Glycative Stress Research

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Review article

Glycative stress and the inner ear disorder

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Abstract

Sensorineural hearing loss is reported to be frequently associated with diabetic patients; however, it has not been widely recognized as a complication. Renal disorders are well known as one of the three major complications of diabetes, and the renal glomerulus has a histological similarity to the cochlear stria vascularis of the inner ear. It is well known that ototoxicity and nephrotoxicity are common adverse events in administration of aminoglycoside antibiotics and platinum-based antineoplastic agents. As just described, the relationship between inner ear disease and renal disease has been pointed out before. In order to elucidate the mechanism of the onset of hearing loss in diabetes, we conducted a study using Tsumura Suzuki Obese Diabetes (TSOD) mouse, a type 2 diabetes mellitus (T2DM) model. It was confirmed that TSOD mice developed deafness earlier than those of the control, and histopathologically, a significant reduction in the capillary distribution density was observed in the stria vascularis. This is considered to be a finding of diabetic microangiopathy in the inner ear. In order to prevent hearing loss caused by diabetes, caloric restriction and Kampo administration (Bofutsushosan and Daisaikoto) were conducted with TSOD mice, resulting in the hearing loss being significantly ameliorated and the cochlear blood flow being maintained. On the other hand, the administration of metformin, drawing attention in the field of anti-aging medicine, was not effective in TSOD mice, while, in the presbycusis model DBA/2, the hearing loss was suppressed from the early stage in an interesting issue. The background of T2DM is often associated with the metabolic syndrome caused by excessive accumulation of visceral fat. Various factors are involved in its pathogenesis in a complicated manner. Further studies are needed to clarify the mechanism of diabetic inner ear disorder.

***KEY WORDS*:** diabetes mellitus type 2, hearing loss, auditory brainstem response, cochlea, stria vascularis, metformin

Introduction

Due to changes in lifestyle, such as diet and exercise, the diabetic population in Japan has been increasing; however, sensorineural hearing loss is not widely recognized as a diabetic complication. Deafness induced by diabetes, often early onset from past epidemiologic studies, impairs the low and mid range, which is important for conversation, and thus significantly reduces quality of life (QOL). In recent reports, it has been shown that hearing loss is a risk of dementia in the elderly and, with growing public interest in this issue, attention is being paid to prevention as well as treatment for hearing loss. Based on our study using type 2 diabetes mellitus (T2DM) model mice, this article describes changes of the inner ear in diabetes-induced auditory disorder and its prevention.

Anatomy of the inner ear

The structure of the ear consists of the outer ear, middle ear, and the inner ear, which contains the cochlea, which controls auditory sense, and the vestibular and semicircular canals, which control the balance sense. The cochlea is a peripheral auditory sensory organ with a two-and-a-half-turn snail-like structure. The cross section of the cochlea is divided into three sections. The central section is called the cochlear canal, with the endolymph fluid inside and the outer section includes scala vestibule and scala tympani with the perilymph fluid inside. The composition of perilymph is a high Na^+ and low K^+ concentration similar to extracellular fluid, while the composition is different in the endolymph with high K^+ and low Na^+ as well as intracellular fluid.

The sound that is input to the outer ear as air vibrations

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vibrates the eardrum and auditory ossicles of the middle ear and vibrates the labyrinthine fluid in the cochlea of the inner ear, followed by conversion into electrical energy as the action potential of neurons, and then is transmitted from the cochlear nerve afferently to the central nervous system. Similarities exist between the inner ear cochlear striatum and renal glomeruliis region that supplies the energy required for conversion.

The cochlear stria vascularis, rich in capillaries, is located on the outer wall of the cochlear duct and spirally surrounds the cochlea, producing an endolymphatic potential of +80 to +90 mV (*Fig. 1*)[1].

Similarities between the cochlear stria vascularis and the renal glomeruli

The kidney includes one million units called nephrons, which are composed of glomeruli and renal tubules. The glomerulus consists of inside capillaries with a thread-ball-like structure and outer surrounding Bowman's capsule with a bag structure. The blood in the capillaries is filtered by the glomerulus and Bowman's capsule receives the primitive urine filtrated. NaCl, Ca^{2+}, and Mg^{2+} are reabsorbed from the glomerulus to the proximal tubule and then NaCl, Ca^{2+}, and water are reabsorbed by the distal tubule, followed by, further in the collecting duct, the reabsorption of Na^{+}, water, K^{+} and the secretion of H^{+}. The cochlear stria vascularis also has many ion transporters, *i.e.*, K^{+} channels and Na, Cl, K cotransporters. K^{+} in the endolymph is said to be recycled, where K^{+} that has flowed into the auditory hair cells circulate from the stria vascularis to the endolymphatic space.

Relationship between labyrinth disease and renal disease

The relationship between labyrinth disease and renal disease has been pointed out before. Alport's syndrome is a disease that causes progressive hereditary nephritis, which is caused by mutation of type 4 collagen gene in the glomerular basement membrane. This disease is known to be often associated with progressive bilateral sensorineural hearing loss. It is also well known that ototoxicity and nephrotoxicity are common adverse events when aminoglycoside antibiotics or platinum-based antineoplastic agents are administered. These reports suggest a similarity between the inner ear and the kidney.

