Antihyperglycemic and Antioxidant Properties of Caffeic Acid in *db/db* Mice

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**ABSTRACT**

This study investigated the blood glucose-lowering effect and antioxidant capacity of caffeic acid in C57BL/KsJ-*db/db* mice. Caffeic acid induced a significant reduction of the blood glucose and glycosylated hemoglobin levels than the control group. The plasma insulin, C-peptide, and leptin levels in caffeic acid group were significantly higher than those of the control group, whereas the plasma glucagon level was lower. Increased plasma insulin by caffeic acid was attributable to an antidegenerative effect on the islets. Caffeic acid also markedly increased glucokinase activity and its mRNA expression and glycogen content and simultaneously lowered glucose-6-phosphatase and phosphoenolpyruvate carboxykinase activities and their respective mRNA expressions, accompanied by a reduction in the glucose transporter 2 expression in the liver. In contrast to the hepatic glucose transporter 2, adipocyte glucose transporter 4 expression was greater than the control group. In addition, caffeic acid significantly increased superoxide dismutase, catalase, and glutathione peroxidase activities and their respective mRNA levels, while lowering the hydrogen peroxide and thiobarbituric acid reactive substances levels in the erythrocyte and liver of *db/db* mice. These results indicate that caffeic acid exhibits a significant potential as an antidiabetic agent by suppressing a progression of type 2 diabetic states that is suggested by an attenuation of hepatic glucose output and enhancement of adipocyte glucose uptake, insulin secretion, and antioxidant capacity.

Type 2 diabetes is characterized by pancreatic β-cell dysfunction accompanied by insulin resistance. Normal pancreatic β-cells can compensate for the insulin resistance by increasing insulin secretion; however, extensive exposure of pancreatic β-cells to high glucose levels causes β-cell dysfunction that is associated with impaired insulin secretion and biosynthesis (Robertson et al., 1992). Insulin resistance contributes to increasing glucose output in the liver and decreasing glucose uptake in adipose tissues (Ferre et al., 1996; Abel et al., 2001). In particular, liver is an insulin-sensitive tissue and plays a major role in maintaining glucose homeostasis by regulating the interaction between the glucose utilization and gluconeogenesis (Ferre et al., 1996). Indeed, resistance to insulin-stimulated glucose transport in adipose tissue is one of the defects in insulin resistance states such as obesity and type 2 diabetes (Abel et al., 2001). Thus, a suitable antidiabetic agent should improve glucose-induced insulin secretion, hepatic glucose metabolism, and peripheral insulin sensitivity.

There is an increasing evidence indicating that oxidative stress produced under hyperglycemia can cause or lead to insulin resistance and diabetes complications (Matsuoka, 1997). Moreover, several studies have shown that antioxidant ameliorates a number of altered physiological and metabolic parameters that occur as a result of type 2 diabetes (Kaneto et al., 1999; Balasubashini et al., 2004). Phenolic compounds, widely distributed in food plants, act as a primary antioxidant and can be helpful for improving or preventing a number of chronic diseases (Scalbert et al., 2005). However, there is a growing interest in several biological properties of phenolic compounds in addition to their antioxidant effects, and the evidence suggests that certain dietary polyphenolic compounds may result in an altered glucose metabolism (Okutan et al., 2005; Scalbert et al., 2005). Among various phenolic compounds, caffeic acid [3,4-di(OH)-cinnamate], found in many types of fruit and coffee in high concentrations, has exhibited pharmacological antioxidant,

**ABBREVIATIONS:** GK, glucokinase; G6Pase, glucose-6-phosphatase; PEPCK, phosphoenolpyruvate carboxykinase; SOD, superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidase; FOX 1, ferrous oxidation with xylenol orange; TBARS, thiobarbituric acid-reactive substances; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; bp, base pair(s); GLUT, glucose transporter; ROS, reactive oxygen species.
Antidiabetic Effects of Caffeic Acid

Materials and Methods

Animals and Diets. Twenty male C57BL/KsJ-db/db mice were purchased from Jackson Laboratory (Bar Harbor, ME) at 5 weeks of age (23 g). They were fed a pelleted commercial chow diet for acclimation from the arrival for 2 weeks, then randomly divided into two groups with 10 mice and fed an AIN-76 semisynthetic diet with or without a caffeic acid supplementation (0.02%; 3,4-dihydroxybenzilic acid, 3-(3,4-dihydroxyphenyl)-2-propenoic acid; Sigma-Aldrich, St. Louis, MO) for 5 weeks.

