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Research article

Chlorogenic acid rescues sensorineural auditory function in a diabetic animal model

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HIGHLIGHTS

- CA may improve damaged peripheral and central auditory function in the DM mouse model.
- CA may aid in the recovery of OHCs damage in the DM mice cochlea and otic hair cells damage in AIZL.
- The efficacy of CA in diabetic sensorineural auditory dysfunctions seems to be an independent action of CA, rather than an hypoglycemiant effect.

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ABSTRACT

Recently, many studies have reported that sensorineural hearing impairment related to neurological disorders may be caused by diabetes mellitus. However, to date, only a small number of studies have investigated the treatment of sensorineural hearing impairment. In the present study, the effects of chlorogenic acid on diabetic auditory pathway impairment were evaluated by neuro-electrical physiological measurements and morphological investigations. We have shown that CA efficiently prevents the progression of auditory pathway dysfunction caused by DM using auditory brainstem responses and auditory middle latency responses in mice. Additionally, using transient-evoked otoacoustic emissions measurement and scanning electron microscope observation of hair cells in DM mice, we found that CA may aid in the recovery from outer hair cell and otic hair cell damage. In conclusion, CA has beneficial effects for the management of diabetic sensorineural auditory dysfunction.

1. Introduction

Recently, many studies have reported that sensorineural hearing impairment is a neurological disorder possibly caused by diabetes mellitus (DM) [1]. Additionally, DM was shown to be an independent risk factor for hearing impairment in a large population-based dataset study [2].

DM causes neuropathy and microvascular damage, especially affecting the peripheral arteries and nerves, kidneys, and retinas [3]. The cochlea and auditory nerves are likewise at risk [4]. Previous studies have reported thickened vessels of the stria vascularis, atrophy of the stria vascularis, and loss of outer hair cells (OHCs) in the cochlea [5]. Auditory neural changes have also been demonstrated by histologic studies revealing auditory nerve demyelination and spiral ganglion loss [6].

But few studies on preventing or treating diabetic sensorineural hearing impairments have been reported. Accordingly, this study investigated the anti-diabetic sensorineural auditory dysfunction efficacy of chlorogenic acid (CA) administration in a DM mouse model and a zebrafish model of alloxan-induced neuromast damage.

To demonstrate the effect of CA in diabetic sensorineural auditory dysfunction, we performed electrophysiological auditory functional studies in a DM mouse model. Neurological evaluation of the auditory brainstem responses (ABRs) served to assess the integrity of the peripheral auditory nerve and the lower part of the brain. Furthermore, assessment of the auditory middle latency responses (AMLRs) provided useful insight into the neurological function of the higher central auditory nervous system. We also performed transient evoked otoacoustic emissions (TEOAEs) testing to directly measure cochlear function and used scanning

Abbreviations: DM, diabetes mellitus; CA, chlorogenic acid; ABRs, auditory brainstem responses; AMLRs, auditory middle latency responses; TEOAE, transient evoked optoacoustic emission; SEM, scanning electron microscope; GLM, glimepiride; AIZL, alloxan induced zebrafish larvae.

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electron microscope (SEM) observation to directly measure the outer hair cells in DM mice. We confirmed the ameliorative action of CA using live image observation of neuromasts and otic hair cells in a zebrafish larvae model of alloxan-induced neuromast damage. Finally, in a comparative experiment with hypoglycemic agents, we verified whether the efficacy of CA was related to its glucose-lowering effects.

2. Materials and methods

All of the experimental procedures described herein were performed in accordance with the Principles of Laboratory Animal Care (NIH publication, #80-23, revised 1996) and the Animal Care and Use Guidelines of Nambu University, Republic of Korea. Seven-week-old male *Lepr (+/+)C57BL/KsJ (dbdb)* mice and *Lepr(+/−)C57BL/KsJ* mice *(dbh)* as the appropriate control were purchased from Jung-Ang Lab Animal (Seoul, Republic of Korea). The mice were housed individually at a 12-h light/dark cycle with food and water *ad libitum*.

Normal hearing mice were divided into four groups (n = 10/group) as follows: eight-week-old adult male *Lepr(+/−)C57BL/KsJ (dbh)* littermates (Nor), *Lepr (+/+)C57BL/KsJ (dbdb)* mice (DM), and *Lepr (+/+)C57BL/KsJ (dbdb)* mice treated with 10 mg/kg (C10) and 20 mg/kg (C20) CA. CA treatments were performed once daily for 8 weeks beginning in mice that were 8 weeks old.