Quick CA *et al.*[2] showed in their pathological study that anticochlear antibodies react with renal tubular epithelium in rats and guinea pigs, and that anti-renal glomerular basement membrane antibodies react with the cochlear stria vascularis and the capillaries of spiral ligament (ligamentum spirale cochleae).

Arnold W *et al.*[3] demonstrated, using a rat glomerulonephritis model induced by an anti-basement membrane antibody, that both stria vascularis and renal glomeruli were impaired in basement membrane and

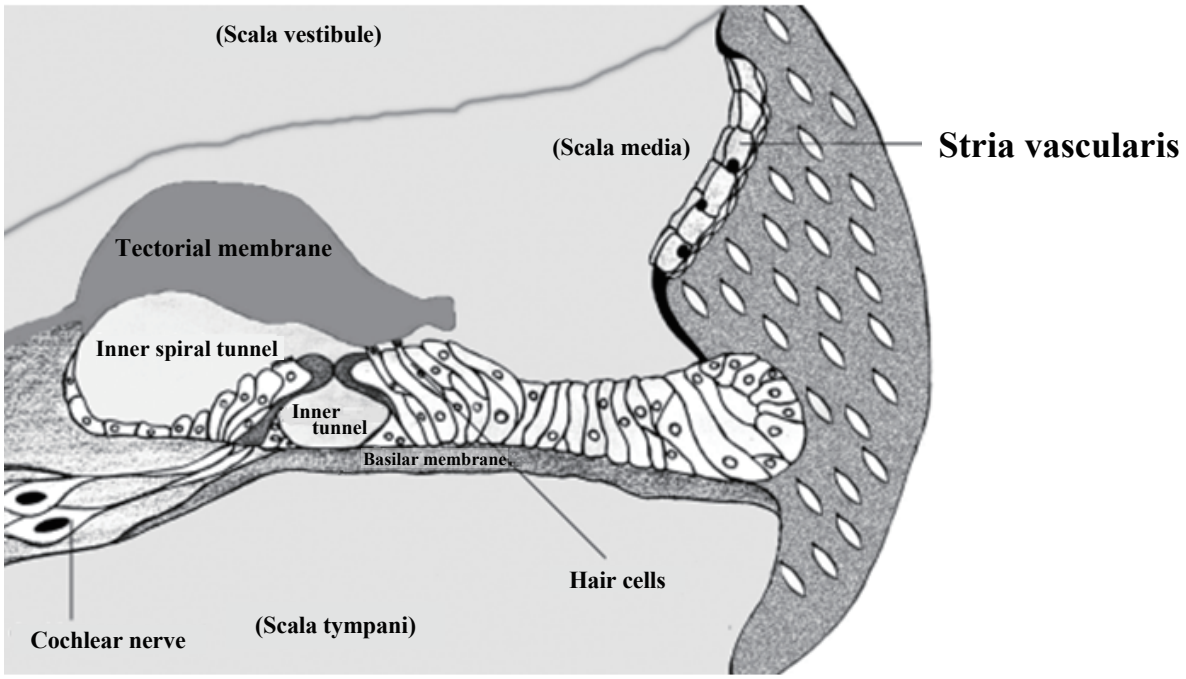

Fig. 1. **Cross section of cochlear.**

The stria vascularis is located on the lateral wall of the cochlea and is involved in endolymph production and maintenance of electric potential. It is rich in capillaries and composed of three types of cells: marginal cells, intermediate cells, and basal cells. Quoted from reference 1).

endothelial cells. It is considered that the inner ear, especially stria vascularis, and the renal glomeruli have similar antigenicity.

Typical histopathological changes in diabetic nephropathy include the glomerular basement membrane thickening, mesangial enlargement, tubular basement membrane thickening, interstitial enlargement, and microvascular exudative lesions. It has been reported that this cause is persistent hyperglycemia exposing to endothelial cells in microcirculation, *i.e.*, glomerular capillaries, peritubular capillaries, and arterioles [4].

The cochlear stria vascularis has a structure composed of epithelial cells and capillaries and is rich in vascularity. Therefore, if there are factors that affect the vasculature, the tissue may be vulnerable to disability

Diabetes and hearing loss

The number of people with diabetes in Japan has been increasing since 1997 [5], and according to the results of the National Health and Nutrition Survey conducted by the Ministry of Health, Labour and Welfare (MHLF) in 2017, the prevalence of diabetes was 18.1% for men and 10.5% for women, with an estimated 10 million people. Although retinopathy, neuropathy, and nephropathy are well known as the three major complications of diabetes, sensorineural hearing loss is not recognized as a major complication. Hearing loss may actually be underestimated.