All mice were maintained under a controlled light/dark cycle (12:12 h, lights on at 8:00 AM) and constant temperature (24°C). They were given free access to food and distilled water, and the food consumption and body weight gain were measured daily and weekly, respectively. At the end of the experimental period, all the mice were anesthetized with ketamine after a 12-h fast, and blood samples were collected from the inferior vena cava into heparin-coated tube. The livers and adipose tissues were separated. The livers and adipose tissues were washed and homogenized in 5 volumes of a 30% (w/v) KOH solution and dissolved at 100°C for 30 min. The protein concentration was measured using the method of Bradford (1976) using bovine serum albumin as the standard. In addition, the hemoglobin concentration was estimated in an aliquot of the hemolysate using a commercial assay kit (Sigma-Aldrich).

Blood Biomarkers. The blood glucose concentration were measured at 7, 8, 9, 10, 11, and 12 weeks of age after 0, 1, 2, 3, 4, and 5 weeks of caffeic acid supplementation, respectively. The blood glucose concentration was measured with whole blood obtained from the tail veins after withholding food for 6 h using a glucose analyzer based on the glucose oxidase method (Glucocard test strip; Arkray, Kyoto, Japan), and the glycated hemoglobin was measured with a analyzer (Roche Diagnostics, Basel, Switzerland). Plasma insulin (DSL-1600 Insulin RIA kit; Diagnostic Systems Laboratories, Webster, TX), C-peptide (C-peptide RIA kit; Diagnostic Systems Laboratories), glucagon (Glucagon RIA kit; PerkinElmer Life and Analytical Sciences, Boston, MA), and leptin (Mouse leptin RIA kit; Linco Research, St. Charles, MO) levels were measured based on a radioimmunometric assays.

Hepatic Glycogen Assay. The hepatic glycogen concentration was determined as described previously by Steiffer et al. (1950) with modification. In brief, the liver tissue was homogenized in 5 volumes of a 30% (w/v) KOH solution and dissolved at 100°C for 30 min. The glycogen was determined by treatment with an anthrone reagent [2 g of anthrone/1 liter of 95% (v/v) H2SO4] and measuring the absorbance at 620 nm.

