9&lt;sup&gt;2,3&lt;/sup&gt;</td>
<td>64.3±4.4&lt;sup&gt;2,3&lt;/sup&gt;</td>
<td>97.3±4.3&lt;sup&gt;2&lt;/sup&gt;</td>
</tr>
<tr>
<td>AcvGlc 50</td>
<td>41.8±1.4</td>
<td>7.4±0.1</td>
<td>348.0±28.0&lt;sup&gt;2&lt;/sup&gt;</td>
<td>102.6±5.2&lt;sup&gt;2&lt;/sup&gt;</td>
<td>118.0±7.5</td>
</tr>
<tr>
<td>AcvGlc 100</td>
<td>40.5±0.8</td>
<td>7.6±0.4</td>
<td>277.9±12.0&lt;sup&gt;2,3&lt;/sup&gt;</td>
<td>61.7±4.2&lt;sup&gt;2,3&lt;/sup&gt;</td>
<td>100.1±9.0</td>
</tr>
<tr>
<td>Baseline</td>
<td>41.5±1.7</td>
<td>7.3±0.2</td>
<td>479.9±12.8</td>
<td>125.3±7.4</td>
<td>113.5±5.1</td>
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</table>

<sup>1</sup>Data are the mean (mg/dL)±SE. Acarbose 50 and 100 are 1-week interventions with 50 and 100 mg acarbose/100 g diets, respectively. AcvGlc 50 and 100 are 1-week interventions with 50 and 100 mg AcvGlc/100 g diets, respectively, C57, C57BL/6J mice; DB, db/db mice. <sup>2</sup>p<0.05 compared with baseline; <sup>3</sup>p<0.05 compared with the 50 mg/100 g diet treatment; <sup>4</sup>p<0.05 compared with acarbose treatment.

The glucose removal rate was significantly improved after AcvGlc treatment, and this result was dramatic in db/db mice. The K<sub>G</sub> between 30 and 60 min was 3.9%/min in vehicle-treated db/db mice, and glucose elimination was significantly improved after AcvGlc treatment (11.4%/min). This effect was blunted in control mice, but still remained significant (7.0%/min with AcvGlc vs. 3.9%/min with vehicle). Similar findings were observed in db/db mice.
Effect of AcvGlc on the expression of GLUTs in the liver and small intestine. GLUT expression was examined since the hypoglycemic effects of AcvGlc could alter cellular glucose uptake in the liver and the small intestine. GLUT10, which is abundant in the liver, was initially identified in a survey of expressed sequences in the Type II diabetes-linked region of human chromosome 20q12-13.1 (17) and is the highest-affinity transporter identified in the GLUT family with a $K_M$ of approximately 0.3 mM (18). Under a normal diet, GLUT10 levels were markedly increased in diabetic mouse livers compared with controls. After the AcvGlc 100 diet, GLUT10 levels decreased in both mice (18% reduction, $p<0.01$ in db/db; 51% reduction, $p<0.01$ in C57BL/6j). In C57BL/6j mice, GLUT10 was nearly undetectable.

GLUT12 is specifically expressed in insulin-sensitive tissues and is abundant in the small intestine (19). Its expression was induced after AcvGlc treatment in both strains of mice (Fig. 3). Similar results were observed for the expression of GLUT5, especially in db/db mice, which is a fructose transporter highly expressed in the small intestine (data not shown). These results suggest that AcvGlc administration may induce monosaccharide transporters in the small intestine, probably to ensure the sufficient uptake of monosaccharide energy sources.

Effect of AcvGlc on the expression of molecules involved in lipid transport in the liver. The LDL receptor and LDL receptor related protein (LRP) are two major receptors for the removal of apoB-containing lipoproteins, including triglyceride-rich chylomicron remnants and very-low-density lipoprotein (VLDL) (20). The hepatic secretion of apoE facilitates triglyceride-rich lipoprotein removal (21), thus its expression after triglyceride lowering by AcvGlc was examined. The levels of the LDL receptor, LRP, and apoE were not altered by AcvGlc treatment in either the control or the db/db mice after AcvGlc treatment.

This is one of the few studies to investigate the expression of GLUTs after treatment with α-glucosidase inhibitor. We examined the expression of two novel glucose transporter proteins that may be associated with diabetes, GLUT10 and GLUT12, both of which have physiological roles and regulation that are not well understood in vivo.

GLUT10 was identified in a survey of expressed sequences in the Type II diabetes-linked region of human chromosome 20q12-13.1 (17), and is the highest-affinity transporter in the GLUT family with a $K_M$ of approximately 0.3 mM (18). GLUT12 is an insulin-sensitive glucose transporter protein in the small intestine, as is GLUT4 in adipocytes and muscle (19). We found that both hepatic GLUT10 and intestinal GLUT12 were markedly increased in diabetic mice and our data clearly showed that the overexpression of these two GLUTs was indeed associated with diabetes status, at least in mice. The etiological implications and the mechanism of the overexpression of these two GLUTs in diabetes should be studied further. It will be also interesting to examine GLUT10 and GLUT12 expression in human tissue samples, if possible.

In the intestine, we showed that GLUT12 is induced in both db/db and control animals after feeding an AcvGlc containing diet. Paive et al. (22) previously reported that acute treatment with acarbose induced intestinal GLUT1, but did not change the levels of intestinal Na⁺/glucose cotransporter-1 (SGLT-1) and GLUT2. Total glucose uptake
in the intestine was not altered in the study. Since GLUT2 is a low affinity but high capacity glucose transporter, it is probably a major shuttle system for intestinal glucose uptake with SGLT-1. Other intestinal GLUTs including GLUT1 and GLUT2 may participate in the fine tuning of glucose uptake. Therefore, it may be possible that the upregulation of GLUT2 as well as GLUT1 may help to preserve the total glucose absorption capacity of the small intestine in the presence of α-glucosidase inhibitor.

In the liver, we found that GLUT10 expression was dramatically repressed in db/db mice treated with AcpGlc. GLUT10 was nearly undetectable after feeding the AcpGlc diet. GLUT2 is also abundant in the liver as well as in the small intestine; however, it cannot be the major hepatic GLUT for glucose homeostasis due to its high Km (15-20 mM). Since the increased rates of glucoseogenesis and glycogenolysis in diabetic patients (24) are normalized in response to improved glucose tolerance, we speculate that the down-regulation of GLUT10 after AcpGlc treatment may be associated with improved glucose tolerance.

Additionally, we investigated the expression of receptors for triglyceride-rich lipoproteins. The LDL receptor and LRP are two major receptors for triglyceride rich lipoproteins and remove more than 90% of chylomicron remnants in a single-pass liver perfusion experiment (23, 24). Apolipoprotein E is a major ligand of the LDL receptor and LRP regarding the transport of triglyceride rich lipoproteins and is produced by the liver (21). We found that LDL receptor, LRP and apoE expression were not altered after AcpGlc treatment, thus the triglyceride lowering effects of AcpGlc may operate via mechanisms other than those enhancing receptor-mediated lipoprotein uptake.

In conclusion, our results show that AcpGlc improved plasma triglyceride and glucose metabolism as effectively as acarbose. GLUT10 was markedly increased in diabetic mice. AcpGlc treatment reduced hepatic GLUT10 and induced intestinal GLUT2 expression. These data suggest that the regulation of GLUT10 and GLUT2 may be important in the improvement of plasma glucose metabolism.

Acknowledgments

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References