A meta-analysis by the Lancet International Commission team reported that hearing loss was a significant risk factor for dementia in all three studies, with a high combined relative risk of 1.94 (95% confidence intervals [CI]: 1.38-2.73) [6]. In 2017, the Commission recognized hearing loss as the first factor that could prevent the onset and development of dementia with medical interventions, and it quickly gained worldwide attention. The WHO guideline 2019 "Risk reduction of cognitive decline and dementia" also recommends early response and intervention to hearing loss [7].

The prevalence of hearing loss in patients with diabetes has been extensively reported, including in the 2008 US Large Scale Epidemiologic Survey. According to this report, the prevalence of auditory disorder is significantly higher in diabetic adults, and the risk of hearing loss is higher not only in the high range but also in the mid and low range [8]. A large-scale epidemiological survey was conducted at the National Center for Geriatrics and Gerontology in 2010, and the relationship between diabetes and auditory disorder was investigated in 2,306 middle-aged and elderly local residents. As a result, it was reported that diabetes has a significant negative effect on audibility, and its effect is more pronounced in middle-aged than in elderly [9]. Therefore, it is suggested that hearing loss due to diabetes develops from an early age, and the low- and mid-frequency range, which is important for conversation, is also impaired, which significantly reduces QOL.

Morphological changes in the cochlea due to diabetes have been studied so far using human temporal bones and animal models. However, there still remain many unclear points, not reaching a common conclusion.

Fukushima H *et al.* [10] examined the human temporal bone pathology and pointed out that the cochlea with T2DM had a significantly thickened capillary wall of the stria vascularis compared with the non-diabetic case. They concluded that this is a finding of diabetic microangiopathy in the cochlea.

As an animal model, Smith TL *et al.* [11] reported a thickening of the basement membrane of capillaries of the stria vascularis in streptozocin–treated rats, which is thought to be involved in diabetic microangiopathy. Leptin gene-deficient ob/ob mice are considered to be T2DM models. Lee HS *et al.* [12] investigated auditory sense using this model, and pointed out that the threshold of the auditory brain stem response (ABR) increased from the earlier stage than that of control, and histologically degeneration of outer hair cells and disappearance of ganglion spiral cochleae cell.

Thus, many studies suggest that diabetes causes hearing loss early in life with aging, and microangiopathy occurs in the cochlea. However, because many factors are involved in diabetes, auditory assessment and histological changes in the inner ear are not consistent among models and reporters. It can be said that diabetic inner ear disorder is a complicated pathogenesis in which various findings are mixed.

Study of hearing loss in T2DM model TSOD mouse

The TSOD (Tsumura Suzuki Obese Diabetes) mouse we used is a T2DM model mouse established by sibling mating of male ddY mice that exhibits obesity and diabetes [13]. Manifestation of this model includes overeating, severe obesity, hyperglycemia, hyperinsulinemia, and hyperlipidemia, and as a diabetic complication, peripheral neuropathy has also been reported. Thickening of the glomerular loop wall, enlargement of the mesangial region, and nodule formation have been reported in the kidney. Dyslipidemia has also been confirmed, in addition to elevated total cholesterol and triglyceride levels, and hyper-LDL-cholesterolemia. From the examination of CT images, model rats showed a relatively small amount of subcutaneous fat, but excessive accumulation of visceral fat, which was accompanied by visceral fat type obesity, impaired glucose tolerance, hyperlipidemia, and hypertension. Therefore, this mouse is considered to correspond to the metabolic syndrome model.

Using TSOD mice and a control strain, TSNO (Tsumura Suzuki Non-obesity) mice, we measured body weight, blood glucose level and auditory brainstem response (ABR) over time as an evaluation of auditory sense [14]. Furthermore, each temporal bone was excised and examined histopathologically. In TSOD mice, body weight and blood glucose levels significantly increased from 2 months of age, and were significantly higher than those of TSNO (***Fig. 2***). The ABR threshold, an assessment of hearing, significantly increased in TSOD mice from 8 months of age, resulting in early hearing loss (***Fig. 3***). Histopathologically, in TSOD mice, narrowing of the stria vascular capillaries on the lateral wall of the cochlea was observed, and the additional vascular staining revealed reduction of the blood vessel density compared to that of TSNO (***Fig. 4***). These findings are considered to be diabetic microangiopathy in the cochlea, suggesting a chronic decrease in blood flow in the diabetic inner ear. As mentioned above, the stria vascularis is rich in capillaries, so it is considered to be morphologically