Enzyme Activities. Glucokinase (GK) activity was determined in the hepatic cytosol using a spectrophotometric assay as described by Davidson and Arion (1987) with a slight modification, whereby the formation of glucose 6-phosphate at 37°C was coupled to its oxidation by glucose-6-phosphate dehydrogenase and NADP+. The reaction mixture contained, in a final volume of 1 ml: 50 mM sodium Hepes, pH 7.4, 100 mM KCl, 7.5 mM MgCl2, 5 mM ATP, 2.5 mM dithioerythritol, 10 mg/ml albumin, 1 mM NADP+, 5.5 U of glucose-6-phosphate dehydrogenase (Leuconostoc mesenteroides), hepatic cytosol, and 10 mM glucose, Glucose-6-phosphatase (G6Pase) activity was determined in the hepatic microsome using a spectrophotometric assay according to the method Alegre et al. (1988) with a slight modification, which contained 100 mM sodium Hepes, pH 6.5, 26.5 mM glucose-6-phosphate, 1.8 mM EDTA, both previously adjusted to pH 6.5, 2.5 mM NADP+, 0.6 KIU/ml mutarotase, and 6 KIU/ml glucose dehydrogenase. Phosphonopropyruvate carboxykinase (PEPCK) activity was monitored in the direction of oxaloacetate synthesis using the spectrophotometric assay developed by Bentle and Lardy (1976) with a slight modification. The reaction mixture contained the following in 1-ml final volume: 50 mM sodium Hepes, pH 6.5, 1 mM inosine diphosphate, 1 mM MnCl2, 1 mM dithiothreitol, 0.25 mM NADH, 2 mM phosphoenolpyruvate, 50 mM NaHCO3, 7.2 U of malic dehydrogenase, and hepatic cytosol. Enzyme activity was determined at 25°C for 2 min by decrease of absorbance at 340 nm. Superoxide dismutase (SOD) activity was spectrophotometrically measured by the inhibition of pyrogallol autoxidation at 420 nm for 10 min according to the method of Marklund and Marklund (1974). One unit was determined as the amount of enzyme that inhibited the oxidation of pyrogallol by 50%. Catalase (CAT) activity was measured using Aebi’s (1974) method with a slight modification, in which the disappearance of hydrogen peroxide was monitored at 240 nm for 5 min using a spectrophotometer. Ten microliters of the solution was added to a cuvette containing 2.89 ml of a 50 mM potassium phosphate buffer, pH 7.4, then the reaction was initiated by adding 0.1 ml of 30 mM H2O2 to make a final volume of 3.0 ml at 25°C. The decomposition rate of H2O2 was measured at 240 nm for 5 min using a spectrophotometer. Glutathione peroxidase (GSH-Px) activity was measured using the spectrophotometric assay at 25°C, as described previously by Paglia and Valentine’s (1967) method with a slight modification. The reaction mixture contained 2.525 ml of a 0.1 M Tris-Cl/HCl buffer, pH 7.2, 75 µl of 30 mM glutathione, 100 µl of 6 mM NADPH, and 100 µl of glutathione reductase (0.24 U). One hundred microliters of the solution was added to 2.8 ml of the reaction mixture and incubated at 25°C for 5 min. The reaction was initiated by adding 100 µl of 30 mM H2O2, and the absorbance measured at 340 nm for 5 min. The protein concentration was measured by the method of Bradford (1976) using bovine serum albumin as the standard. In addition, the hemoglobin concentration was estimated in an aliquot of the hemolysate using a commercial assay kit (Sigma-Aldrich).

Hydrogen Peroxide and Lipid Peroxidation Assay. The hydrogen peroxide levels in erythrocyte and liver were measured by Wolf’s method (1994). FOX 1 reagent was prepared as following mixture with 100 µM xylenol orange, 250 µM ammonium ferrous sulfate, 100 mM sorbitol, and 25 mM H2SO4. Fifty microliters of test sample was added to 950 µl of FOX 1 reagent, vortexed, and incubated at room temperature for a minimum of 30 min at which color development is virtually complete. The absorbance was read at 560 nm, and the standard was linear in the 0 to −5 µM concentration range. The erythrocyte and hepatic thiobarbituric acid-reactive substances (TBARS) concentration, as a marker of lipid peroxide production, was measured spectrophotometrically by the method of Ohkawa et al. (1979).

Northern Blot Analysis. Total RNA was isolated from the livers by the guanidine thiocyanate-phenol method of Chomzynski and Sacchi (1987). The total RNA (20 µg) was separated on a 0.9% agarose gel containing 2.2 M formaldehyde and transferred to Nyt-
ran-Plus membranes (Schleicher and Schuell, Dassel, Germany). The membranes were then hybridized with a 32P-labeled cDNA probe, washed at room temperature with 2× sodium chloride sodium citrate containing 0.1% SDS followed by two washes at 65°C with 0.2× sodium chloride sodium citrate containing 0.1% SDS, and exposed to X-ray film with an intensifying screen at -70°C. Thereafter, DNA probes were visualized using ECL kit (Upstate Biotechnology, Lake Placid, NY) and anti-mouse GLUT4 (1:50,000; Biogenesis) and then probed with rabbit anti-mouse GLUT2 (1:50,000; Biogenesis, San Antonio, TX). The homogenates were then centrifuged at 1000 g for 10 min and then immunostained with the primary antibody, monoclonal mouse anti-insulin. The antigen-antibody complex was visualized by an avidin-biotin peroxidase complex solution using an ABC complex no. M32599). The intensities of the mRNA bands were quantified using a Bio Image Whole Band Analyzer (50S; B.I. System Co., Jackson, MI) and subsequently normalized based on the intensity of the respective GAPDH mRNA bands.

