

## A new animal model for Ménière's disease

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### Abstract

*Conclusion.* A new mouse model for Ménière's disease has been developed by the treatment with both LPS and aldosterone. The induction of vestibular dysfunction in the hydropic animals model may require additional stress such as reduced inner ear blood flow, acute changes of endolymph volume and/or pressure, etc. *Objective.* The aim of this study is to develop a more suitable animal model, which shows more resemblance to the pathophysiological process in Ménière's disease. *Materials and methods.* Adult CBA/J mice were treated with intratympanic injection of LPS, intraperitoneal injection of aldosterone or injection of both LPS and aldosterone. Morphological analysis was performed in the cochlea and endolymphatic sac. *Results.* All experimental animals showed mild to moderate endolymphatic hydrops. The animals treated with both LPS and aldosterone showed reversible vestibular dysfunction after the intratympanic injection of epinephrine.

**Keywords:** *endolymphatic hydrops, lipopolysaccharide, aldosterone, mice, Ménière's disease*

### Introduction

Since the discovery of endolymphatic hydrops in temporal bones of patients with Ménière's disease, endolymphatic hydrops has been accepted as the pathological substrate of Ménière's disease (1,2). Hydrops may arise as a result of the destabilization of natural regulation through overproduction of endolymph and/or reduced absorption of endolymph (3). Serious attempts using various methods have been made to develop an animal model for Ménière's disease. Endolymphatic hydrops was observed after the injection of distilled water, horse serum, tuberculin, or sodium

chloride into the middle ear cavity, and after injection of pilocarpine or acetylcholine around the eighth nerve trunk in the internal auditory canal. When the function of the stria vascularis was altered by subcutaneous injection of arsenic or Atoxyl, or intraperitoneal injection of ethacrynic acid, hydrops developed in a few instances (4). Injection of purified cholera toxin into the scala media to increase endolymph secretion from the stria vascularis by stimulating adenyl cyclase resulted in a high incidence (81%) of hydrops (5).

Since its introduction by Kimura and Schuknecht in 1965 (6), surgical induction (surgical obliteration of endolymphatic duct and sac) of endolymphatic hydrops in the guinea pig has become the standard model for the study of Ménière's disease. This procedure has been readily adopted by some investigators because it reliably produces both histological hydrops and hearing loss. However, this animal model does not result in anything resembling attacks of vertigo, even though a predictable low-tone hearing loss occur (7,8). In this standard surgical obliteration guinea pig model, the hydrops is induced by destruction of the endolymphatic sac (ES) and obliteration of the endolymphatic duct (ED) with bone wax (6). Recently, Dunnebier et al. (9) induced mild endolymphatic hydrops through total dissection of the extraosseous part of the ES adjacent to the sigmoid sinus in order to obstruct the venous outflow to the sigmoid sinus and produce mild fibrosis of the most distal portion of the sac. Although several modifications of the standard animal model including the model of Dunnebier et al. (9) were developed, which produced hydrops in a variable degree, were still too destructive or could not be standardized enough to be superior in relation to the standard model (4).

In contrast to the surgical obliteration model, "over-production" models have been developed that, like the surgical model, produce both histological hydrops and hearing loss (4,7,8). These models include the introduction of cholera toxin into the inner ear (5), chronic administration of vasopressin (10) or aldosterone (9), and various techniques for the introduction of inner ear autoimmune disease (8,11). Because the mechanism of hydrops production is different in overproduction models than in the surgical model, it is possible that the pathophysiology of hearing loss is also different.

Recently, the two-phase hydrops guinea pig model has been developed (9). This model is based on a combination of chronic ES dysfunction, induced by slight destruction of the most distal part of the ES, and acute stress-induced endolymph production by stimulation of the Na/K-ATPase in the stria vascularis with aldosterone.

At this time it is still unclear whether one of these models better replicates the auditory and cochlear pathologic changes in Ménière's disease, so it is important to continue the work of characterizing all animal models as thoroughly as possible. It is

not clear as well that the characteristic vestibular symptoms of Ménière's disease are reproduced in the animal model, or even whether the basic physiology of hydrops production is the same as in the human condition (4,7,8).

The aim of this study is to develop a more suitable animal model, which shows more resemblance to the pathophysiological process in Ménière's disease. For this aim, we used a combination of both a reduced absorption of endolymph and an enhanced production of endolymph fluid. An acute reduction of absorption of endolymph is induced by the intratympanic injection of lipopolysaccharide (LPS) (12,13). A mild increase of endolymph production is induced by administration of aldosterone, to stimulate the Na/K ATPase in the stria vascularis and the dark cells (9).

### **Materials and methods**

Twelve healthy, otomicroscopically normal adult CBA/J mice with 20-25g body weight and a normal Preyer's reflex were used in this study. Care and use of the animals was approved by the Animal Experimentation Committee, Hiroshima University School of Medicine (permit no. A06-68) and was carried out in accordance with the Guide to Animal Experimentation, Hiroshima University and the Committee on Research Facilities for Laboratory Animal Science, Hiroshima University School of Medicine.

The animals were randomly divided into 3 groups. The group 1 animals (N=5) were inoculated with once-daily injection of 1mg LPS extracted from *Escherichia Coli* (Sigma Chemical Co., St. Louis, USA) dissolved in 0.1 ml of sterile saline for five days. The toxin was instilled into the left tympanic cavity through the tympanic membrane using a sterile 27-gauge needle. The right side non-injected ear was served as a control. The group 2 animals (N=2) received a once-daily intraperitoneal injection of aldosterone in a dose of 100µg/100 g/day (Acros organics, New Jersey, USA) for 5 days. The group 3 animals (N=5) were inoculated with both LPS in the left side ear and aldosterone intraperitoneally in the same way for 5 days. Twenty-four hours after the last injection, the animals were deeply anesthetized with pentobarbital and fixed by cardiac perfusion with 4% paraformaldehyde in 0.1 M phosphate buffer solution, pH 7.4. The temporal bones were excised and immersed in the same fixative for another 2 h. They were decalcified with 0.1M buffered Na-EDTA for 14 days. The specimens were then dehydrated with graded ethanols and embedded in a water-soluble resin (JB-4<sup>®</sup>). Sections were cut about 4 µm thick and stained with toluidine blue for light microscopy.

The light microscopic specimens were viewed in a Nikon photo microscope (Eclipse E600). Analogue images were obtained using an intensified digital color

charge-coupled device camera (C4742-95; Hamamatsu Photonics) and stored as digital images using IP lab Spectrum software (version 3.0; Signal Analytics Corporation).

#### *Quantitative assessment of volumetric changes of the scala media*

For the quantitative assessment of volumetric changes of the scala media, the change ratios of the cross-sectional area of the scala media were measured from the mid-modiolar sections of the cochlea (10). For this analysis, the following 2 parameters were measured in the lower and upper turns, not including the hook portion; 1) the cross-sectional area of the scala media (S), enclosed by the distended Reissner's membrane, and 2) the cross-sectional area of the original scala media (S\*), enclosed by a straight line segment, which represents the normal position of the idealized Reissner's membrane connecting the normal lateral attachment of Reissner's membrane at the upper margin of the stria vascularis to its normal medial attachment at the spiral limbus. The anatomical measurements were carried out in a blind fashion using the IP Lab Spectrum software.

