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Original Research

Digoxin induces inner ear damage

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Abstract

Auditory neuropathy is a hearing disorder in which the inner ear can detect sounds, but has a problem with sending sounds to the brain. The main pathology might be the disorder between inner hair cell synapse and cochlear nerve. Although it is an indication for cochlear implants, there is no radical treatment yet. Therefore, a neuropathy model using animals is needed for the development of new treatment. So, we investigated digoxin-induced inner ear disorders using guinea pigs. As the results, when the digoxin was administrated into the cochlea, the number of cochlear spiral ganglion cells decreased. However, no obvious damage was observed to the cochlear hair cells. As the result of ABR (Auditory Brainstem Response), physiological dysfunction was also confirmed. As for the effect on the vestibule, the vestibular ganglion cells were damaged, but the hair cells in the otoliths and in the ampulla were not damaged. These results suggest that digoxin might be useful drug for the generation of the animal model to auditory neuropathy.

Key words: digoxin, ganglion cells, hair cells

Introduction

Cardiac glycosides such as digitoxin and digoxin extracted from the leaves of digitalis, and K-strophantin and G-strophantin (ouabain) from the seeds of Apocynaceae plants. It is a powerful systolic force enhancer with a structure in which sugar is coordinated to the steroid skeleton, and is used for the treatment of congestive heart failure and so on. It is said to have an inhibitory effect on Na, K-ATPase. Ouabain is known to be toxic to inner ear (especially cochlear stria vascularis and

spiral ganglion cells), and is used as a model for inner ear disorders (Schmiedt et al., 2002; Lang et al., 2005; Fu et al., 2012. Yuan et al., 2014). However, the grade of damage was not constant, and hair cell damage may also be observed. (Schomann et al., 2018). Here we use guinea pigs to investigate whether the same cardiac glycoside, digoxin, also causes inner ear damage. In experiments with mice, digoxin has already been reported to cause damage to vestibular ganglion cells (Taura et al., 2021), but its effect on hair cells has not been clarified. In this study, we also investigated the effect of digoxin on hair cells.

Materials & Methods

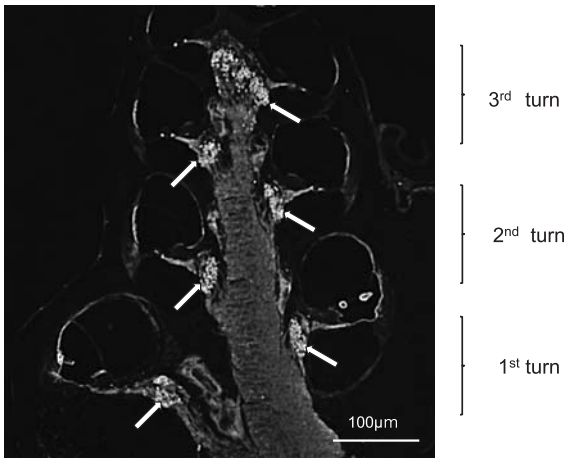
In order to find appropriate condition, we tried two ways as follows: 2 μL of digoxin solution (DIGOSIN Injection 0.25 mg /ml: TAIYO Pharma, Co., Ltd.Tokyo, JAPAN) was administered for 10 minutes to a 4-week guinea pig (purchased from Japan SLC Inc. Hamamatsu, Japan) through a left round window. As for the administration route, fused silica (Acom) was inserted through a left round window and administered directly to the inside of the scala tympani. As a control, saline was inserted through a right round window of the same animal. Five days later, the animal was fixed with 4 % PFA and the temporal bone was removed. The same amount of digoxin was similarly administered over 2 hours and fixed after 5 weeks. The number of each animal damaged model was one. The animal was fixed with 4 % PFA and removed temporal bones were decalcified with EDTA, embedded in OCT, and sectioned with a cryostat to a thickness of 20 μm. Spiral ganglion cells were identified by immunostaining in the area where the cochlear axis was visible. Samples were permeabilized with 5 % Triton X-100 in phosphate-buffered saline after 3×phosphate-buffered saline wash. Explants were then exposed to a blocking solution containing 10% bovine serum albumin, and then incubated with anti- myosin7A rabbit polyclonal antibodies (25–6790; Proteus Bioscience, Ramona, CA, USA; 1: 500). Alexa Fluor 488 goat anti-rabbit IgG (A-11011, Invitrogen, CA, USA; 1:100) was used as the secondary antibody. At the same time, specimens were incubated with anti- Tuj1 mouse antibodies (801213; BioLegend, San Diego, CA, USA; 1: 500). Alexa Fluor 568 goat anti-mouse antibody (A-11011, Invitrogen, CA, USA; 1: 500) was used as the secondary antibody. Finally, the explants were incubated with DAPI for 15 min. Specimens were examined using a KEYENCE all-in-one microscope (BZ-X810, KEYENCE, Japan). For the number of spiral ganglion cells, the area of the Rosenthal canal in the section was determined using Image J, and the number of Tuj1 positive spiral ganglion cells were counted to calculate the density of the spiral ganglion cells. Vestibular ganglion cells and vestibular hair cells were also identified by HE staining (Hematoxylin & Eosin staining). For the evaluation of spiral ganglion cells, the number of cells per unit area was measured in any three regions, and averaged. To evaluate cochlear function, ABR (Auditory Brainstem Response) was performed 2 days after administration of digoxin, and auditory function was also examined.