**Western Blot Analysis.** The livers and adipose tissues were prepared according to Nathan et al. (2001) with a slight modification. In brief, the tissue was homogenized with a buffer containing 250 mM sucrose, pH 7.4, 10 mM triethanolamine, 10 mM acetic acid, 1 mM EDTA, 1 mM phenylmethylsulfonyl fluoride, 1 mM benzamidine hydrochloride hydrate, and 1 mM dithiothreitol. The homogenates were then centrifuged at 100,000 g for 1 h at 4°C in an ultracentrifuge (Beckman, Fullerton, CA). The pellet resuspended as a membrane fraction for a glucose transporter 4 (GluT4) Western blot analysis (Fig. 2). In contrast, the plasma leptin level was positively correlated (r = 0.748, p < 0.01) (Fig. 2). In contrast, the plasma leptin level was positively correlated (r = 0.748, p < 0.01) (Fig. 2). In contrast, the plasma leptin level was positively correlated (r = 0.748, p < 0.01) (Fig. 2).

**Pancreas Histology and Immunohistochemistry.** Removed pancreas was fixed in 10% (v/v) buffered formalin, processed routinely, and embedded in paraffin wax. Paraffin sections were cut at 4-μm thickness and deparaffinized in xylene for 5 min and rehydrated through the graded ethanol. The section were stained with H&E, and for immunohistochemistry, rehydrated sections were treated with 3% (v/v) H2O2 in methanol for 30 min to block endogenous peroxidase and washed with 0.01 mM phosphate buffer for 10 min and then immunostained with the primary antibody, monoclonal mouse anti-insulin. The antigen-antibody complex was visualized by an avidin-biotin peroxidase complex solution using an ABC kit (Vector Laboratories, Burlingame, CA) with 3,3-diaminobenzidine (Zymed Laboratories, San Francisco, CA). The membranes were then hybridized with a 32P-labeled cDNA probe, washed at room temperature with 2× sodium chloride sodium citrate containing 0.1% SDS followed by two washes at 65°C with 0.2× sodium chloride sodium citrate containing 0.1% SDS, and exposed to X-ray film with an intensifying screen at -70°C. Thereafter, DNA probes were visualized using ECL kit (Upstate Biotechnology, Lake Placid, NY) and anti-mouse GLUT4 (1:50,000; Biogenesis) and then probed with rabbit anti-mouse GLUT2 (1:50,000; Biogenesis, San Antonio, TX). The homogenates were then centrifuged at 1000 g for 10 min and then immunostained with the primary antibody, monoclonal mouse anti-insulin. The antigen-antibody complex was visualized by an avidin-biotin peroxidase complex solution using an ABC kit (Vector Laboratories, Burlingame, CA) with 3,3-diaminobenzidine (Zymed Laboratories, San Francisco, CA).

**Statistical Analysis.** All data are presented as the mean ± S.E. Statistical analyses were performed using the SPSS program (SPSS, Inc., Chicago, IL). Student’s t test was used to assess the differences between the groups. Statistical significance was considered at p < 0.05. Pearson correlation coefficients were calculated to examine the association of the plasma leptin with the blood glucose, plasma insulin, body weight, and adipose tissue weight.

**Results**

**Body Weight Gain, Relative Organ Weight, and Food Intake.** The body weight of the caffeic acid group increased throughout the experimental period, whereas that of the control group decreased after 3 weeks. Thus, the body weight was significantly higher in the caffeic acid group than in the control group at weeks 3, 4, and 5 of the experimental period (Fig. 1). Food intakes and relative organ weights were about the same for all groups (data not shown).

