Short Communication

Protective effects of alpha-lipoic acid on hair cell damage in diabetic zebrafish model

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\textbf{ABSTRACT}

Hearing impairment is one of the complications in diabetes mellitus; however, there are very few therapeutic studies on it. In this study, we investigated the protective effect of alpha-lipoic acid (ALA) on hearing loss in diabetic transgenic zebrafish and confirmed that ALA protects the loss of hair cells (HCs) caused by hyperglycemia. The data indicated that ALA has a protective effect on the damage to HCs in diabetic zebrafish.

1. Introduction

Diabetes mellitus (DM), characterized by high levels of blood glucose, causes several complications, including hearing loss, neuropathy, cardiomyopathy, and nephropathy. Diabetic hearing loss is a prevalent complication, similar to peripheral neuropathy, and is characterized by changes in cochlear structure and the loss of hair cells (HCs) that be caused by hyperglycemic damage to the vasculature and neural system in the inner ear \cite{1–3}. In addition, hyperglycemia could directly cause HC loss via the glucose toxicity-induced intracellular stress, because HCs use glucose as an energy source and glucose is transported by several glucose transporters (GLUTs), such as GLUT3 and GLUT10; glucose is required for mechanotransduction as well as ATP synthesis \cite{4,5}. However, hearing loss as a diabetes complication is poorly understood in clinical settings, and there are no specific treatment for this condition.

Alpha-lipoic acid (ALA), also known as thioctic acid, is one of the treatments for diabetic neuropathy. It is considered an effective antioxidant \cite{6–8} and can serve as a potential treatment for hearing impairment in patients with diabetes. Several studies have demonstrated that ALA has protective and therapeutic effects against ototoxicity. \cite{9,10}. Therefore, we hypothesized that ALA could protect HCs from damage under diabetic conditions. To test this hypothesis, we generated transgenic zebrafish that exhibit hyperglycemic loss of HCs, using chemogenetic ablation, and examined the protective effects of ALA on HCs.

2. Materials and methods

2.1. Zebrafish maintenance and ethics

Wild-type (AB strain) and \textit{Tg(ins:nfsB-mCherry)} lines were used in the present study. All experimental procedures were approved by the Korea University Institutional Animal Care and Use Committee and performed in accordance with the animal experiment guidelines of the Korea National Veterinary Research and Quarantine Service.

2.2. Generation of \textit{Tg(ins:nfsB-mCherry)} zebrafish

To establish the \textit{Tg(ins:nfsB-mCherry)} zebrafish line, we used multi-site Gateway cloning with 5′-\textit{ins} [11], middle-\textit{nfsB-mcherry} [12], 3′-polyA entry clones, and LR clonase II (Invitrogen, CA, USA), according to the manufacturer’s instructions [13]. The \textit{Ins:nfsB-Mcherry} plasmid was co-injected with transposase mRNA into wild-type embryos at the one-cell...
2.3. Synthesis of antisense RNA probe and whole-mount in situ RNA hybridization

We amplified the ins cDNA using 2–5 days-post-fertilization (dpf) zebrafish cDNA and PCR primers designed from the GenBank sequence.
3.1. Ablation of pancreatic \( \beta \)-cells causes hyperglycemia in Tg(ins:nfsB-mCherry) zebrafish

To establish a hyperglycemic zebrafish model, we generated Tg(ins:nfsB-mCherry) zebrafish that express nitroreductase (nfsB)-fused mCherry proteins in pancreatic \( \beta \)-cells under the control of the ins promoter (Fig. 1A). nfsB converts a prodrug MTZ into a cytotoxic product, resulting in specific ablation of pancreatic \( \beta \)-cells. We observed that mCherry\(^{+} \) pancreatic \( \beta \)-cells were ablated at 4 dpf in MTZ-treated transgenic larvae compared to those in DMSO-treated transgenic larvae (control) (Fig. 1B, G). Apoptosis of mCherry\(^{+} \) pancreatic \( \beta \)-cells was detected using the TUNEL assay (Fig. 1C, D), and the expression of the ins mRNA was found to be reduced, as assessed using in situ RNA hybridization (Fig. 1E, F) and qRT-PCR (Fig. 1H). We further confirmed that glucose levels were higher in MTZ-treated transgenic larvae than in controls (Fig. 1J). These data indicate that the impairment of pancreatic \( \beta \)-cells causes hyperglycemia in zebrafish larvae.