From these parameters, the increase ratios (%) of the cross-sectional area of the scala media (IR-S) of an upper or lower turn was calculated according to the equation as described below.

$$\text{Increase ratio (\% of the cross-sectional area of the scala media (IR-S))} = 100 \times \frac{\sum(S_x - S^*_x)}{\sum S^*_x} \quad (x: \text{upper or lower turn})$$

#### *Quantitative assessment of luminal size change of the ES*

The luminal sizes of the ES in the intraosseous rugose portion as well as the extraosseous distal portion were calculated in the following way. One light micrograph from the stored digital images was prepared at the mid portion of the intraosseous rugose portion of each ES. On these photographs, the lumen of the sac and the bony lining of the vestibular aqueduct were outlined. The relative size of the lumen as compared with the bony lining of the vestibular aqueduct was calculated again using the IP Lab Spectrum software. In the distal portion of the ES, one light micrograph from the stored digital images was prepared at the distal portion of each ES. On these photographs, the short diameter of the lumen of the sac and that of the outer lining of whole ES was measured. The relative diameter was calculated again using the IP Lab Spectrum software (14).

#### *Induction of vestibular dysfunction*

In order to create the vestibular dysfunction, some animals in each group were

inoculated with 1:10,000 epinephrine into the middle ear cavity through the tympanic membrane. The behavior was recorded with VTR. The nystagmus was recorded in darkness using a Frenzel's glass with an infrared CCD camera with a video monitor.

## Results

### *Endolymphatic hydrops*

In the control ears, in which were not inoculated LPS nor treated with aldosterone, no hydrops was detected in any of the turns of these cochleas (Fig. 1a).

In the ears treated with aldosterone only, all cochleas demonstrated a mild to moderate degree of EH. Intracochlear variation in the severity of hydrops was observed in this group. Three of nine cochleas showed more severe hydrops in the lower turn and six revealed more severe hydrops in the upper turn. Elongation and folding of the Reissner's membrane was noted in four of nine cochleas, especially in the lower turn (Fig. 1b).

In the ears inoculated with LPS, but not treated with aldosterone, a slight or moderate severity of hydrops was observed in the cochlea. All cochleas showed more severe hydrops in the upper turn. Folding of Reissner's membrane was noted in one of five cochleas (Fig. 1c).

In the ears, in which LPS was inoculated and aldosterone was administered, all cochleas showed mild to moderate EH. Intracochlear variation in the severity of hydrops was also demonstrated in this group. The combined treatment with both LPS and aldosterone did not clearly demonstrate a shift to a more severe degrees of hydrops when compared with only LPS or aldosterone treatment. However, aldosterone did increase the severity of hydrops in the lower turn. The severity of hydrops was more distinct in the upper turn of the cochlea in general. Elongation and folding of the Reissner's membrane was noted in two of five cochleas, especially in the lower turn (Fig. 1d).

The increase ratios (IR) of the cross-sectional area of the SM of upper and lower turns were calculated. In the control ears, IR was  $97 \pm 6.1$  % (mean  $\pm$  SD, N=5) in the upper turn and  $100 \pm 5.2$  % in the lower turn. In the aldosterone-treated ears, IR was significantly increased both in the upper turn ( $122 \pm 8.2$  %, N=9,  $p < 0.01$ ; Student's t-test) and in the lower turn ( $117 \pm 10.4$  %,  $p < 0.01$ ). IR was also significantly increased in the LPS-treated ears in the upper turn ( $126 \pm 6.7$  %, N=5,  $p < 0.01$ ) and in the lower turn ( $115 \pm 6.5$  %,  $p < 0.01$ ), and in the LPS+aldosterone-treated ears in the upper turn ( $131 \pm 8.4$  %, N=5,  $p < 0.01$ ) and in the lower turn ( $115.4 \pm 10.0$  %,  $p < 0.05$ ) (Fig. 2). The

significant difference in IR was noted between the upper and lower turns in the LPS-treated ears ( $p < 0.05$ ) and aldosterone+LPS-treated ears ( $p < 0.01$ ), whereas no significant difference was noted in the aldosterone-treated ears. No significant difference was observed between the experimental groups.

#### *Endolymphatic sac*

Light microscopic examination of the normal and experimental animals was performed to assess the effect of LPS inoculation, administration of aldosterone, or both LPS and aldosterone on the ES.

In the control ears, the intra-osseous portion of the ES was easily identified by its cylindrical cells, which protrude into the lumen as irregular papillae. The epithelial lining of the distal portion of the ES was cuboidal, except at the extreme end, where it was squamous. In the intra-osseous portion of the ES, numerous, distended lateral intercellular spaces (LIS) were seen in the epithelial cell lining of the ES. The epithelial cells were columnar in general. In the distal portion of the ES, LIS was not so distended as in the intra-osseous portion, but were nevertheless evident (Fig. 3a,b).

In the aldosterone-treated ears, the lumen of the distal portion of the ES was markedly dilated. The epithelial cells became thinner and the LIS had collapsed. Inside the lumen, the ES contained clear endolymph without macrophages. The intra-osseous portion of the ES was also dilated. The LIS had also collapsed and the looseness of the perisaccular tissue was no longer observed. The epithelial cells were often of the cuboidal or flat type (Fig. 3c,d).

In the LPS-treated ears, the lumen of the distal portion of the ES was narrower and had collapsed. Distended LIS were observed between the cuboidal epithelial cells. Inside the lumen, a number of macrophages were observed. In the intra-osseous portion of the ES, the lumen of the ES had collapsed and contained a stainable substance with a number of macrophages. The LIS appeared distended showing a general increase in size (Fig. 4a,b).

In the LPS+aldosterone-treated ears, intrasaccular variation in the morphological features was observed. The distal portion of the ES showed wide variations from near collapse to marked dilation. The LIS appeared distended in general. The intra-osseous portion of the ES showed a wide variation too, while the lumen of the ES was slightly dilated in general. The LIS were generally distended (Fig. 4c,d).

The relative luminal size of the ES was judged using the ratio between the size of the ES lumen and that of the bony vestibular aqueduct in the intermediate, rugose portion as well as the ratio between diameter of the ES lumen and that of the whole ES

in the distal portion. In the normal condition the relative size of the lumen of the ES was  $47 \pm 7.1$  % (mean $\pm$ SD, N=5) in the intra-osseous portion and  $68 \pm 3.6$  % in the distal portion. In the aldosterone-treated ears, the luminal size of the ES was significantly increased to  $61 \pm 2.8$  % (mean $\pm$ SD, N=9) in the intra-osseous portion ( $p < 0.01$ ) and  $82 \pm 1.7$  % in the distal portion ( $p < 0.01$ ), while it was significantly decreased to  $35 \pm 1.4$  % (N=5) (intra-osseous portion) ( $p < 0.01$ ) and  $58 \pm 3.4$  % (distal portion) ( $p < 0.01$ ) in the LPS-treated ears. In the LPS + aldosterone-treated ears, the luminal size of the ES in the intra-osseous portion ( $43 \pm 2.8$  %) was similar to that of the control, while the distal portion ( $71 \pm 5.1$  %) showed wide variations in size from normal to a marked dilation (Fig. 5). In the aldosterone-treated ears, relative size of the ES lumen was significantly greater than that of the LPS- or aldosterone+LPS-treated ears ( $p < 0.01$ ).