**Blood Glucose and Glycosylated Hemoglobin Levels.** All db/db mice were diabetic when the experiment began, as indicated by the blood glucose level (≥21.47 mM). Caffeic acid significantly lowered the blood glucose level compared with the control group at weeks 3, 4, and 5 of the experimental period (Fig. 1). The glycosylated hemoglobin level was also significantly lower in the caffeic acid group than the control group (Table 1).

**Plasma Insulin, C-Peptide, Glucagon, and Leptin Levels.** The plasma insulin, C-peptide, and leptin levels of the caffeic acid group were significantly higher than those of the control group, whereas the plasma glucagon level of the caffeic acid group was significantly lower than that of the control group (Table 1). The plasma leptin and blood glucose levels were inversely correlated (r = 0.748, p < 0.01) (Fig. 2). In contrast, the plasma leptin level was positively correlated with body weight (r = 0.819, p < 0.001) and plasma insulin level (r = 0.835, p < 0.001) (Fig. 2).
Hepatic Glucose-Regulating Enzyme Activities and Glycogen Concentration. Caffeic acid significantly elevated hepatic GK activity compared with the control group by approximately 28% (Fig. 3). In contrast, G6Pase and PEPCK activities were markedly lower in the caffeic acid group by 29 and 19%, respectively (Fig. 3). The hepatic glycogen concentration was significantly higher in the caffeic acid group (Table 1).

Erythrocyte and Hepatic Antioxidant Enzyme Activities, Hydrogen Peroxide, and Lipid Peroxidation Levels. The erythrocyte SOD, CAT, and GSH-Px activities were significantly higher in the caffeic acid group than in the control group (Table 2). Caffeic acid also markedly elevated the hepatic SOD, CAT, and GSH-Px activities (Table 2). The hydrogen peroxide levels were significantly lower in the erythrocyte, hepatic cytosolic, and mitochondrial fraction from the caffeic acid-supplemented db/db mice (Table 2). In addition, the caffeic acid significantly lowered the lipid peroxidation levels in erythrocyte and liver (Table 2).

Hepatic Enzyme mRNA Expression. The mRNA levels of the hepatic glucose metabolic and antioxidant enzymes were monitored using a Northern blot analysis. As a loading control, the glucose regulating enzyme and antioxidant enzyme mRNA signals were normalized to the GAPDH mRNA signal for each group. The mRNA level of GK was significantly elevated in the caffeic acid-supplemented group than in the control group (Fig. 4). However, the mRNA levels of

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**TABLE 1**
Concentrations of plasma insulin, c-peptide, glucagon, leptin, blood glycosylated hemoglobin, and hepatic glycogen in C57BL/KsJ-db/db mice fed diet supplemented with caffeic acid

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Caffeic Acid</th>
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<tbody>
<tr>
<td>Insulin (pM)</td>
<td>202.10 ± 12.62</td>
<td>328.62 ± 17.04*</td>
</tr>
<tr>
<td>C-Peptide (pM)</td>
<td>199.80 ± 2.35</td>
<td>233.10 ± 2.35*</td>
</tr>
<tr>
<td>Glucagon (ng/l)</td>
<td>136.64 ± 3.62</td>
<td>98.46 ± 3.39*</td>
</tr>
<tr>
<td>Leptin (µg/l)</td>
<td>49.10 ± 3.16</td>
<td>77.10 ± 2.78*</td>
</tr>
<tr>
<td>Glycosylated hemoglobin (%)</td>
<td>13.48 ± 0.11</td>
<td>11.11 ± 0.06*</td>
</tr>
<tr>
<td>Glycogen (mg/g liver)</td>
<td>56.15 ± 1.51</td>
<td>70.23 ± 0.48*</td>
</tr>
</tbody>
</table>

Values are mean ± S.E., n = 10. *p < 0.001 vs. control group as determined by Student’s t test.