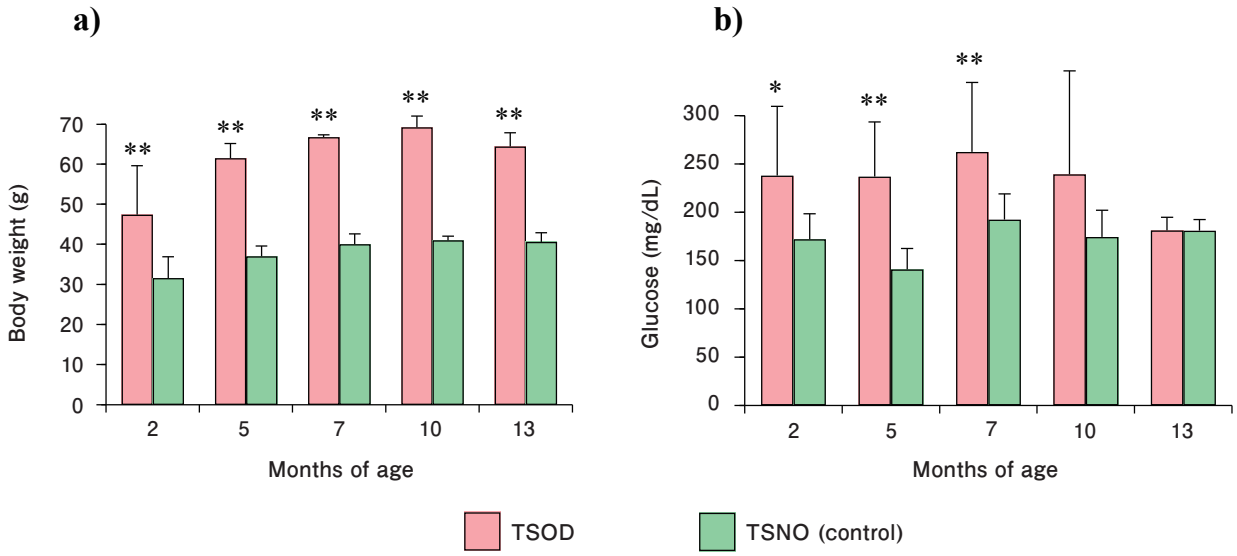

Fig. 2. **Changes in body weight and blood glucose level in TSOD and TSNO mice.**

a) Body weight. Diabetic model TSOD mice showed significant weight gain from 2 months of age, and exhibited severe obesity of more than 60 g at 5 to 10 months of age. Significant weight gain was observed compared to control TSNO mice. **b)** Diabetes model TSOD mice showed a blood glucose level of over 200 mg/dL from 2 months of age, and hyperglycemia persisted up to 10 months of age. Results are expressed as mean ± SEM, * $p < 0.05$, ** $p < 0.01$ vs. TSNO (control), n = 5. TSOD, Tsumura Suzuki Obese Diabetes; TSNO, Tsumura Suzuki Non-obesity = control for TSOD; SEM, standard error mean. Quoted from reference 14).

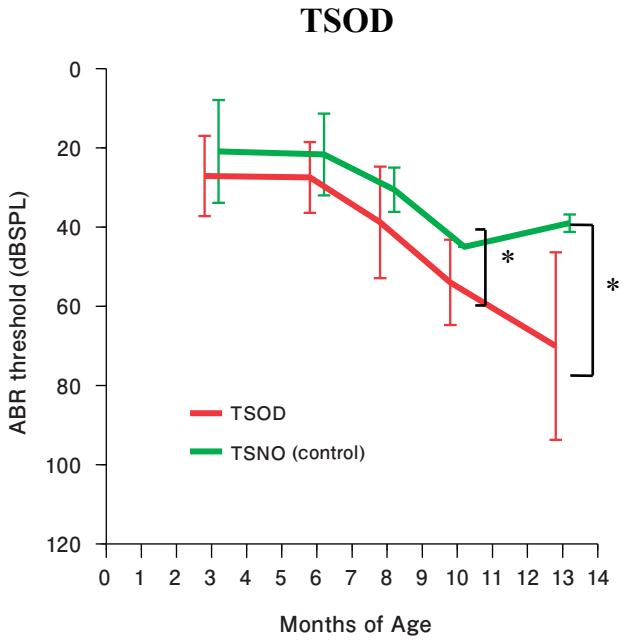

Fig. 3. **Auditory evaluation in TSOD and TSNO mice.**

Auditory sense was evaluated by measuring the threshold of auditory brainstem response (ABR) at 8 kHz. Diabetes model TSOD mice showed a significant threshold increase from 10 months of age, and hearing loss occurred with aging earlier compared with control TSNO mice. Results are expressed as mean ± SEM, * $p < 0.05$ vs. TSNO (control), n = 5. TSOD, Tsumura Suzuki Obese Diabetes; TSNO, Tsumura Suzuki Non-obesity = control for TSOD; SEM, standard error mean. Quoted from reference 14).