#### *Behavioral data*

Treatment with epinephrine did not induce any behavioral changes in the animals treated with aldosterone or LPS alone, whereas treatment with both aldosterone and LPS had drastic effects. Four of five animals in this group showed signs of severe vestibular dysfunction. Following epinephrine injection, the vestibular effects started to manifest within 5 min. In the most affected stage, the animals could not maintain a stable posture but leaned to the left (injected) side (Fig. 6). They were unable to swim and when walking showed a tendency to turn to left. At the same time faint but distinct nystagmus toward the right (non-injected) side was noted with infrared Frenzel's glass. This behavior reached its maximum after 10 min and continued for about 30min. The animals then gradually recovered and after 1 h, all animals displayed normal vestibular function. Signs of ataxia, weakness of muscle tonus were no longer observed at any given time.

#### **Discussion**

Even though obliteration of the ED and ES in the guinea pig is most the ideally available procedure for producing hydrops, this model is not perfect for Ménière's disease, because after such procedures these animals rarely show episodic vestibular symptoms (4,7,8). Furthermore, this model requires the surgical obstruction of ED and ES. Several modifications of this animal mode, which produced hydrops to a variable degree, were still too destructive and were a not physiological accurate model for Ménière's disease. In the present study, we succeeded in producing hydrops without any surgical procedures on the ED and ES. Another interesting point was that we were

able to induce EH in the mice. By surgical obliteration of the ES, EH has been produced in the guinea pig with 100% accuracy. Another reliable species for production of EH by this procedure is the rabbit. In the cat, EH can be produced in 80% of animals. The sac blockage procedure was not successful in monkeys and the success rate was poor in chinchillas (4). In mice, surgical obliteration of the ES is difficult because of its size and location. Actually, mice have several advantages as an animal model, compared with the guinea pig. Mice have now been widely used for inner ear research, have a number of antibodies available, and also have more technical advantages for the investigation of genetical problems.

In the present investigation, we used a combination of overproduction of endolymph and reduced absorption of endolymph (or ES dysfunction) to induce EH.

Endolymph overproduction was induced by stimulation of the Na/K ATPase in the stria vascularis with aldosterone (9). The specific chemical composition of endolymph and the generation of the transepithelial positive potential are considered to be regulated by membrane-bound sodium-potassium activated adenosine triphosphatase (Na/K ATPase) in the marginal cells of the stria vascularis and the dark cells of the vestibular labyrinth (15). In the guinea pig, large amounts of Na/K ATPase were detected in several inner ear structures (16,17) and a relationship between circulating adrenal steroid and Na/K ATPase activity in the inner ear have been demonstrated (18,19,20). Depletion of aldosterone after bilateral adrenalectomy of the rat reduces ATPase activity in the stria vascularis, which induced morphological changes within the cellular structure of the cochlea have also been observed (18). Re-establishment of an endogenous level of aldosterone restores the cellular morphology and increase the Na/K ATPase activity (19). The Na/K ATPase level in the stria vascularis increases as a result of enhanced aldosterone levels induced by low-sodium high-potassium diets (20). Aldosterone levels may also be increased by emotional stress, which has been suggested as a precipitating factor in Ménière's disease (21). The strial Na/K ATPase activation by aldosterone may cause an increased secretion of potassium ions in the endolymphatic compartment and excess production of endolymph. This may contribute to the development of EH as seen in Ménière's disease (9).

In the present investigation, all aldosterone-treated cochleas demonstrated mild to moderate EH. This aldosterone-induced EH has already been reported in the guinea pig (9). In mice, as in guinea pigs (9), some aldosterone-treated cochleas showed more severe hydrops in the lower turn. This is a characteristic finding for aldosterone-induced hydrops, as in the standard surgical model, the hydrops is concentrated in the apical turns of the cochlea (6). Concerning the mechanisms of the



aldosterone-induced EH, the localization and properties of ATPase in the inner ear has been put forward (15). It has been reported that there was a marked decrease in stria enzyme activity from the base to the apex of the cochlea. Stimulation of this enzyme activity by aldosterone stimulates the basal turn more to develop hydrops, probably because of the relatively intense enzyme activity in this part of the stria.

The present study also revealed that aldosterone-treated ES showed a marked dilation of the luminal size of the ES, in both the intra-osseous and in the extra-osseous portion. A similar finding was also noted in the acute EH induced by injecting distilled water into the middle ear cavity in mice (22). The dilation of the ES lumen may reflect a sudden volume increase in the endolymph, which could thus be an additional support for the overproduction of endolymph by aldosterone.

The reduced absorption of endolymph or ES dysfunction was induced by the intratympanic injection of LPS. Intratympanic injection of LPS induces otitis media. EH has been observed in animal studies of otitis media (4,12,13). Fluctuating sensorineural hearing loss in chronic otitis media has led to the hypothesis that the latter can cause hydrops (23). Histopathologic studies on human temporal bones have found EH to be a common occurrence in human cases of otogenic or meningogenically induced suppurative or serous labyrinthitis. Paparella and Djalilian (23) reported that in a study of 560 temporal bones, 75 of 194 bones with otitis media were affected by EH. The frequency of the presence of both disease processes supports the hypothesis that the two diseases may be related. In the guinea pigs (13) and chinchilla (12), mild EH has been observed after injecting LPS. The most common pathological findings in the cochleas after LPS inoculation were inflammatory cell infiltration and bleeding in the perilymphatic and endolymphatic spaces, distension of the intercellular space of stria vascularis, hair cell damage, vacuolation of supporting cells, i.e. Hensen's cells and Deiter's cells, and mild EH (12,13). In the ES, inflammatory cell infiltration was frequently seen in the lumen but also to a small extent in the subcellular connective tissue. The ES lumen was generally filled with stainable substance (13).

The present study also demonstrated mild EH in the cochlea. In the ES, the lumen contains stainable substances and inflammatory cell infiltration. The luminal size of the ES was diminished in both the intra-osseous and the distal portion. Similar findings, i.e. an increase in stainable substance and decrease in luminal size of the ES, have also been reported following administration of glycerol in mice (14). These changes were suggested to reflect a reduced absorption of endolymph, thus possibly indicating that inoculation of LPS causes the reduced absorption of endolymph as well.

By using the combination of these two methods, we hoped that a new experimental

mouse model could be developed, showing closer resemblance to the pathophysiological process in Ménière's disease. The present study revealed that treatment with aldosterone, LPS, or both aldosterone and LPS, induced EH in mice. Our findings may mean that EH is the result of either insufficient resorption of or excess production of endolymph. The combined treatment with both LPS and aldosterone did not clearly demonstrate a shift to a more severe degrees of hydrops, when compared with only LPS or aldosterone treatment. However, aldosterone did increase the severity of hydrops in the lower turn. Elongation and folding of the Reissner's membrane, as observed in the present study, may indicate a previous occurrence of EH. As a result, combined treatment induced a uniform pattern of hydrops.