**TABLE 2**
The activities of erythrocyte and hepatic antioxidant enzyme and the levels of hydrogen peroxide and TBARS in C57BL/KsJ-db/db mice fed diet supplemented with caffeic acid

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>Caffeic Acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>SOD (Ug Hb)</td>
<td>898.25 ± 16.49</td>
<td>1037.96 ± 16.93***</td>
</tr>
<tr>
<td>CAT (µmol/g Hb/min)</td>
<td>93.40 ± 16.23</td>
<td>143.80 ± 9.57*</td>
</tr>
<tr>
<td>GSH-Px (µmol/g Hb/min)</td>
<td>28.16 ± 1.86</td>
<td>42.55 ± 2.34**</td>
</tr>
<tr>
<td>H2O2 (µmol/g Hb)</td>
<td>23.52 ± 0.56</td>
<td>21.68 ± 0.09*</td>
</tr>
<tr>
<td>TBARS (nmol/g Hb)</td>
<td>2.68 ± 0.01</td>
<td>2.27 ± 0.01***</td>
</tr>
<tr>
<td>Liver</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SOD (µmg protein)</td>
<td>7.73 ± 0.57</td>
<td>15.51 ± 0.97***</td>
</tr>
<tr>
<td>CAT (µmol/mg protein/min)</td>
<td>4.79 ± 0.16</td>
<td>5.82 ± 0.28*</td>
</tr>
<tr>
<td>GSH-Px (µmol/mg protein/min)</td>
<td>42.22 ± 2.01</td>
<td>57.35 ± 1.80***</td>
</tr>
<tr>
<td>mH2O2 (nmol/mg protein)</td>
<td>7.77 ± 0.32</td>
<td>5.17 ± 0.35**</td>
</tr>
<tr>
<td>mH2O2 (nmol/mg protein)</td>
<td>66.32 ± 2.15</td>
<td>52.10 ± 2.26**</td>
</tr>
<tr>
<td>TBARS (nmol/g liver)</td>
<td>4.88 ± 0.33</td>
<td>2.49 ± 0.42**</td>
</tr>
</tbody>
</table>

Values are mean ± S.E., n = 10. *p < 0.05 vs. control group as determined by Student’s t test. **p < 0.01 vs. control group as determined by Student’s t test. ***p < 0.001 vs. control group as determined by Student’s t test.
G6Pase and PEPCK were markedly lower in the db/db mice supplemented with caffeic acid (Fig. 4). The mRNA levels of SOD, CAT, and GSH-Px were significantly elevated in the caffeic acid group (Fig. 4). Thus, the changes in the glucose metabolic and antioxidant enzymes mRNA expressions were similar to the respective enzyme activities in the liver.

**Glucose Transporter Protein Expression.** The changes in the hepatic GLUT2 and adipose tissue GLUT4 protein expressions were examined by the Western blotting analysis (Fig. 5). Caffeic acid significantly lowered the hepatic GLUT2 protein level compared with the control group. In contrast, the expression of GLUT4 protein in adipose tissue was markedly increased in the caffeic acid group.

**Histology and Immunohistochemistry for Pancreas.** The control db/db mice exhibited islet boundary definition loss and degeneration, whereas db/db mice supplemented with caffeic acid preserved islet architecture (Fig. 6). When β-cells were stained with anti-insulin antibodies, caffeic acid-supplemented db/db mice exhibited strong staining compared with the control db/db mice (Fig. 6).

**Discussion**

Caffeic acid significantly lowered the fasting blood glucose level compared with the control db/db mice, which is in agreement with previous studies done by others (Hsu et al., 2000; Cheng et al., 2003; Park and Min, 2006). The level of glycosylated hemoglobin, a well recognized marker of chronic glycemic control, was also markedly lower in the db/db mice supplemented with caffeic acid. This antihyperglycemic action of caffeic acid is likely associated with a marked en-
enhancement of the GK mRNA expression and activity in the liver. Hepatic GK has a major effect on glucose homeostasis and is a potential target for pharmacological treatment of type 2 diabetes, as evidenced by the fact that liver-specific GK-knockout mice exhibited mild hyperglycemia (Pustie et al., 1999) and rats overexpressing GK in the liver had reduced blood glucose (Ferre et al., 1996). The increase of hepatic GK can cause an increased utilization of the blood glucose for energy production or glycogen storage in the liver (Iynedjian et al., 1988). This study showed that hepatic glycogen content was significantly higher in the caffeic acid-supplemented group.