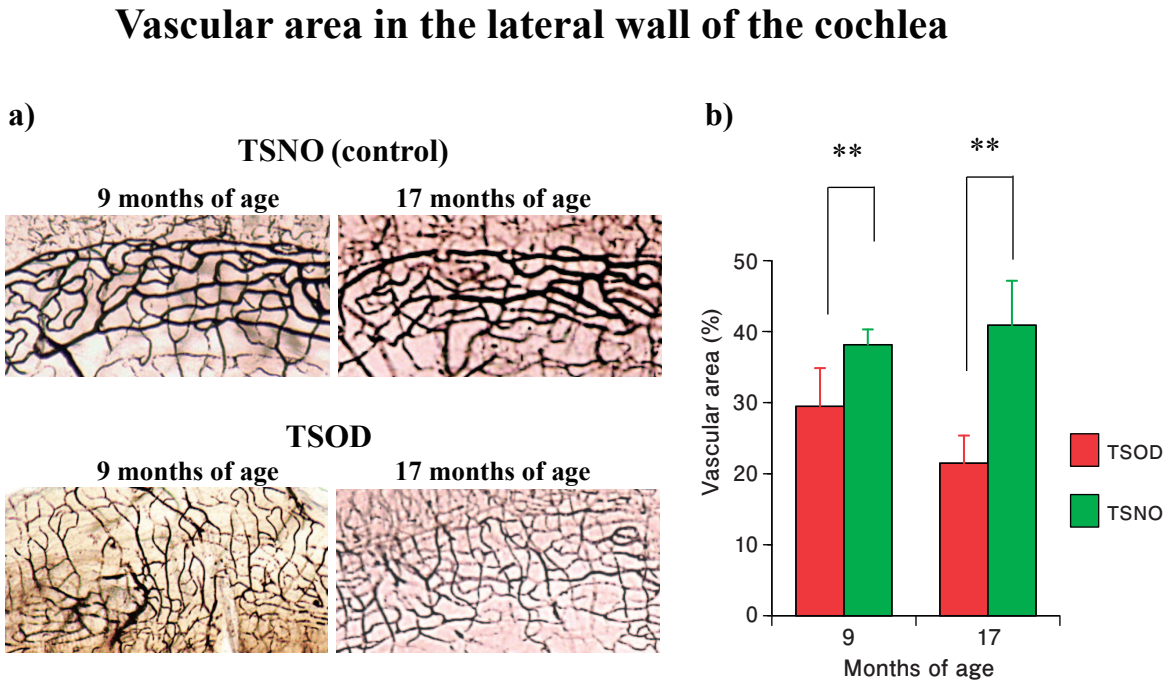

Fig. 4. **Evaluation of blood vessel area of outer wall of cochlea by blood vessel staining (India ink) in TSNO and TSOD mice.**

a) At 9 and 17 months of age, capillaries in the cochlear stria vascularis were observed by blood vessel staining. In TSOD mice, a decrease in the distribution density of capillaries was observed. **b)** When the blood vessel area was evaluated using the image processing software "ImageJ," the TSOD mice showed a significant reduction in the vessel area at any age compared to the TSNO mice. Results are expressed as mean ± SEM, ** $p < 0.01$ vs. TSNO (control), $n = 2$. TSOD, Tsumura Suzuki Obese Diabetes; TSNO, Tsumura Suzuki Non-obesity = control for TSOD; SEM, standard error mean. Quoted from reference 14).

susceptible to microangiopathy. In the 1970s, based on pathological findings in the cochlea, Schuknecht HF [15] classified senile deafness into four types as follows: 1) Sensory presbycusis, 2) Neural presbycusis, 3) Strial presbycusis, and 4) Cochlear conductive presbycusis. TSOD mice are considered to belong to 3) Strial presbycusis. As mentioned above, the outer wall of the cochlea mainly composed of stria vascularis has the function of maintaining the homeostasis of the ion concentration in the inner ear. Therefore, once damaged, hair cells are thought to be unable to function normally and produce sensorineural hearing loss.

Concurrently, gene expression analysis of visceral fat in TSOD mice (12 months of age) revealed high expression of TNF-α and IL-6, inflammatory cytokines [16]. We also performed gene expression analysis in the cochlea of TSOD mice (14 months of age) and found that 176 gene expression increased and 345 gene expression decreased ($n = 3$, $p < 0.01$, expression ratio > 1.5 [= Log2 ratio > 0.585] and expression ratio < 0.66 [= Log2 ratio < 0.585]). There were, in addition to Igf1, decreased expression of many growth factors and receptors such as Igfbp6, Tgfb2, Fgfr2, Fgfr2, Ctgf, Fgf7, Fgf12, and increased expression of many inflammatory cytokines such as IL1a and Icam4. From the above findings, it was confirmed that TSOD mice, a T2DM model, had an early hearing loss with aging, and in the cochlea, chronic blood flow failure due to diabetic microangiopathy was confirmed as in the human temporal bone specimen. Gene comprehensive analysis suggested that chronic inflammation may be involved in the cochlea.

Anti-aging medical trials for the inner ear

As an attempt to protect the inner ear against hearing loss in T2DM model mouse TSOD, we administered calorie restriction and traditional Chinese medicine (Kampo) using Bofutsushosan and Daisaikoto [17]. The experimental animals were divided into 4 groups with 10 animals in each group. In the control group, TSOD mice were fed an ad libitum standard solid diet. In the calorie restricted group, the solid feed was administered every other day. Both the Bofutsushosan group and the Daisaikoto group were raised by allowing the animals to freely ingest a solid feed containing 3% each component. ABR test was performed periodically to evaluate auditory sense, and the cochlea was excised and examined histopathologically. As a result, weight gain was significantly suppressed in the calorie-restricted group, and blood glucose level was decreased in the calorie-restricted group and the Kampo group (Bofutsushosan group, Daisaikoto group). Compared with the control group, the ABR threshold increase was suppressed and the age-related hearing loss was ameliorated in the calorie restricted group and the Kampo group. Similarly, in the histological examination, the distribution density of capillaries on the