3.2. ALA protects against HC loss due to hyperglycemia

We observed that the number of HCs in the MTZ-treated transgenic larvae was lower than that in the controls, as evidenced by YO-PRO1 staining results (Fig. 1K, P, R). Conversely, there were no effects on HCs in the DMSO, ALA, DMSO and ALA co-treated transgenic larvae (Fig. 1L, M, R), or MTZ-treated wild-type larvae (Fig. 1N, R). In addition, the number of HCs was higher in larvae co-treated with MTZ and ALA than in those treated with MTZ alone (Fig. 1P, Q, R). These findings indicate that ALA protects HCs from hyperglycemia-induced cell loss.

4. Discussion

Hyperglycemia promotes the formation of reactive oxygen species (ROS) through intracellular signaling pathways, such as the polyl pathway [19], and then directly damages the cochlea to induce hearing impairment [1–3]. In the present study, we demonstrated that ALA, a metabolic antioxidant, exerts protective effects against hyperglycemic loss of HCs in a diabetic zebrafish model. Our results are supported by previous studies showing that ALA effectively prevents HC damage from cisplatin-induced ototoxicity in mice as well as in cell cultures by scavenging the ROS [8,9]. In addition, a previous study reported that ALA attenuates kanamycin-induced ototoxicity in mice by inhibiting the expression of p38 and p-JNK involved in apoptosis [15]. Therefore, we suggest that ALA has protective effects against hyperglycemia-induced HC loss and might be effective in clearing ROS and inhibiting apoptotic molecules.

Several studies have reported that ALA can restore the conduction velocity of peripheral nerves following cisplatin-induced peripheral neurotoxicity in rats and reduces the apoptosis of neurons after L-hydroxyglutaric acid-induced neurotoxicity in the zebrafish brain [16,17]. These data indicate that ALA exerts neuroprotective effects. Hong et al. also have shown that the necrosis of pancreatic \( \beta \)-cells causes sensorineural hearing loss and loss of HCs following hyperglycemia in chemical-induced diabetic zebrafish and mouse models [18]. Therefore, ALA could have neuroprotective effects against diabetic hearing loss in zebrafish.

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References

[1] H. Fukushima, S. Cureoglu, P.A. Schachern, T. Kununoki, M.F. Oktay, N. Fukushima, M.M. Paparella, T. Harada, Cochrane changes in patients with type 1 diabetes mellitus, Otolaryngol. Head Neck Surg. 133 (1) (2005) 100–106, https://doi.org/10.1016/j.otohns.2005.02.004.

[2] H. Fukushima, S. Cureoglu, P.A. Schachern, M.M. Paparella, T. Harada, M.F. Oktay, Effects of type 2 diabetes mellitus on cochlear structure in humans, Arch. Otolaryngol. Head Neck Surg. 132 (9) (2006) 934–938, https://doi.org/10.1001/archotol.132.9.934.

[3] M. Kishio, K. Tanaka, Pathological changes of the inner ear and central auditory pathway in diabetes, Ann. Otol. Rhinol. Laryngol. 80 (2) (1971) 218–228, https://doi.org/10.1177/000348497108002028.

[4] N. Huerzeler, V. Petkovic, M. Sekulic-Jablanovic, K. Kucharava, M.B. Wright, D. Bodmer, Insulin receptor and glucose transporters in the mammalian cochlea, Audiol. Neurootol. 24 (2) (2019) 65–76, https://doi.org/10.1159/000499561.

[5] B. Chen, Y. Wang, M. Geng, X. Lin, W. Tang, Localization of glucose transporter 10 to hair cells’ cuticular plate in the mouse inner ear, Biomed. Res. Int. 2018 (2018) 1–7, https://doi.org/10.1155/2018/9951764.