A low hepatic GK activity is also reported to favor the release of glucose synthesized by gluconeogenesis into the circulation (Hers and Hue, 1983). Hepatic gluconeogenesis is also crucial to the maintenance of fasting hyperglycemia and is observed high in db/db mice (Friedman et al., 1997). The G6Pase and PEPCK are the key enzymes that control gluconeogenesis and glucose output from the liver, and their gene expressions were increased in db/db mice (Friedman et al., 1997). In the study, caffeic acid caused a marked reduction in the hepatic PEPCK and G6Pase activities and their mRNA levels in db/db mice, indicating a decreased hepatic glucose production. Along this line, hepatic GLUT2 protein expression was also lowered in the caffeic acid group than in the control group. The decrease in GLUT2 expression is known to be related with a decrease in hepatic glucose output (Oka et al., 1990). Based on these results, the caffeic acid seemed to suppress the hepatic glucose output by enhancing hepatic glucose utilization and inhibiting glucose over-production in db/db mice.

Hepatic GK, G6Pase, and PEPCK activities are reported to be controlled primarily at the level of transcription, being regulated by insulin and glucagon. High insulin levels have been shown to inhibit hepatic glucose production by means of stimulation of GK gene transcription and glycogen synthesis and inhibition of gluconeogenesis (Iynedjian et al., 1988; Friedman et al., 1997). In contrast, glucagon induces an inhibition of GK gene transcription and a stimulation of hepatic PEPCK gene transcription, and even a small increase of glucagon level may induce a relative increase in the gluconeogenesis (Iynedjian et al., 1995; Friedman et al., 1997). In our study, the changes in hepatic glucose-regulating enzymes could be partly attributed to insulin and glucagon levels because plasma insulin level was significantly elevated, whereas plasma glucagon level was lowered in the caffeic acid-supplemented db/db mice than in the control db/db mice at 12 weeks old.

Plasma insulin levels of db/db mice are known to be age-dependent. The initial adaptation to the insulin resistance is one of islet β-cell hyperplasia resulting in marked hyperinsulinemia (Orland and Permutt, 1987). However, when the db/db mice reach at 12 to 24 weeks old, islet develops β-cell necrosis, hyperinsulinemia is diminished, and the mice manifest symptoms of insulin deficiency (Orland and Permutt, 1987). We observed that islet surface area in pancreas is relatively greater in caffeic acid-supplemented db/db mice than in the control group. Caffeic acid also preserved islet and β-cell architecture relatively better compared with the control group. Moreover, caffeic acid significantly increased the levels of C-peptide that has a longer half-life than insulin and thus may better represent insulin secretion than insulin levels do (Doda, 1996). Taken together, these data suggest that the plasma insulin level in the db/db mice may be gradually declined after reaching the peak point, whereas caffeic acid is considered to slow the age-dependent insulin decline by a reduction of β-cell mass. Similar effects of ferulic acid, a phenolic acid, on pancreas of diabetic rats were reported by Balasubashini et al. (2004).
Another possible mechanism by which caffeic acid mediates its antidiabetic action may be due to enhanced transport of blood glucose to adipose tissue. In general, glucose transport in liver and adipocytes are regulated by different mechanisms. Hepatic GLUT2 expression is higher in human and rodent with type 2 diabetes (Friedman et al., 1997); however, adipose GLUT4 overexpression is known to alleviate insulin resistance and pancreatic defects in db/db mice, resulting in a markedly improved glycemic control (Gibbs et al., 1995). Conversely, selective elimination of GLUT4 expression in adipose tissue impairs insulin action in liver (Abel et al., 2001). The present study showed that caffeic acid significantly enhanced the GLUT4 protein expression in adipose tissue compared with the control group. This result can be supported by Pinent et al.'s (2004) findings that procyanidins, a polyphenolic compound, increased the amount of insulin-sensitive GLUT4 and stimulated glucose uptake in adipose tissue. In other words, caffeic acid has a dual mechanism of action that enhances insulin release from the pancreas and also improves insulin resistance in the liver and adipose tissue.

