Preventive Effect of Chlorogenic Acid on Lens Opacity and Cytotoxicity in Human Lens Epithelial Cells

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The present study reveals the pharmacological effect of chlorogenic acid, a major phenolic compound in plants, food, and coffee. Chlorogenic acid shows various biological properties, such as anti-oxidant, anti-carcinogenic, anti-inflammatory activities, and modulating effects on lipid and glucose metabolism under metabolic dysregulation conditions.1–3) Chlorogenic acid methyl ester, one of the constituents of Platycodon grandiflorum, exerts inhibitory effects on advanced glycation end products (AGEs) formation and aldose reductase (AR) activity and may be useful as a potential therapeutic agent for diabetic complications.4)

AR (EC 1.1.1.21), the first rate-limiting enzyme in the polyol pathway, is located in the eye, kidney, and also in other tissues involved in diabetic complications. Increased glucose enters the polyol pathway and is reduced by AR to sorbitol.5,6) The intracellular accumulation of sorbitol and its metabolite eventually results in a loss of osmotic integrity, which leads to lens hydration and swelling followed by cell damage, resulting in the diabetic cataract.9,10) One approach to prevent or delay the onset of diabetic complications is genetic deletion or inhibition of AR.9) AR inhibitors (ARIs), such as epalrestat, 3,3-tetramethyleneglutaric acid (TMG) and fidarestat, have been developed, and some have been shown to prevent diabetic cataract in animal models or patients.5,10,11) ARIs from natural products have been shown to provide important information concerning the role of epithelium in cataract formation.16) Furthermore, studies have shown that osmotic stress in the lens caused by sorbitol accumulation induces apoptosis in lens epithelial cells, leading to the development of cataracts.17) Fas-mediated apoptosis was observed in lens epithelial cells of human and diabetic rat cataracts rat by immunohistochemical study.18,19) Diabetic condition such as high glucose trigger cell apoptosis. Puerarin decrease lens epithelial cell apoptosis induced by peroxynitrite in diabetic rats.20)

Although chlorogenic acid has various biophysiological effects, its effect on diabetic cataract formation was not well known until recently. The aim of this study was to investigate the effect of chlorogenic acid on cataractogenesis. Our findings demonstrate the protective effects of chlorogenic acid on AR activity, xylose-induced lens opacity, and cytotoxicity in HLE-B3 cells.

Key words chlorogenic acid; aldose reductase; lens opacity; cytotoxicity; human lens epithelial cell

Chlorogenic acid is one of the most abundant polyphenol compounds in plants, food, and coffee. Chlorogenic acid shows various biological properties, such as anti-oxidant, anti-carcinogenic, anti-inflammatory activities, and modulating effects on lipid and glucose metabolism under metabolic dysregulation conditions.1–3) Chlorogenic acid methyl ester, one of the constituents of Platycodon grandiflorum, exerts inhibitory effects on advanced glycation end products (AGEs) formation and aldose reductase (AR) activity and may be useful as a potential therapeutic agent for diabetic complications.4)

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MATERIALS AND METHODS

Materials Chlorogenic acid was obtained from Carl Roth GmbH & Co (KG, Karlsruhe, Germany). The CCK-8 kit (Cell Counting Kit-8) was obtained from Dojindo Laboratories (Tokyo, Japan). M199 medium and fetal bovine serum (FCS) were purchased from Gibco BRL (Grand Island, NY, U.S.A.). All other reagents were from Sigma-Aldrich (St. Louis, MO, U.S.A.).