Despite the successful induction of EH, none of the present experimental animal showed any signs of vestibular dysfunction as did previous animal models (4,7,8). Although it is being extensively studied, the standard guinea pig model does not reliably reproduce vestibular symptoms. Importantly, hydrops is produced by under-resorption of the endolymph, a mechanism that might not directly imitate the production of hydrops in Ménière's disease. Other induced models, i.e. by delayed perilymphatic infusion of keyhole limpet hemocyanin (11), anti-CB11 monoclonal antibodies, cholera toxin (5), chronic administration of vasopressin (10), and the two-phase hydrops guinea pig model (9), all appear to have the same limitations inherent to the standard guinea pig model (4,7,8). Based on the present findings and previous investigations, the vestibular dysfunction might not result from the existence of EH alone. Actually in the human temporal bone studies, EH was observed in the temporal bones of persons showing symptoms and signs of Ménière's disease (1,2,23). Indeed, hydrops is both the histological hallmark of Ménière's disease and the working concept of its pathogenesis. Paradoxically, not all people with symptoms of Ménière's disease have hydrops, and not all people with hydrops discovered at autopsy had symptoms during life (23).

Numerous investigations have suggested that the symptoms of Ménière's disease derive from a disturbance in the volume/pressure relationship of the endolymph. It is not yet known whether hydrops is the cause of the symptoms or simply a side effect of the disorder. In fact, Ménière's disease patients never suffer from incessant vertigo. Typically, the vertigo begins fairly quickly and builds in intensity over minutes or hours. When severe, nausea and vomiting may occur, movement during a vertigo attack exacerbate the nausea, so patients quickly learn to stay motionless during an attack. The vertigo usually lasts more than 20 minutes and rarely more than 24 hours. Vertigo attacks occurred episodically, elicited by additional factors such as emotional stress (8).

In another way, all animal models-including the present one- might be a model of an asymptomatic period of Ménière's disease. Based on this hypothesis, we injected epinephrine into the middle ear cavity as an additional stressor. As a result, we succeeded in inducing reversible vestibular dysfunction in our animal model treated with both aldosterone and LPS. It has been reported that after topical epinephrine application, cochlear blood flow immediately decreased to 20% of baseline and maintained this level for about 5 min before returning toward baseline (24). It is therefore suggested that the vestibular dysfunction observed in the present investigation may have been induced by the reduced inner ear blood flow in the hydropic ear. Similar reversible vestibular dysfunction was noted after injecting glycerol in the guinea pig surgical obliteration model (25). These results may indicate that the occurrence of vestibular dysfunction in hydropic animals models may require additional stress such as reduced inner ear blood flow, sudden changes in endolymph volume and/or pressure. The present study also revealed that the animals treated with LPS or aldosterone alone did not show any vestibular dysfunction after the intratympanic injection with epinephrine. This may indicate that the overproduction of endolymph or reduced absorption of endolymph (ES dysfunction) alone is not enough to produce vestibular dysfunction after additional stress.

Based on the previous and present investigation, we consider the pathogenesis of Ménière's disease as follows. EH is caused by many extrinsic and intrinsic factors (23). These include hypopresia of the vestibular aqueduct and sac, racial genetic factor, autoimmunity, otitis media, trauma, otosclerosis, vasopressin, allergy, and viral infection. Patients with EH alone are generally free from vertigo. However, additional stress such as sudden changes in endolymphatic pressure, restricted inner ear blood flow, rupture of the endolymphatic membrane (Reissner's or the saccular wall), may cause a vertigo attack. It seems logical that both physical and chemical mechanisms can concomitantly cause the symptoms. Chemical factors characteristically include osmotic and hydrostatic pressure alterations and, most likely, changes in membrane permeability, causing egress of inappropriate ions across the membrane barrier so as to incite sensory dendrites leading to cochlear and vestibular symptoms.

In conclusion, the new murine model explains some of the shortcomings of the classical model, and may relate to many observations in Ménière's disease. Although many more morphological and pathophysiological investigations with this new model need to be performed, the present results indicate that it may contribute to a better understanding of the pathogenesis. Consequently, this study may contribute to a more sophisticated therapeutical approach to Ménière's disease, in particular by restricting

endolymph production.

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## Figure Legends

### Fig. 1

- a. In the control ears, no hydrops is detected in any of the turns of the cochlea.
- b. In the aldosterone treated ears, mild endolymphatic hydrops is observed (asterisks). Elongation and folding of the Reissner's membrane is noted in the lower turn (arrow).
- c. In the LPS inoculated ears, a slight or moderate degree of hydrops has been observed (asterisks). Hydrops is more severe in the upper turn. Folding of the Reissner's membrane is noted (arrow).
- d. In the LPS+aldosterone treated ears, moderate endolymphatic hydrops is noted (asterisks). The endolymphatic hydrops is more uniform in this group.

### Fig. 2

The increase ratios (IR) of the cross-sectional area of the scala media of upper and lower turns are calculated. In the control ears, IR is  $97\pm 6.1\%$  in the upper turn and  $100\pm 5.2\%$  in the lower turn. In the aldosterone treated ears, IR is significantly increased both in the upper turn ( $122\pm 8.2\%$ ,  $p<0.01$ ) and in the lower turn ( $117\pm 10.4\%$ ,  $p<0.01$ ). IR is also significantly increased in the LPS treated ears in the upper turn ( $126\pm 6.7\%$ ,  $p<0.01$ ) and in the lower turn ( $115\pm 6.5\%$ ,  $p<0.01$ ), and in the LPS+aldosterone treated ears in the upper turn ( $131\pm 8.4\%$ ,  $p<0.01$ ) and in the lower turn ( $115.4\pm 10.0\%$ ,  $p<0.05$ ). The significant difference of IR is noted between the upper and lower turns in the LPS treated ears ( $p<0.05$ ) and aldosterone+LPS treated ears ( $p<0.01$ ).

### Fig. 3

- a. In the control ears, the intraosseous portion of the ES is identified by its cylindrical cells, which protrude into the lumen. Numerous, widened LIS are seen between the epithelial cell lining of the ES (arrows).
- b. In the distal portion of the ES, the epithelial lining is cuboidal. LPS is not obvious compared to the intraosseous portion.
- c. In the aldosterone treated ears, the intraosseous portion of the ES is dilated. The LIS are collapsed and the looseness of the perisaccular tissue is no longer observed. The epithelial cells are flat type.
- d. The lumen of the distal portion of the ES is markedly dilated. The epithelial cells become thinner and the LIS are collapsed. In side the lumen, the ES contains clear endolymph without macrophages (asterisk).



**Fig. 4**

a: In the LPS treated ears, the lumen of the intraosseous portion of the ES is collapsed and contained a stainable substance with a number of macrophages (asterisk). The LIS appear widened showing a general increase in size (arrows).

b: The lumen of the distal portion of the ES is narrower and collapsed. The widened LIS are observed. Inside the lumen, a number of macrophages are observed.

c: In the LPS+aldosterone treated ears, the intraosseous portion of the ES shows a wide variation as well, while the lumen of the ES is slightly dilated in general. The LIS are widened in general (arrows).

d: The distal portion of the ES shows wide variations from almost collapse to marked dilation.