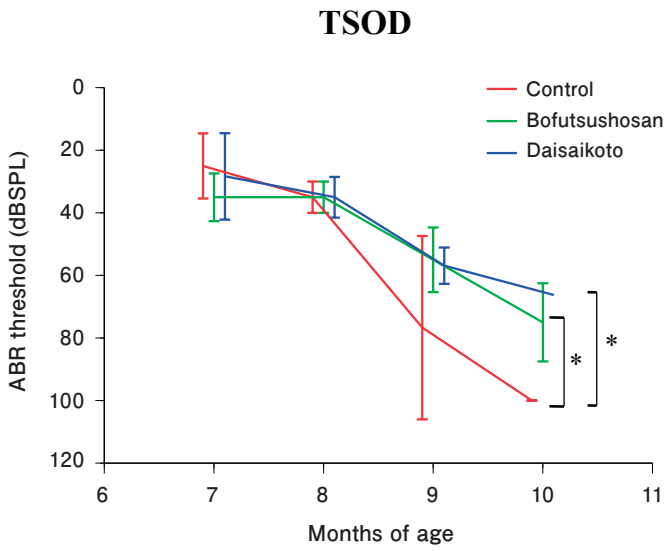

Fig. 5. **Auditory evaluation of Kampo administration to TSOD mice.**

Auditory sense was evaluated by measuring the threshold of auditory brainstem response (ABR) at 8 kHz.
Compared with the control group, the increase in ABR threshold with age was ameliorated in the group treated with Kampo medicine: Bofutsushosan and Daisaikoto. Results are expressed as mean ± SEM, * $p < 0.05$ vs. control (without Kampo medicine), n = 10. TSOD, Tsumura Suzuki Obese Diabetes; SEM, standard error mean. Quoted from reference 17).

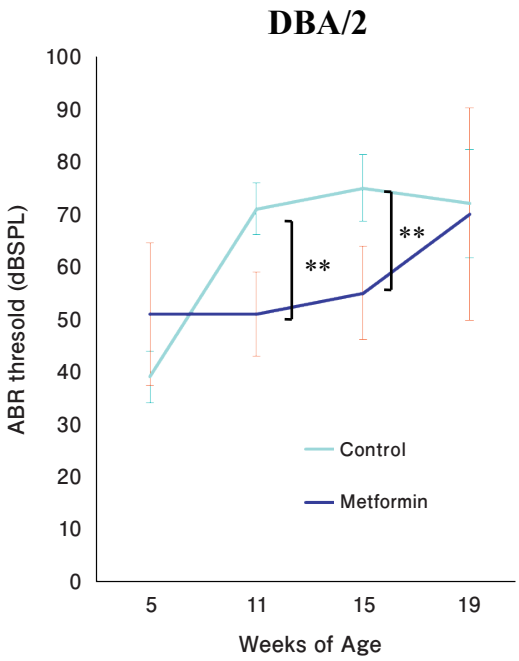

Fig. 6. **Auditory evaluation of metformin administration to DBA/2 mice.**

Auditory sense was evaluated by measuring the threshold of auditory brainstem response (ABR) at 8 kHz.
Compared with the control group, the ABR threshold was significantly lower in the metformin-administered group from 11 weeks of age, indicating a protective effect. Results are expressed as mean ± SEM, ** $p < 0.01$ vs. control (without metformin medicine), n = 5. DBA/2, presbycusis model; SEM, standard error mean.

outer wall of the cochlea was well maintained in the calorie-restricted group and the Kampo group as compared with the control group, suggesting that the blood flow failure in the inner ear due to diabetes improved (*Fig. 5*).

Additional studies were conducted using the antidiabetic drug "metformin." Metformin has been widely used as a therapeutic agent for T2DM widely in the world since the 1950s, and furthermore, many longevity effects and antitumor effects have been reported in recent years, and anti-aging effects have been attracting attention. It is reported that long-term administration of metformin mixed feed to C57BL/6 mice showed a lifespan extension effect of 5.83% as compared with the control group[18]. In addition, 78,241 diabetic patients treated with metformin had a 15% reduction in mortality compared to 90,463 non-diabetic patients[19]. Therefore, a mixed feed of metformin was administered to TSOD mice (T2DM model) for a long term from 4 to 30 weeks of age, and a comparison was made with the control group. Administration was also performed in the same manner in presbycusis model mice C57BL/6J and DBA/2. As a result, in TSOD mice, there was no significant difference in the ABR threshold between the metformin group and the control group. On the contrary, in the C57BL/6 mouse for presbycusis, although there was no significant difference in the metformin group, the increase in ABR threshold tended to be lower than that in the control group at early stage of 10 weeks of age. Furthermore, in the DBA/2 mouse, the ABR threshold was significantly lower in the metformin group from 11 weeks of age, and the inner ear protective effect was observed (*Fig. 6*). An additional histological study of the cochlea in DBA/2 mice showing a significant improvement revealed that ganglion spiral cochleae cells and hair cells under the tectorial membrane were retained well in the metformin group compared to the control group. Observation of SA-β-gal activity, a marker of cellular aging, in various organs throughout the body showed reduced activity in the metformin group at the liver, lungs and brain compared with the control group, suggesting that cellular aging is suppressed (*Fig. 7*).