Experimental protocols were approved by the Animal Research Committee of Shiga Medical Center Research Institute.

Statistical analysis was performed by analysis of variance followed by the least significant difference two-sided t-test (Excel). Differences associated with P-values of 0.05 were considered to be statistically significant. All data are presented as mean±S.D.

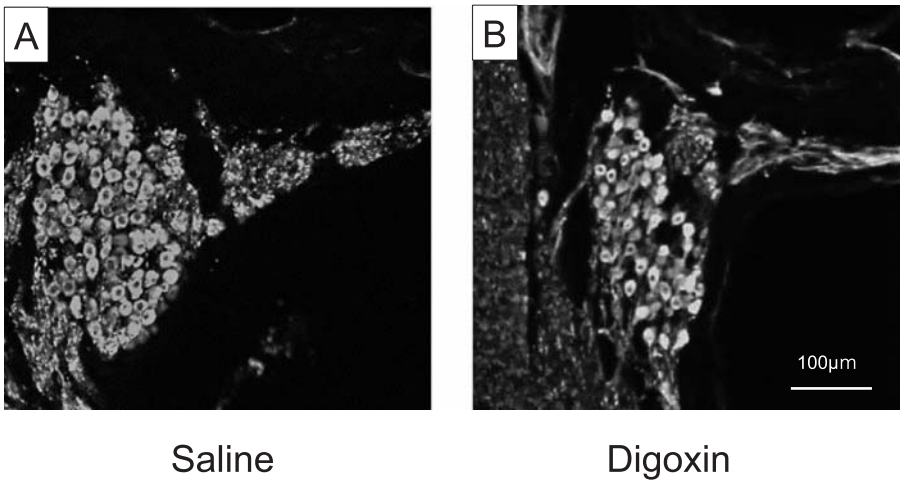
Results

In the overall image of the cochlea treated with digoxin for 10 min, damage to the spiral ganglion cells was observed as shown by the arrows (Figure 1). In all turns (1st turn / 2nd turn / 3rd turn), the spiral ganglion cells were sparse and seemed to be damaged. The spiral ganglion cells were damaged on the left ear of the digoxin administration for 10 min, compared to the right ear without digoxin (Figure 2). When the



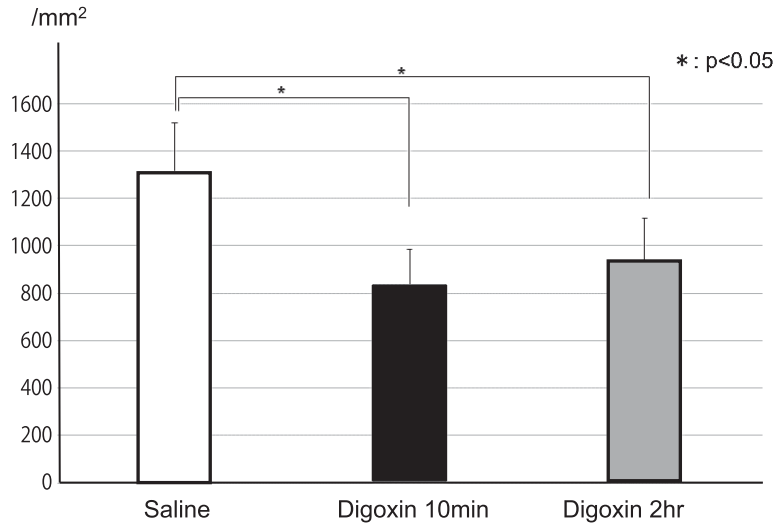
Spiral ganglion neurons (White arrow) were sparse and damaged almost all turns