Body weights and blood glucose levels were evaluated in 16-week-old mice of four groups (n = 10/group) using a method described by Hong and Kang [7]. At 16 weeks, the ABR, AMLR, and TEOAE measurements of four groups (n = 10/group) were measured on anesthetized mice after *i.m.* administration of xylazine 0.43 mg/kg (Bayer Korea) and ketamine 4.57 mg/kg (Yuhan Co., Korea) using a method described by Hong and Kang [7]. Peripheral auditory functions were assessed through measurements of ABRs and AMLRs using GSI Audera (Viasys Healthcare Inc., USA) and cochlear function was assessed with TEOAEs using ILO analyzer (Otodynamics Co. Ltd., UK). After the auditory tests, all mice of four groups (n = 10/group) were sacrificed and the cochleae were prepared for scanning electron microscopy (SEM) measurements, as described previously [8]. The hair cells of the organ of Corti were observed using a Hitachi S-500 SEM (Tokyo, Japan).

Adult zebrafish or larvae maintenance and embryo production were performed as described previously [9]. The zebrafish larvae were divided into four groups (n = 10/group) as follows: no treated zebrafish larvae (Nor), alloxan-induced zebrafish larvae (AIZL) (DM), AIZL treated with 10 μM glimepiride (GLM), and AIZL treated with 10 μM CA (CA).

Five days post-fertilization (dpf), the zebrafish larvae of DM, GLM, and CA groups were treated with 100 μM alloxan for 15 min, and then placed in 0.03% sea salt solution. After 6 h, the zebrafish larvae of GLM or CA groups, respectively, were treated with 10 μM glimepiride (GLM) or 10 μM CA for 12 h. Following treatment, the zebrafish larvae of four groups (n = 10/group) were stained with 0.1% YO-PRO for 30 min to identify apoptotic cells and the morphologies of the lateral line neuromasts and otic hair cells were analyzed under a fluorescence microscope using a method described [9].

Data were analyzed using the Prism 5 Statistical Software package (GraphPad, San Diego, CA, USA). All data are expressed as mean ± standard error of the mean. Statistical comparisons between all groups were performed using a one-way ANOVA test with a Dunnett post hoc test. *P* values of <0.05, 0.01, and 0.001 were considered significant.

3. Results

To investigate the anti-diabetes efficacy of CA, we evaluated body weights and blood glucose levels in 16-week-old mice (Fig. 1). The DM group exhibited significantly increased body weights and blood glucose levels compared with the Normal group. However, the body weights in the C20 group showed significantly decreased compared with DM group. Mice in the CA treatment groups exhibited decreased blood glucose levels compared with DM group. These results demonstrate that CA improved the hyperglycemia observed in the diabetic mouse model.

To detect any improvement mediated by CA in peripheral auditory function damaged by DM, hearing threshold and latency tests were performed using ABRs. Hearing thresholds or latencies in response to clicks, 4-kHz TBs and 8-kHz TBs in the DM group were significantly higher or delayed compared with the Normal group, respectively. Hearing thresholds or latencies of the CA treatment groups decreased significantly compared to the DM group (Fig. 2A-F). These data indicate that CA may improve damaged peripheral auditory function in the DM mouse model.

In order to investigate any protective effects of CA against abnormalities in central auditory functions, AMLR amplitudes and latencies were measured. Pa latencies and the Na-Pa amplitudes of the AMLRs in the DM group were significantly delayed and lower compared with the Normal groups. However, in the CA treatment groups, Pa latencies decreased and Na-Pa amplitudes increased compared with DM group to an extent similar to that observed in the Normal group. These data indicate that the central auditory pathway may become affected in diabetic mice over time, whereas CA may suppress central auditory pathway dysfunction in DM mice models (Fig. 3A, B).

To assess the efficacy of CA against the normality of cochlear function and morphological differences in the DM mouse model, TEOAE tests and SEM measurements were performed. TEOAE SNRs of the DM group were significantly lower compared with the Normal group. The results of the morphological analysis of hair cells in the organ of Corti performed using SEM were similar to the physiological findings. Damage to the stereocilia of OHCs occurred in the DM mice. In the CA treatment groups, TEOAE SNRs were increased significantly in a dose-dependent manner excluding 4 kHz TB stimulus (Fig. 4A, B, C). Also, the morphologic results of OHCs in mice treated with CA were similar to those of the Normal mice (Fig. 4D). The concordance between these morphological and physiological data suggests that CA may aid in the recovery of OHC damage in the cochlea.

In order to morphologically confirm the efficacy of CA, lateral line neuromasts and otic hair cells were observed in AIZL using live images obtained with a fluorescence microscope. In AIZL, damage to lateral line neuromasts and otic hair cells was observed. However, in the CA-treated zebrafish, number of trunk neuromasts increased significantly compared with the number in DM zebrafish (Fig. 5A, B). The number of otic hair cells also increased and the increased distance between each hair cell decreased significantly compared with DM zebrafish (Fig. 5C–E). We confirmed the ameliorative action of CA on alloxan induced damage to neuromast and otic hair cells. In order to determine whether the effect of the CA was mediated through a glucose-lowering effect, a comparative experiment using zebrafish larvae treated with GLM, a hypoglycemic agent, was performed. GLM-treated zebrafish larvae showed no significant difference compare with animals in the alloxan-treated group with respect to the number of trunk neuromasts and the number of otic hair cells or the increased distance between hair cells.