Rat Lens Aldose Reductase Activity AR activity was measured as described previously.12,21,22) All animal procedures were approved by the committee on animal care at our institute and were conducted according to institutional guidelines. Rat lenses were isolated from the eyes of 8-week-old Sprague-Dawley rats (SD rats, Orient Co., Seongnam, South Korea), and homogenized in 12 volumes of 135 mM Na, K-phosphate buffer (pH 7.0), and 10 mM 2-mercaptoethanol. The homogenate was centrifuged at 14000 rpm for 30 min, and the supernatant was used as crude rat lens AR. The incubation mixture contained 135 mM Na, K-phosphate buffer (pH 7.0), and 10 mM 2-mercaptoethanol. The homogenate was centrifuged at 14000 rpm for 30 min, and the supernatant was used as crude rat lens AR. The incubation mixture contained 135 mM Na, K-phosphate buffer, 100 mM lithium sulfate, 0.03 mM reduced nicotinamide adenine dinucleotide phosphate (NADPH), 1 mM D-glucosamine, and 50 µl of enzyme substrate, with or without 25 µl chlorogenic acid or positive control, in a total volume of 1.0 ml. The reaction was initiated by the addition

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of NADPH at 37 °C and stopped by the addition of 0.3 ml of 0.5 m HCl. Next, 1 ml of 6 m NaOH containing 10 mM imidazole was added, and the solution was heated at 60 °C for 10 min to convert NADP to a fluorescent product. The fluorescence (ex. 360 nm/em. 460 nm) was assayed using a spectrofluorometric detector (Synergy HT, Bio-Tek, Winooski, VT, U.S.A.).

The concentration of each test sample giving 50% inhibition of activity (IC50) was estimated from the least-squares regression line of the logarithmic concentration plotted against the remaining activity.

**Lens Organ Culture** The lenses of SD rats were carefully removed and extracted using a posterior approach. Lenses were cultured in 24-well dishes. Each well contained 1 ml of M199 medium containing Earle’s salts, t-glutamine, and 2200 mg/l sodium bicarbonate without phenol red for mimicking a hyperglycemic condition caused by diabetes. All lenses were allowed to equilibrate in the incubator (37 °C, 5% CO2) for 3 d. Sugar cataracts were induced by adding 20 mM d(+)-xylose to the medium with gentamycin (5 mg/l) and fungizone (0.5 mg/l). Medium was changed every day and supplemented with chlorogenic acid or epalrestat, as a positive inhibitor.

**Analysis of Lens Opacification** Lenses were photographed under an optical microscope with a CCD camera. The opaque area of the lens was analyzed using an imaging system program (NIH Image J, Bethesda, MD, U.S.A.). Data are expressed as the percentage of opaque area compared to the entire area of the lens.

**Cytotoxicity Assay** Cytotoxicity was evaluated in vitro by determining cell viability with the CCK-8 kit. Cells were plated at a density of 1×10⁴ cells/ml in 96-well plates and allowed to attach overnight. The cells were then treated with chlorogenic acid (1—500 μM) or glucose (5.5—200 mM) and incubated for 72 h. After a 4 h incubation with the CCK-8 solution, absorbance was measured with a microtiter plate reader (Bio-Tek) at 450 nm. We calculated the percent viability as optical density of treated sample/optical density of untreated control×100.

**Statistical Analysis** Data are expressed as mean± S.E.M. of multiple experiments. Measurements were evaluated using one-way ANOVA or, where appropriate, two-way ANOVA with Tukey’s test for multiple comparisons, with PRISM software (Graph Pad, San Diego, CA, U.S.A.).

**RESULTS**

**Inhibitory Effect of Chlorogenic Acid on Aldose Reductase Activity in Vitro** The IC50 value of chlorogenic acid (Fig. 1A) in this assay was comparable to that of known ARIs, such as TMG and epalrestat, which suggested that chlorogenic acid appeared to have an inhibitory effect on AR activity. To determine whether chlorogenic acid had a dose-dependent inhibitory effect, rat lens AR was incubated with chlorogenic acid or positive control inhibitors at various concentrations (Fig. 1B). The inhibition of AR by chlorogenic acid and positive control inhibitors gradually increased in a dose-dependent manner.

**Inhibitory Effect of Chlorogenic Acid on Xylose-Induced Lenses Opacity** Next, we tested whether chlorogenic acid prevented xylose-induced lens opacity. Most lenses were covered with opaque rings after 2 d of incubation with 20 mM xylose. The effects of chlorogenic acid were measured every 24 h and compared to those in untreated xylose lenses. Figure 2A shows the opacities of rat lenses incubated in xylose media containing various concentrations of chlorogenic acid (0, 10, 30 or 50 μM). The opacities of the lenses began to increase after 1 d of xylose treatment, and opacities were gradually improved by chlorogenic acid treatment in a dose-dependent manner.