**Fig. 5**

The relative luminal size of the ES was judged using the ratio between the size of the ES lumen and that of the bony vestibular aqueduct in the intermediate portion as well as the ratio between diameter of the ES lumen and that of the whole ES in the distal portion. In the normal condition the relative size of the lumen of the ES is  $47\pm 7.1\%$  in the intraosseous portion and  $68\pm 3.6\%$  in the distal portion. In the aldosterone treated ears, the luminal size of the ES is significantly increased to  $61\pm 2.8\%$  in the intraosseous portion ( $p<0.01$ ) and  $82\pm 1.7\%$  in the distal portion ( $p<0.01$ ), while it is significantly decreased to  $35\pm 1.4\%$  (intraosseous portion) ( $p<0.01$ ) and  $58\pm 3.4\%$  (distal portion) ( $p<0.01$ ) in the LPS treated ears. In the LPS + aldosterone treated ears, the luminal size of the ES in the intraosseous portion ( $43\pm 2.8\%$ ) is similar to that of the control, while the size of the distal portion ( $71\pm 5.1\%$ ) has wide variation. In the aldosterone treated ears, relative size of the ES lumen is significantly larger than that of the LPS or aldosterone+LPS treated ears ( $p<0.01$ ).

**Fig. 6**

After intratympanic injection of epinephrine, the animals treated with both aldosterone and LPS can not keep their position right, and inclined to the left (injected) side.

Figures.