Insulin also stimulates leptin synthesis and release through the regulation of glucose metabolism in adipocytes (Wabitsch et al., 1996). Leptin enhances insulin action by inhibiting hepatic glucose production (Brazilai et al., 1997). These suggest that low levels of leptin with type 2 diabetes could increase insulin resistance and thereby worsening the condition. Interestingly, the present study exhibited a positive correlation between plasma leptin and insulin levels and body weight ($r = 0.835$, $p < 0.001$; $r = 0.819$, $p < 0.001$) and a reverse association between plasma leptin and blood glucose levels ($r = -0.748$, $p < 0.01$), as previously reported by others (Wabitsch et al., 1996; Considine et al., 1996; Moriya et al., 1999). Especially, caffeic acid-supplemented db/db mice continuously gained body weight throughout the study, although the control db/db mice did not gain more after 10 weeks of age. Because db/db mice stops gaining body weight after 10 weeks of age but slowly lose weight along as diabetic phenotype progressed (Orland and Permutt, 1987), it is likely that improvement of hyperglycemia by caffeic acid supplement delays further development of diabetic state and thereby enhances the animal's ability to thrive. Similar result was shown in GLUT4-up-regulated db/db mice that continue to gain body weight until 15 weeks of age and then maintained until at least 35 weeks of age (Gibbs et al., 1995).

Antioxidant was previously been recognized as a means to treat diabetes, whose antioxidants such as vitamin E decrease blood glucose levels through improvement of insulin action in type 2 diabetes (Kaneto et al., 1999). In diabetes, reactive oxygen species (ROS) resulting from hyperglycemia cause cell damage (Matsouka, 1997). Erthrocyte is especially susceptible to oxidative damage resulting from a high concentration of oxygen and hemoglobin (Clemens and Waller, 1987). Liver is also known to undergo free radical-mediated injury in diabetes, and increased ROS is related to the damage of hepatic glucose-regulating enzymes (Lelli et al., 2005). Normally, erthrocyte and liver contain enough scavengers such as SOD, CAT, and GSH-Px to protect against free radical injury. However, prolonged exposure of obese-diabetic db/db mice to hyperglycemic condition reduces the activities of SOD and CAT (Makar et al., 1995).

From our results, caffeic acid supplement resulted in dramatic increase in the antioxidant enzyme activities and mRNA levels in both erythrocyte and liver compared with the control group. The SOD plays an important role in protecting cells from oxidative damage by converting superoxide radicals into hydrogen peroxide, which is then further metabolized by CAT and GSH-Px, where CAT detoxifies hydrogen peroxide and GSH-Px catalyzes the destruction of hydrogen peroxide and lipid hydroperoxide. If the CAT and GSH-Px activity is not sufficiently enhanced to metabolize hydrogen peroxide, this can lead to an increased hydrogen peroxide and TBARS levels (Harou, 1991). As such, a combination of SOD and CAT or GPH-Px may be necessary rather than SOD alone to reduce oxidative stress. It is noteworthy that, in the db/db mice supplemented with caffeic acid, the changes of antioxidant enzymes resulted in a decreased hydrogen peroxide levels in erythrocyte and liver compared with the control group. Furthermore, the erythrocyte and hepatic TBARS levels were significantly lower in the caffeic acid group than in the control group, indicating a decreased rate of lipid peroxidation. As a result, enhanced antioxidant enzyme activities in the erythrocyte and liver by caffeic acid may have a protective role against ROS, thereby preventing the formation of hydrogen peroxide and lipid peroxidation. Thus, it seems reasonable that caffeic acid was effective for preventing erythrocyte and hepatic damage.

In conclusion, the data obtained in this study suggest that caffeic acid is an effective antidiabetic agent via its ability to enhance insulin secretion and to decrease hepatic glucose output along with the increased level of adipocyte glucose disposal in the type 2 diabetic animals. Furthermore, it seems likely that caffeic acid is beneficial against oxidative stress, thereby being helpful in preventing or delaying the development of diabetes and its complications.

References