Figure 1 Cochlear cross section administrated digoxin for 10min



Cells are damaged on the side of the digoxin for 10min administration (A) compared to the side with saline (B)

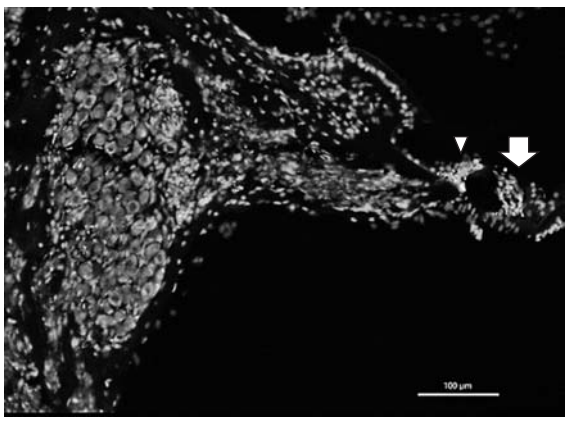
Figure 2 The spiral ganglion cells in 2nd turn



The numbers were significantly reduced in the digoxin treated groups, compared with the saline treated group. Compared two digoxin groups, digoxin for 10min group seemed to be more damaged than digoxin for 2hrs group. But there was no significant difference between them.

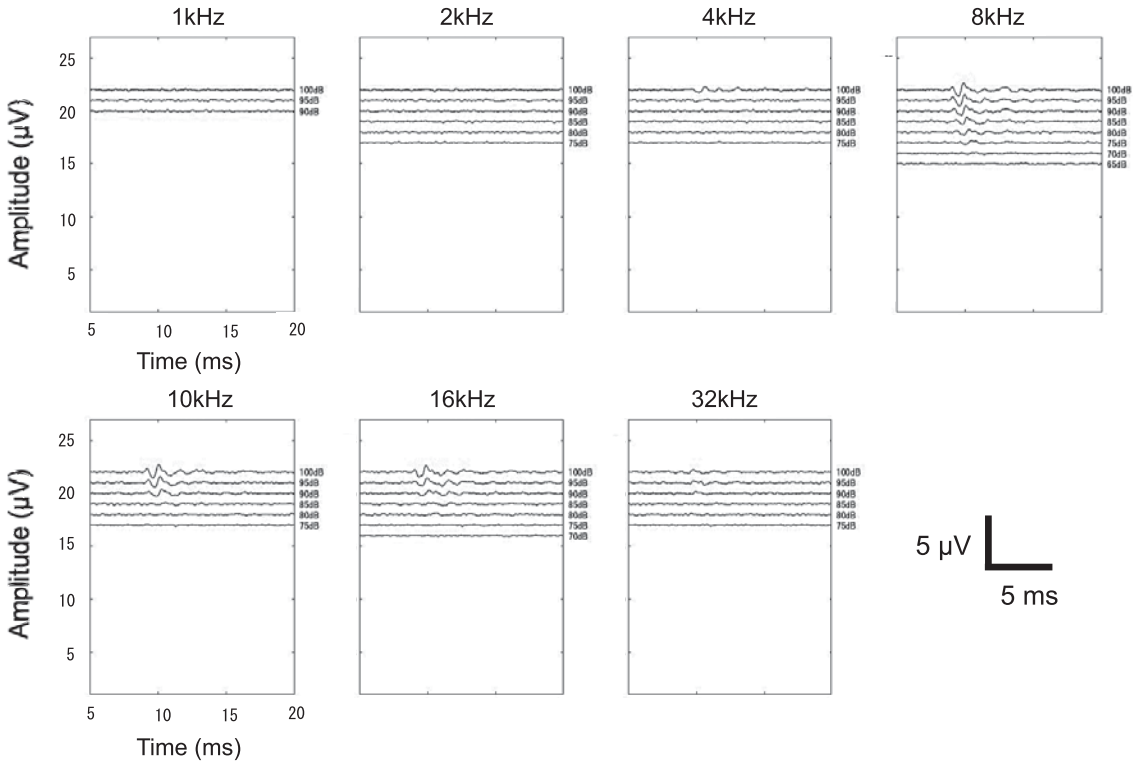
Figure 3 The numbers of spiral ganglion cells (N=3-4)