Fig. 1

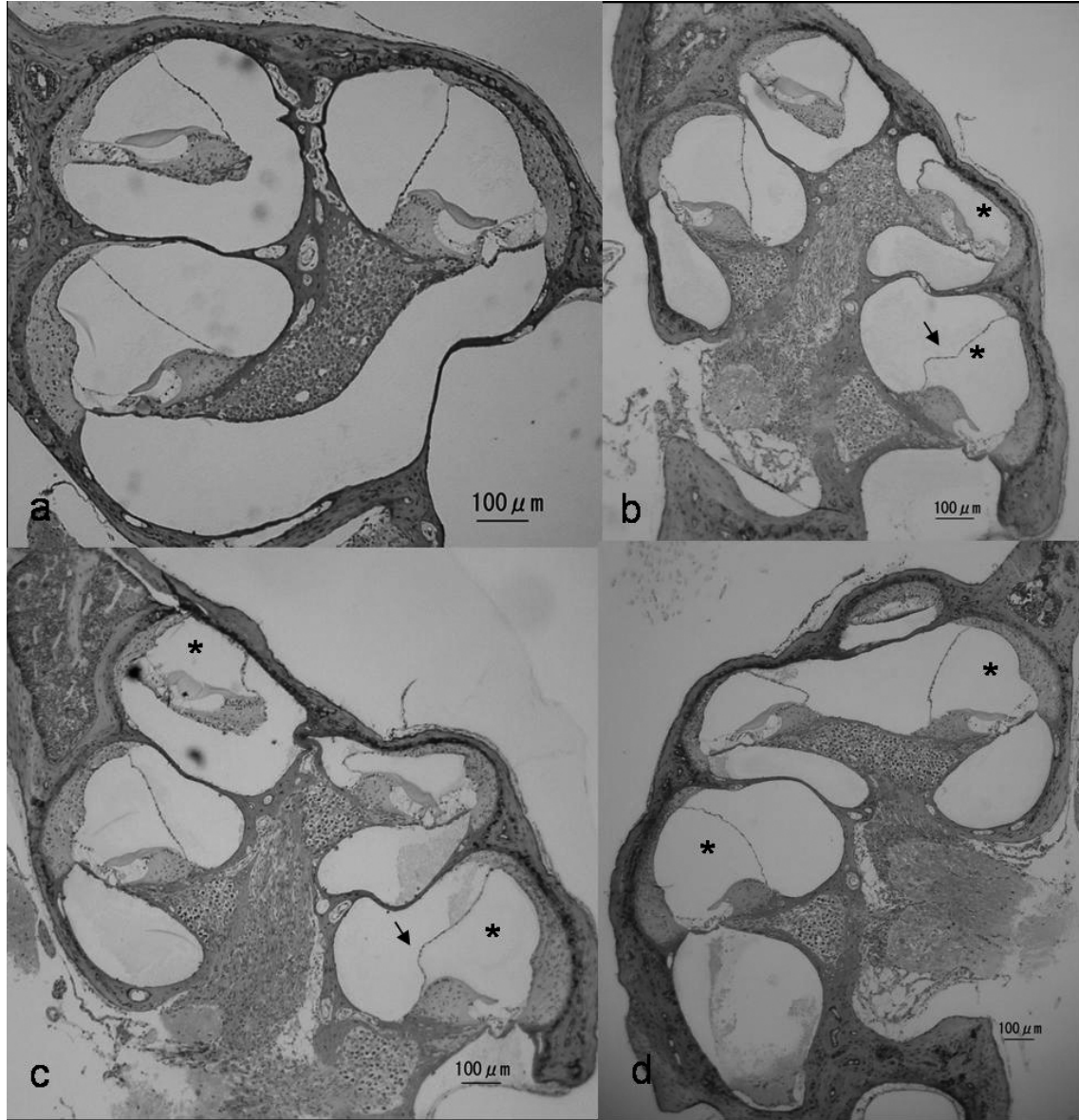


fig. 2

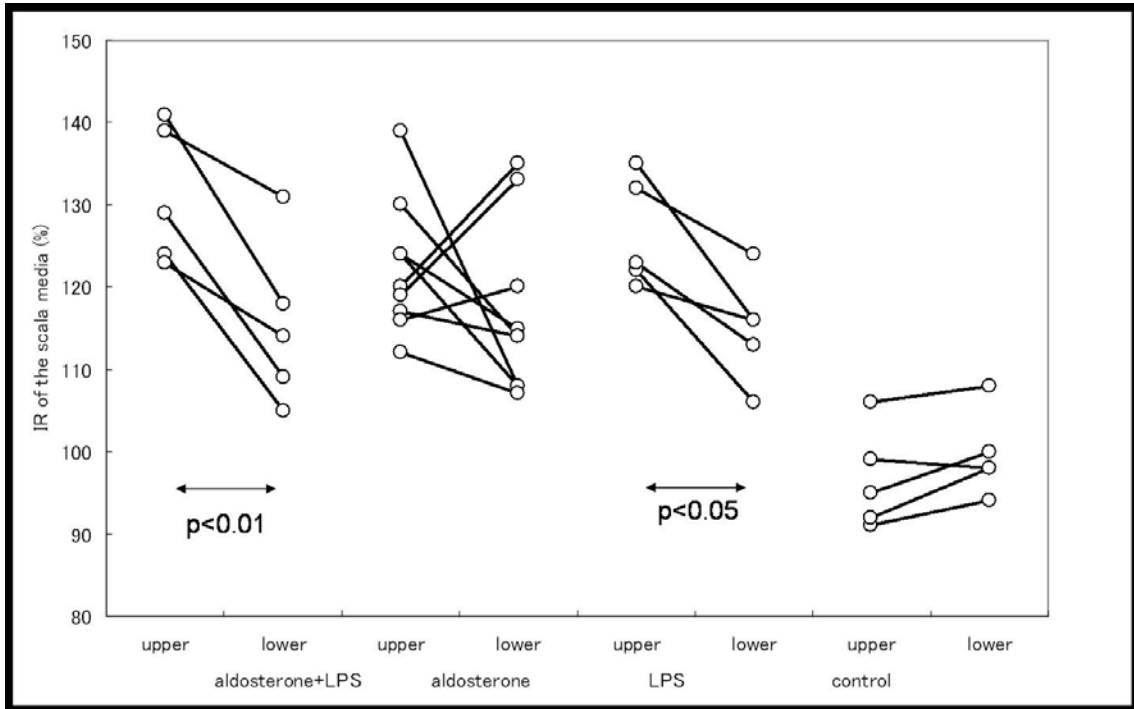


fig. 3

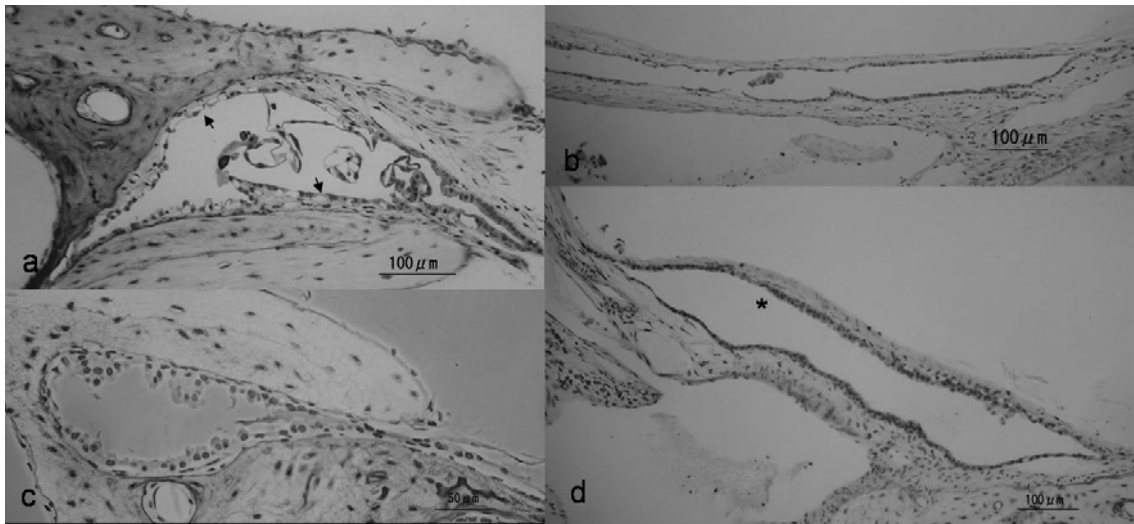


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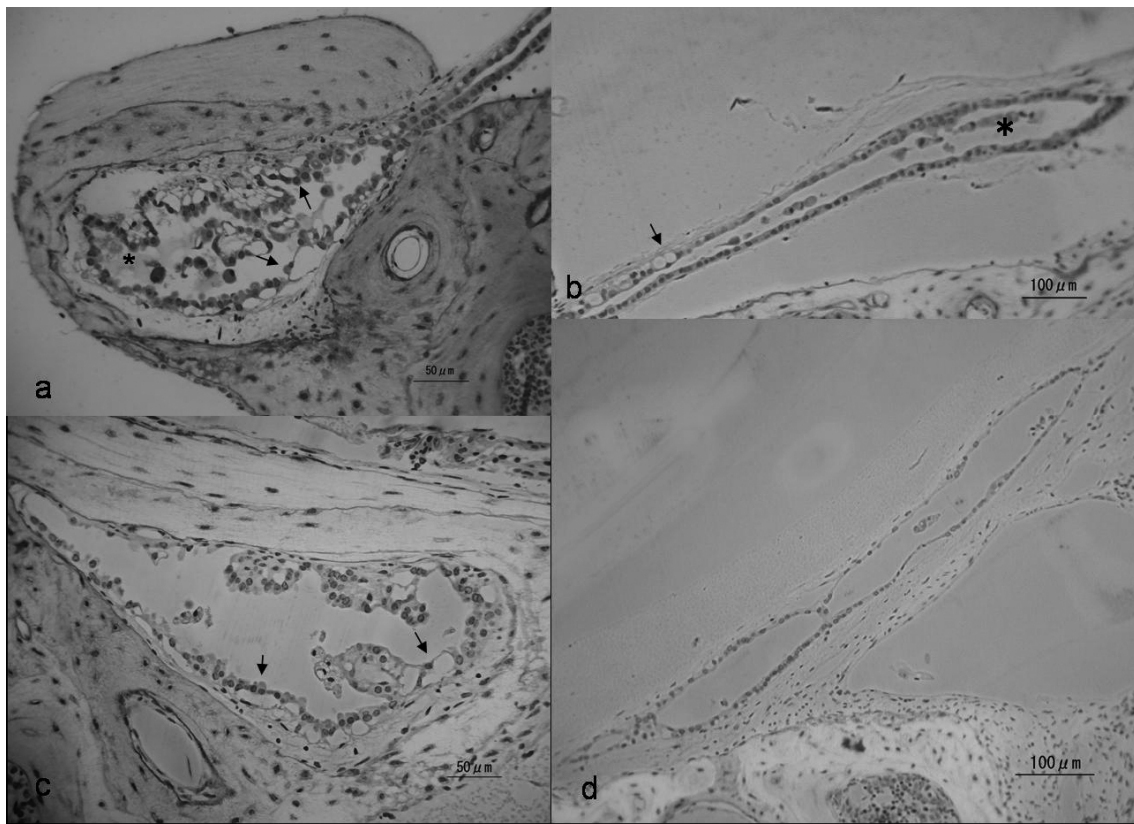