**Preventive Effect of Chlorogenic Acid on Cytotoxicity** To determine the effect of chlorogenic acid on cytotoxicity in HLE-B3 cells under diabetic condition and the optimal condition required for viability, cells were first treated with chlorogenic acid for 72 h. Cells viability is presented as a percentage of the control value. Chlorogenic acid had no cytotoxicity in HLE-B3 cells at concentrations <500 μM (Fig. 3A). Next, cells were treated with various concentration of glucose for 72 h. Cells cultured under high glucose conditions (>50 mM) exhibited significant cytotoxicity (Fig. 3B). Co-treatment with high glucose and chlorogenic acid results in reduced cytotoxicity in a dose-dependent manner (Fig. 3C).

**DISCUSSION**

AR catalyzes the reduction of glucose to sorbitol through the polyol pathway and osmotic stress in the lens caused by sorbitol accumulation induces apoptosis in lens epithelial cells leading to the development of cataracts. Previous studies have suggested that the development of cataract may be prevented by ARIs. In this study, we demonstrated, for the first time, that chlorogenic acid inhibits xylose-induced lens opacity and high glucose-induced cytotoxicity in HLE-B3.
cells. These results show a new treatment possibility for chlorogenic acid against cataract development in the presence of diabetes mellitus.

Cataracts are the leading cause of blindness worldwide. Though the overall outcomes of cataract surgery are excellent, diabetic patients have a higher risk of complications after cataract surgery compared to nondiabetic patients. Surgery may cause a rapid acceleration of retinopathy, induce ruberosis, or lead to macular changes. ARIs and antioxidants have proven beneficial in the prevention or treatment of this diabetic condition in *in vitro* and *in vivo* experimental studies. As phytochemicals are reported to be ARIs, many natural sources have been tested for their ability to provide AR inhibitory activity and prevent diabetic cataract formation *in vivo*. Other ARIs, such as imrestat, ponalrestat, TMG, and epalrestat, also have beneficial effects on diabetic cataract prevention. The main structural features of these ARIs is a polar head group and a hydrophobic ring system.

The present study showed that chlorogenic acid (IC₅₀=0.95±0.28 μM) has stronger AR inhibitory activity than TMG (28.81±1.52 μM), a positive control. However, it was not more effective than epalrestat (0.07±0.01 μM). This result agrees with the observations of Yoshikawa *et al.*, who reported that chlorogenic acid shows inhibitory effects on constituents from *Chrysanthemum indicum*. Recently, we reported that *Aster koraiensis* extract prevents the development of diabetic cataract and nephropathy in streptozotocin-induced diabetic rats. Chlorogenic acid is composed of the two major components of *Aster koraiensis* extract. The AR inhibitory effect of chlorogenic acid may mediate the inhibition of lens opacity. As shown in Fig. 3, chlorogenic acid also prevented cytotoxicity in HLE-B3 cells cultured under high glucose conditions. High glucose induces oxidative stress, and increased oxidative stress causes apoptosis in several cells. Toxic aldehydes generated by oxidative stress in the lens epithelium can also contribute to damage of lens proteins, leading to opacity. Chlorogenic acid shows anti-oxidant effects. Thus, this result indicates that chlorogenic acid is a major compound of *Aster koraiensis* with preventive effects on diabetic cataract formation. These results strongly suggest that further *in vivo* study
of the antitoxic effects chlorogenic acid is necessary to evaluate its mechanism of action and to fully establish its safety profile for the prevention of diabetic cataracts.

In summary, this study demonstrates for the first time that chlorogenic acid inhibits xylose-induced lens opacity and cytotoxicity in HLE-B3 cells under cultured diabetic conditions. These results suggest the usefulness of chlorogenic acid particularly in the treatment of diabetic cataracts.

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REFERENCES