It is reported as a mechanism of the anti-aging effect of metformin that AMP-activated protein kinase (AMPK) is activated to suppress the aging-promoting signal mTOR (mammalian target of rapamycin) and to activate eNOS (endothelial nitric oxide synthase) in vascular endothelial cells, thus increasing NO production and resulting in the improvement of vascular function[20]. AMPK is also a K channel regulator in outer hair cells and is involved in protection of the inner ear from acoustic damage. It is possible that the administration of metformin activated AMPK in the inner ear and exhibited a protective effect, which is

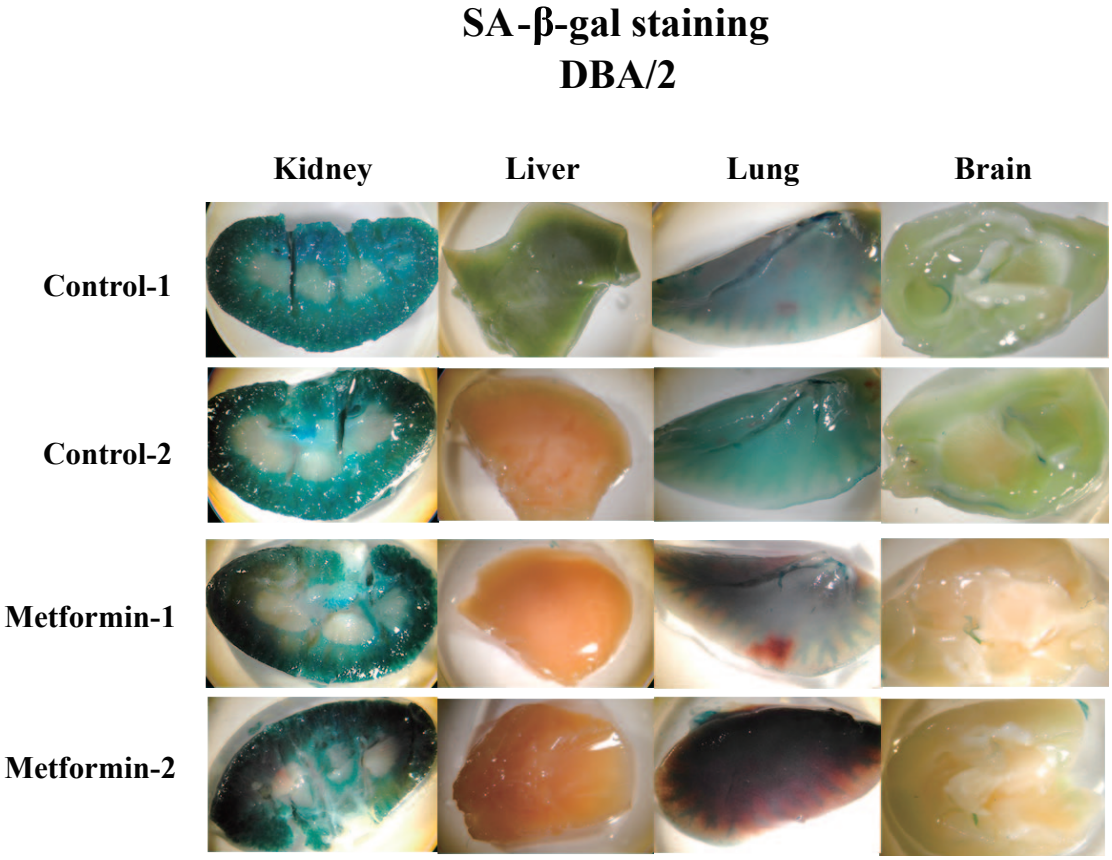

Fig. 7. **Histological evaluation of metformin administration to DBA/2 mice by SA-β-gal staining of various organs.**

The SA-β-gal activity, a marker of cellular senescence, was observed in various organs throughout the body. The stained cells turn blue. In the liver, lung, and brain, the findings suggestive of reduced activity in the metformin group compared with the control group.

necessary for further study. On the other hand, a protective effect was exhibited in the presbycusis model mouse, while not in the T2DM model mouse. As our speculation, since TSOD mice are a model of metabolic syndrome associated with hyperlipidemia, it is possible that oral administration of metformin alone was insufficient to suppress hearing loss associated with severe arteriosclerosis.