numbers of spiral ganglion cells were examined, the numbers were significantly reduced in the digoxin treated group as compared with the saline treated group (Figure 3). When we compared two digoxin groups, digoxin for 10 min group seemed to be more damaged than digoxin for 2hrs group. However, there was no significant difference between them and we could not get enough results for quantification analysis of these two groups yet. In terms of cochlear hair cells, the inner and outer hair cells were remained after digoxin administration for 2 hrs (Figure 4). When ABR was measured for auditory function in the digoxin treated group for 2 hrs, no reaction was observed in the almost all ranges, and functional impairment was confirmed (Figure 5). In addition, when vestibular ganglion cells were also exa-



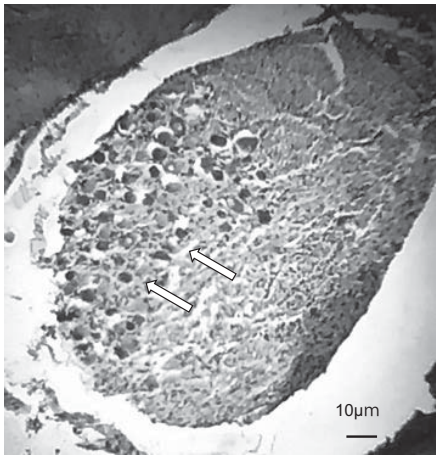
Arrowhead : inner hair cell
Arrow : outer hair cells
Both of inner and outer hair cells were remained

Figure 4 Cochlear hair cells (Myosin 7A) on the side of the digoxin for 2hrs administration



No reaction was observed in the almost all ranges

Figure 5 ABR (Auditory Brainstem Response : ABR) in digoxin treated side for 2hrs



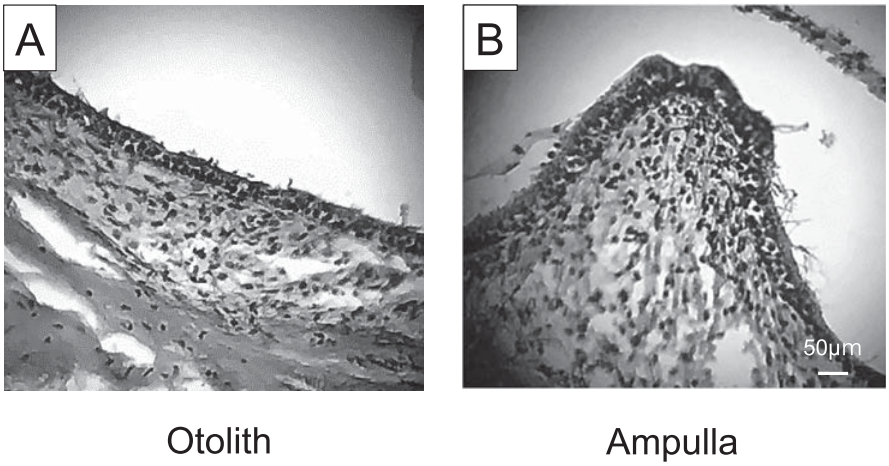
Vestibular ganglion cells were sparse and damaged by administration of digoxin for 2hrs

Figure 6 Vestibular ganglion cells (HE staining : white arrows)

mined with HE staining, vestibular ganglion cells were also damaged by the administration of digoxin for 2 hrs (Figure 6). On the other hand, when the vestibular hair cells administrated digoxin for 10 min were also examined, no obvious damage was found in the otoliths and the ampulla (Figure 7). From these results, digoxin mainly damaged ganglion cells in both the cochlea and the vestibule, although the extent of damage was a little different from the administration time conditions.

Discussion

Auditory Neuropathy (AN) or Auditory Nerve Disease has (1) extremely poor speech clarity compared to hearing level, (2) normal external hair cell function (otoacoustic emission (OAE)), and (3) extremely high level sensorineural hearing loss characterized by findings such as unresponsive auditory brainstem response (ABR), has been reported as a new disease concept (Kaga et al., 1996; Starr et al., 1996). Although no abnormality is found in the function of the outer hair cells, it is said to include various pathological conditions caused by the inner hair cells, the synapse between the inner hair cells and the cochlear nerve, or the disorder of the cochlear nerve. It is also speculated that there is a pathological condition that should be called "vestibular neuropathy" in the vestibular system (Fujikawa et al., 2000). In addition to neonatal hyperbilirubinemia and hypoxia, various related genes have been identified as causes (Kaga et al., 2016; Santarelli et al., 2015; Nishio et al., 2017). The current treatment is cochlear implants, but no