[6] E. Packr, K. Kraemer, G. Rimbach, Molecular aspects of lipic acid in the prevention of diabetes complications, Nutrition 17 (10) (2001) 888–895, https://doi.org/10.1039/s0899-9007(01)00586-x.

[7] G. Winarska, D. Malinska, K. Szymanski, M. Dudziak, J. Bulya, Lipidic acid ameliorates oxidative stress and renal injury in alloxan diabetic rabbits, Biochimie 90 (3) (2008) 450–455, https://doi.org/10.1016/j.biochi.2007.11.010.
[9] J. Kim, H.J. Cho, B. Sagong, S.J. Kim, J.T. Lee, H.S. So, I.K. Lee, U.K. Kim, K.Y. Lee, Y.S. Choo, Alpha-lipoic acid protects against cisplatin-induced ototoxicity via the regulation of MAPKs and proinflammatory cytokines, Biochem. Biophys. Res. Commun. 449 (2) (2014) 183–189, https://doi.org/10.1016/j.bbrc.2014.04.118.

[10] K.H. Kim, B. Lee, Y.R. Kim, M.A. Kim, N. Ryu, D.J. Jung, et al., Evaluating protective and therapeutic effects of alpha-lipoic acid on cisplatin-induced ototoxicity, Cell Death Dis. 9 (8) (2018) 827, https://doi.org/10.1038/s41419-018-0888-z.

[11] H. Pisharath, J.M. Rhee, M.A. Swanson, S.D. Leach, M.J. Parsons, Targeted ablation of beta cells in the embryonic zebrafish pancreas using E. coli nitroreductase, Mech. Dev. 124 (3) (2007) 218–229, https://doi.org/10.1016/j.mod.2006.11.005.

[12] A.Y. Chung, P.S. Kim, S. Kim, D. Kim, I. Jeong, H.K. Kim, et al., Generation of demyelination models by targeted ablation of oligodendrocytes in the zebrafish CNS, Mol. Cell 36 (1) (2013) 82–87, https://doi.org/10.1007/s10059-013-0087-9.

[13] K.M. Kwan, E. Fujimoto, C. Grabher, B.D. Mangum, M.E. Hardy, D.S. Campbell, et al., The Tol2kit: a multisite gateway-based construction kit for Tol2 transposon transgenesis constructs, Dev. Dyn. 236 (11) (2007) 3088–3099, https://doi.org/10.1002/dvdy.21343.

[14] C. Thisse, B. Thisse, High-resolution in situ hybridization to whole-mount zebrafish embryos, Nat. Protoc. 3 (1) (2008) 59–69, https://doi.org/10.1038/nprot.2007.214.

[15] A. Wang, N. Hou, D. Bao, S. Liu, T. Xu, Mechanism of alpha-lipoic acid in attenuating kanamycin-induced ototoxicity, Neural Regen. Res. 7 (35) (2012) 2793–2800, https://doi.org/10.3969/j.issn.1673-5374.2012.35.007.

[16] C. Parng, C. Ton, Y.X. Lin, N.M. Roy, P. McGrath, A zebrafish assay for identifying neuroprotectants in vivo, Neurotoxicol. Teratol. 28 (4) (2006) 509–516, https://doi.org/10.1016/j.ntt.2006.04.003.

[17] S. Tuncer, N. Dalkilic, M. Akif Dunbar, B. Keles, Comparative effects of a lipoic acid and melatonin on cisplatin-induced neurotoxicity, Int. J. Neurosci. 120 (10) (2010) 655–663, https://doi.org/10.3109/00207454.2010.510916.

[18] B.N. Hong, Y.H. Nam, S.H. Woo, T.H. Kang, Chlorogenic acid rescues sensorineural auditory function in a diabetic animal model, Neurosci. Lett. 640 (2017) 64–69, https://doi.org/10.1016/j.neulet.2017.01.030.

[19] S.S. Chung, E.C. Ho, K.S. Lam, S.K. Chung, Contribution of polyol pathway to diabetes-induced oxidative stress, J. Am. Soc. Nephrol. 14 (2003) S233–S236, https://doi.org/10.1097/01.ann.0000077408.15865.06.