Summary

Hearing loss is not widely recognized as a diabetic complication; however, it is often reported that hearing loss occurs with age earlier in patients with diabetes. Hearing impairment occurs in the low and mid range, which is important for conversation, and thus significantly deteriorates QOL. In our examination of T2DM model mouse TSOD, hearing loss was observed from an early stage with aging, and histologically, narrowing of the capillary lumen in the stria vascularis of the lateral wall of cochlea, suggesting the presence of chronic blood flow failure due to diabetic microangiopathy in the inner ear. Administration of Kampo medicine (Boufutsushosan and Daisaikoto) and calorie restriction significantly suppressed age-related hearing loss with maintained cochlear blood flow. Metformin administration was not effective in TSOD mice with severe inner ear vascular disease in the metabolic syndrome model, but in the presbycusis model DBA/2, the hearing loss was suppressed earlier. Metformin administration was not effective in TSOD mice, a metabolic syndrome model, with severe inner ear vascular disease, while in DBA/2 mice, the hearing loss was ameliorated from the earlier stage. Its mechanism was confirmed to have protective effects on cochlear hair cells and spiral ganglion cells.

Conflict of Interest Statement

The authors claim no conflict of interest in this study.

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Reference

1) Akinpelu OV, Ibrahim F, Waissbluth S, et al. Histopathologic changes in the cochlea associated with diabetes mellitus: A review. *Otol Neurotol*. 2014; 35: 764-774.
2) Quick CA, Fish A, Brown C. The relationship between cochlea and kidney. *Laryngoscope*. 1973; 83: 1469-1482.
3) Arnold W. Inner ear and renal diseases. *Ann Otol Rhinol Laryngol Suppl*. 1984; 112: 119-124.
4) Moriya T, Suzuki Y, Inomata S, et al. Renal histological heterogeneity and functional progress in normoalbuminuric and microalbuminuric Japanese patients with type 2 diabetes. *BMJ Open Diabetes Res Care*. 2014; 2: e000029.
5) Ministry of Health, Labour and Welfare. National Health and Nutrition Survey, 2016. (in Japanese) https://www.mhlw.go.jp/content/10904750/000351576.
6) Livingston, G., et al. Dementia prevention, intervention, and care. *Lancet*. 2017; 390(10113): 2673-2734.
7) World Health Organization (WHO). Guidelines for Risk reduction of cognitive decline and dementia. 2019. https://www.who.int/mental_health/neurology/dementia/guidelines_risk_reduction/en/
8) Bainbridge KE, Hoffman HJ, Cowie CC. Diabetes and hearing impairment in the United States: Audiometric evidence from the National Health and Nutrition Examination Survey, 1999 to 2004. *Ann Intern Med*. 2008; 149: 1-10.
9) Uchida Y, Sugiura S, Ando F, et al. Diabetes reduces auditory sensitivity in middle-aged listeners more than in elderly listeners: A population- based study of age-related hearing loss. *Med Sci Monit*. 2010; 16: PH63-68.
10) Fukushima H, Cureoglu S, Schachern PA, et al. Effects of type 2 diabetes mellitus on cochlear structure in humans. *Arch Otolaryngol Head Neck Surg*. 2006; 132: 934-938.
11) Smith TL, Raynor E, Prazma J, et al. Insulin-dependent diabetic microangiopathy in the inner ear. *Laryngoscope*. 1995; 105(3 Pt 1): 236-240.
12) Lee H-S, Kim KR, Chung W-H, et al. Early sensorineural hearing loss in ob/ob mouse, an animal model of type 2 diabetes. *Clin Exp Otorhinolaryngol*. 2008; 1: 211-216.
13) Suzuki W, Iizuka S, Tabuchi M, et al. A new mouse model of spontaneous diabetes derived from ddY strain. *Exp Anim*. 1999; 48: 181-189.
14) Tsuda J, Sugahara K, Hori T, et al. A study of hearing function and histopathologic changes in the cochlea of the type 2 diabetes model Tsumura Suzuki obese diabetes mouse. *Acta Otolaryngol*. 2016; 136: 1097-1106.
15) Schuknecht HF. Pathology of the ear. Harvard University Press, Cambridge, USA, 1974.
16) Nishida T, Tsuneyama K, Fujimoto M, et al. Spontaneous onset of nonalcoholic steatohepatitis and hepatocellular carcinoma in a mouse model of metabolic syndrome. *Lab Invest*. 2013; 93: 230-241.
17) Hori T, Sugahara K, Tsuda J, et al. Oral administration of an herbal medicine to prevent progressive hearing loss in a mouse model of diabetes. *Auris Nasus Larynx*. 2019; 46: 703-708.
18) Martin-Montalvo A, Mercken EM, Mitchell SJ, et al. Metformin improves healthspan and lifespan in mice. *Nat Commun*. 2013; 4: 2192.
19) Bannister CA, Holden SE, Jenkins-Jones S, et al. Can people with type 2 diabetes live longer than those without? A comparison of mortality in people initiated with metformin or sulphonylurea monotherapy and matched, non-diabetic controls. *Diabetes Obes Metab*. 2014; 16: 1165-1173.
20) Salt IP, Hardie DG. AMP-activated protein kinase: A ubiquitous signalling pathway with key roles in the cardiovascular system. *Circ Res*. 2017; 120: 1825-1841.