fig.5

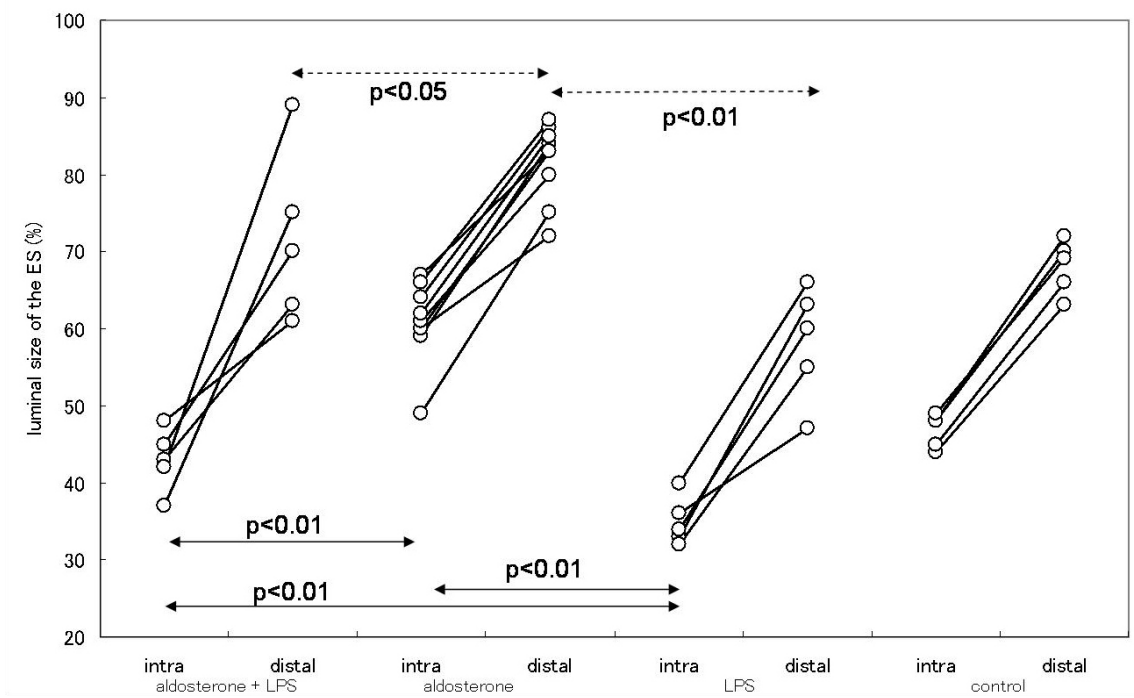


fig. 6



Fig.1

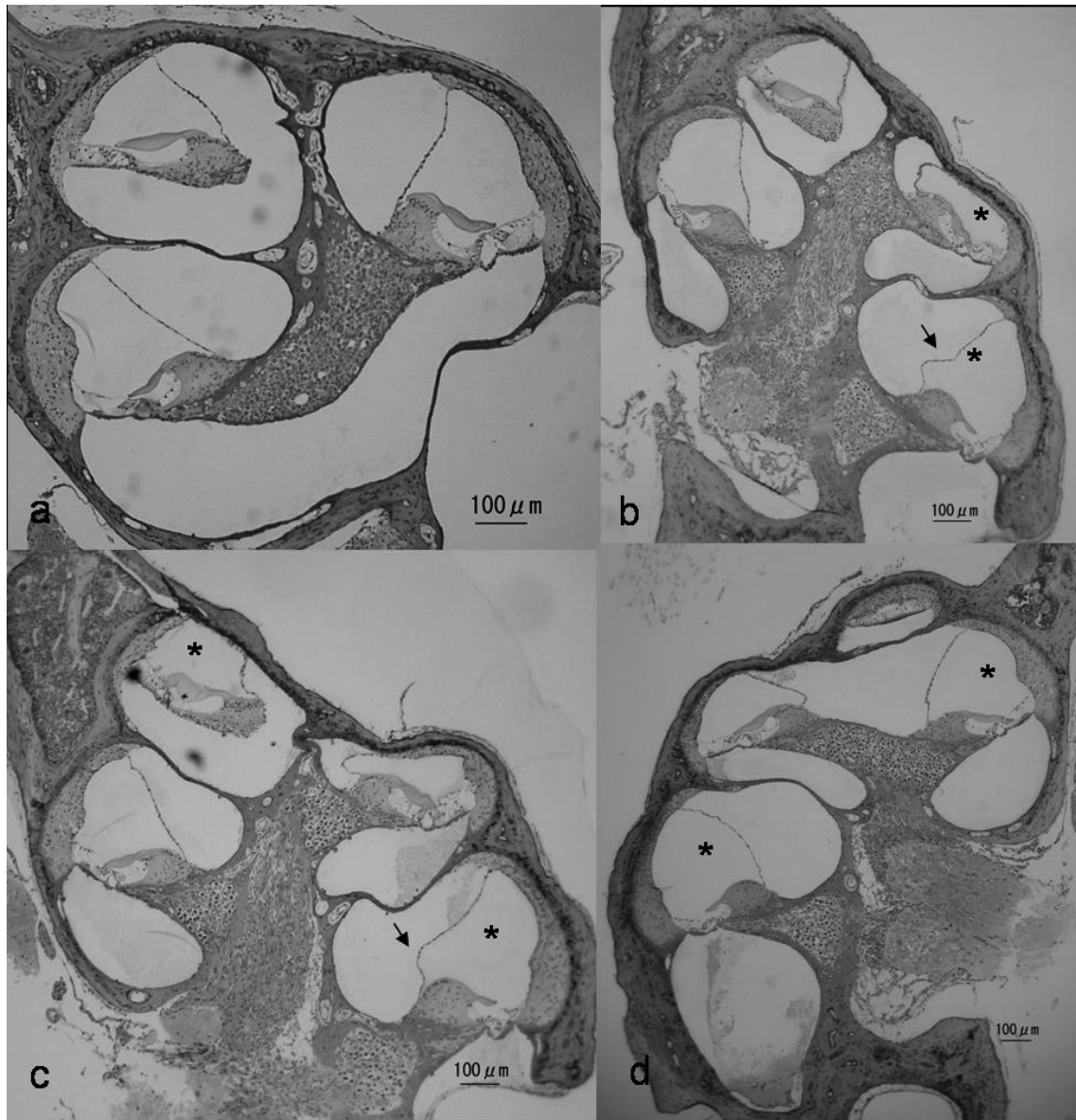


Fig.2

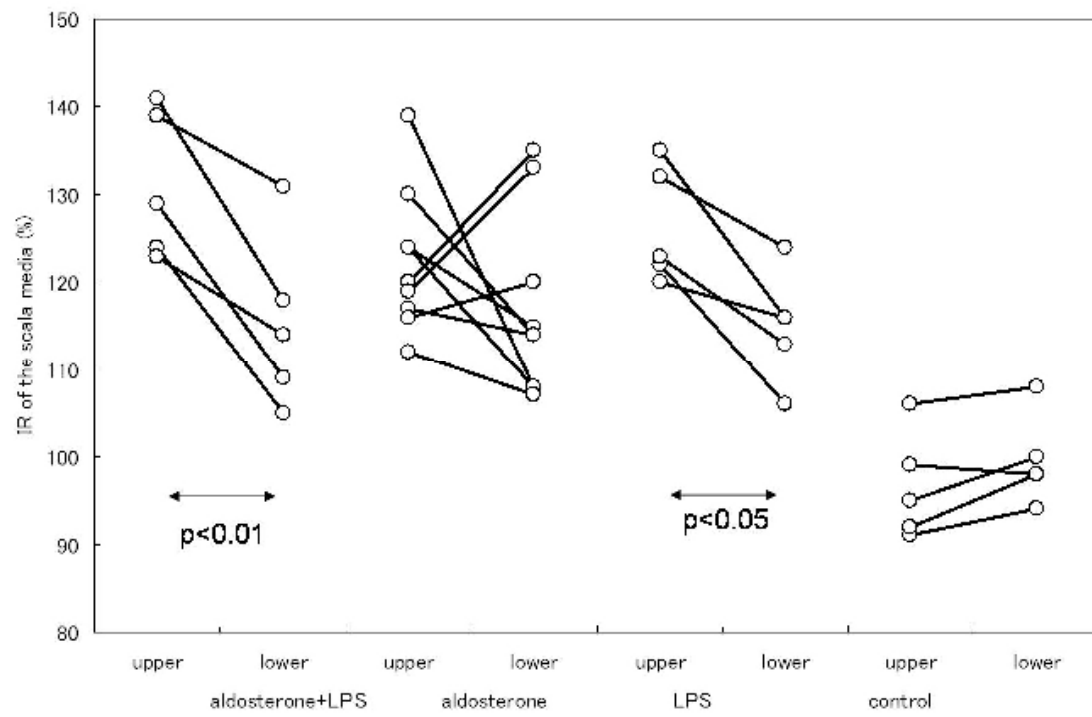