A : Otolith
B : Ampulla
No obvious hair cell damage was found in the otoliths and the ampulla

Figure 7 Vestibular hair cell (HE staining) by administration of digoxin for 10min

radical treatment has been established yet. Therefore, an animal model for cochlear nerve disorder is needed for the development of new treatment. Traditional inner ear studies have mainly focused on hair cell disorders, and disorders models with gentamicin and acoustic trauma have been established. However, in order to develop a treatment for auditory nerve disease, it is necessary to create a disorder model of only synaptic and ganglion cells. In previous reports, it has been reported that ouabain administration mainly damages type I spiral ganglion cells, and it has been used as a model of ganglion cell damage (Lang et al., 2005).

Cardiac glycosides such as digoxin and ouabain are well known for their cardiotonic action and have been used for a long time in history, but their mechanism of action is thought to be the suppression of Na, K-ATPase. It was unclear whether it was related to the enhancement of contraction. When digitalis specifically inhibits Na, K-ATPase, the intracellular Na concentration increases and the intracellular and extracellular Na concentration gradient decreases. Therefore, Ca emission by the Na-Ca exchanger that uses the concentration gradient of Na as energy is reduced. At this time, in the depolarized phase of the action potential of the myocardium, the driving force for Ca discharge is reversed, and Ca inflow occurs through the reverse rotation mode of Na-Ca exchange. It has been interpreted that the intracellular Ca concentration increases by such a mechanism and a cardiotonic effect occurs. However, no confirmation was obtained about the details. Philipson et al examined the action and effect of ouabain using the knockout mouse of Na-Ca exchanger (NCX), and clarified that NCX is indispensable for the cardiotonic action of ouabain. In other words, digitalis is thought to suppress Na, K-ATPase, increase intracellular Ca concentration through Na-Ca exchange, and cause cardiotonic action (Reuter H. et al., 2002). However, in recent years, the following differences have been stated regarding the differences between ouabain and digoxin. Ouabain has a rapid onset of action and stimulates myocardial metabolism, but causes the release of calcium from the intracellular storage via the signal transduction pathway from outside the cell, activating myocardial metabolism. All cardiotonic steroids provide the ion-pumping function of Na, K-ATPase because digitalis enters the cell and acts on ryanodine receptors to increase intracellular calcium concentration by forming transmembrane calcium channels. Some argue that the traditional notion of exerting a therapeutic effect by partially inhibiting it is no longer supported (Fuerstenwerth, 2014).

Digoxin has a molecular weight of 780.9385 and a half-life of 35 to 48 hours for adults, and ouabain (G-strophantin) has a molecular weight of 584.6525, a half-life of 21 hours, is water-soluble and is used in pharmacological experiments, but digestion. It is not often used clinically due to poor absorption by oral administration. In addition, digitalis has a narrow therapeutic range of blood concentration, and side effects such as bradycardia. The cerebral cortex, optic nerve, and retina have been reported as action sites in the visual system of digitalis. The retina is said to have a high affinity for digitalis, and due to the high sensitivity of the photoreceptor cones,

dysfunction of photoreceptor cells has been reported (Madreperla et al., 1994). In the inner ear, it has been reported that ouabain administration mainly damages type I helical ganglion cells (Lang et al., 2005), suggesting that Na, K-ATPase may be highly sensitive. On the other hand, there are individual differences in the disorders caused by ouabain administration, and injuries often lead to fatalities (data not shown). Hair cell damage due to ouabain has also been reported (Schomann et al., 2018). Therefore, the action of digoxin was investigated as a substitute for ouabain. From the results of this experiment, histologically in the digoxin treated cochlea of guinea pigs, the number of cochlear spiral ganglion cells decreased. Digoxin might damage the spiral ganglion cells, but did not cause severe cochlear hair cell damage. Since auditory function was impaired in ABR, it also caused physiological dysfunction. In addition, the vestibular ganglion cells were also found to be damaged, but the hair cells in otoliths and in the ampulla were not clearly damaged. From these, it is considered that digoxin is useful as a model of neuropathy and other neuropathy in the inner ear of guinea pigs, but further verification is required in the future.

The authors declare no conflict of interest.

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