Fig.3

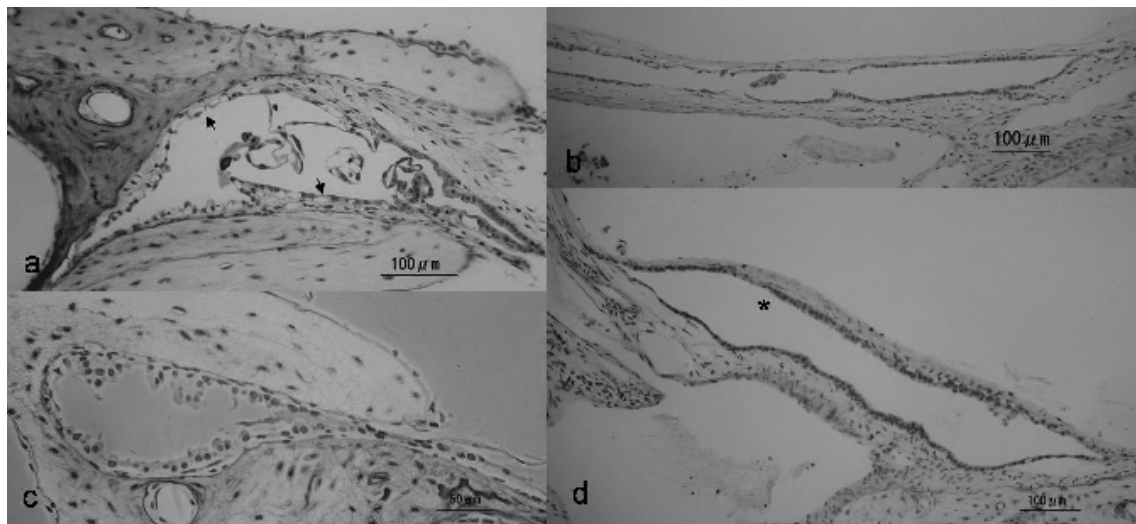


Fig.4

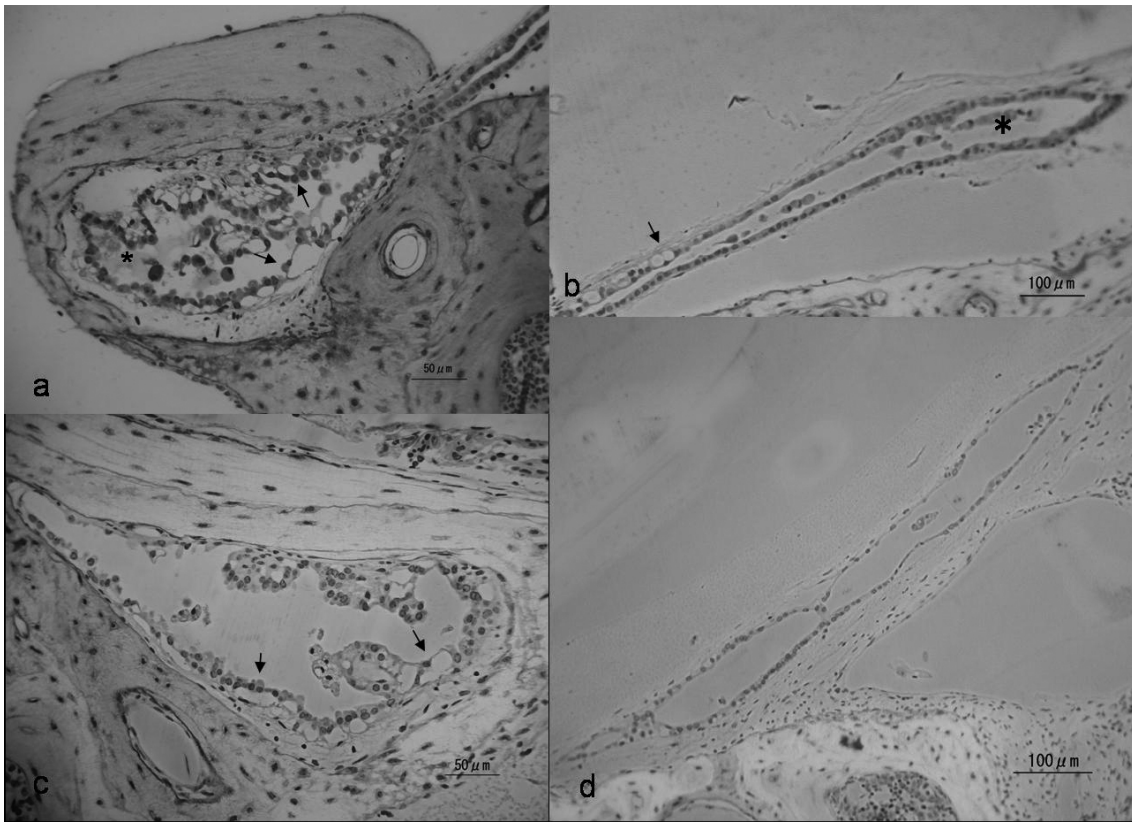


Fig.5

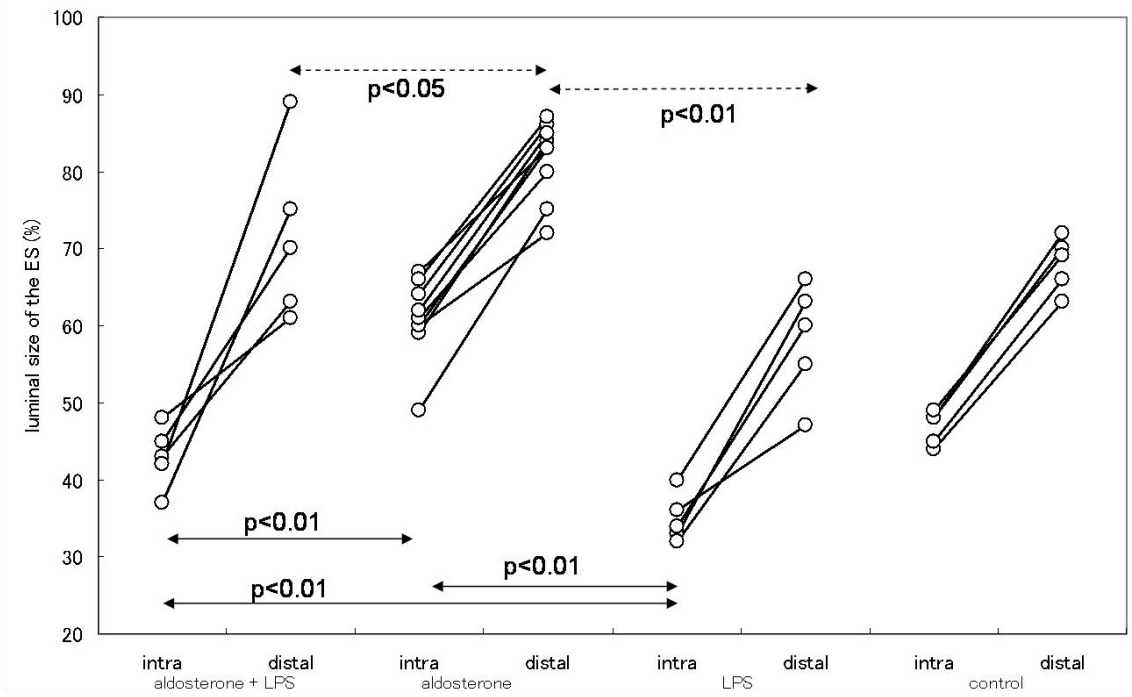




Fig.6

