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Two-phase endolymphatic hydrops
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Endolymphatic hydrops after total dissection or cauterization of the distal portion of the endolymphatic sac

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Introduction

Since Hallpike and Cairns\(^1\) and also Yamakawa\(^2\) in 1938 discovered hydrops of the endolymphatic system in the temporal bones of patients with Menière’s disease, endolymphatic hydrops (Figure 1) has been generally accepted as the basic histopathological substrate of Menière’s disease.

Many animal species have been used to induce endolymphatic hydrops. In 1965 Kimura and Schuknecht\(^3\) consistently produced an experimental endolymphatic hydrops after surgical obliteration of the endolymphatic duct and sac in the guinea pig.

This is, however, a nonphysiological profound model for Menière’s disease in humans. Despite the finding of an abnormal endolymphatic sac and narrowed duct\(^4\), the sac and duct remain patent in patients with Menière’s disease.

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**Figure 1.** Moderate degree of endolymphatic hydrops in the second turn of the cochlea, which is demonstrated by a distension of Reissner’s membrane (arrows), and which was induced by dissection of the endolymphatic sac. Bar=100 µm.
More subtle changes in the absorptive system may result in endolymphatic hydrops. Venous insufficiency of the endolymphatic sac results in an increased venous pressure producing an abnormal absorption and drainage disorder of the endolymphatic fluid unless collateral veins develop\(^7\). Induction of vascular disturbances by blocking the posterior meningeal artery and sigmoid sinus above the external aperture of the vestibular aqueduct has been demonstrated to induce a mild endolymphatic hydrops in the guinea pig, possibly through ischemia of the endolymphatic sac and venous insufficiency\(^6\).

Biopsy specimens of the endolymphatic sac, removed at saccotomy in patients with Menière’s disease, revealed fibrosis of the endolymphatic sac\(^7\). The authors found no relationship between the degree of fibrosis and the duration of the disease, but a correlation between the degree of fibrosis and the extent of hearing loss did exist. Perisaccular fibrosis of the sac in guinea pigs was established by injecting a small amount of 10% silver nitrate solution into the sac\(^8\). The cauterization induced scar tissue and fibrosis of the sac, resulting in extensive hydrops. In some animals, the lumen of the sac was completely filled with dense fibrous connective tissue and the function of the sac seemed to be extensively disturbed. However, the administration of silver nitrate and its spreading through the sac tissue, is uncontrolled, cannot be standardized precisely, and may produce varied damage and dysfunction of both the intraosseous and extraosseous portions of the sac.

We would like to develop a method in which we standardize the damage to the endolymphatic sac, resulting in a mild fibrosis which is restricted to the most distal extraosseous portion of the sac. This method may better emulate the pathophysiology of the endolymphatic hydrops seen in Menière’s disease, than the profound damage created by use of the classical methods of inducing hydrops through destruction of most parts of the endolymphatic sac and vestibular aqueduct.

In this study, we intend to induce a partial dysfunction of the endolymphatic sac as the result of mild fibrosis due to total dissection or controlled cauterization of the extraosseous part of the endolymphatic sac adjacent to the sigmoid sinus.

**Materials and methods**

Seventeen healthy female albino guinea pigs (Harlan, the Netherlands) with a mean weight of 250 g were divided in two groups. Animal care and use were approved by the experimental Animal Committee of the Groningen University, protocol number 0777-1193/1294, in accordance with the principles of the declaration of Helsinki.

In group 1 (n=12) the most distal part of the endolymphatic sac was dissected totally from the sigmoid sinus; in group 2 (n=5) the distal part of the endolymphatic sac was cauterized with silver nitrate in a mild to severe extent. The cochleas of both groups were examined for the presence of endolymphatic hydrops after a mean post-operative follow-up of 23 days using light microscopy.
Surgical procedure

The operation was performed under sterile conditions with a Zeiss operation microscope in a specialized unit of the Central Animal Laboratory. Anesthesia was induced with a combination of halothane, oxygen and N₂O. During surgery, temperature was stabilized by using an electric heating pad.

After visualization of the endolymphatic sac, the interconnections between the sigmoid sinus and the most distal portion of the endolymphatic sac were treated according to two different modalities:

Group 1: The interconnections were totally disrupted, and a small piece of latex was interposed between these two structures and fixed with an absorbable hemostatic sponge (Spongostan®, Ferrosan, Danmark). In some cases, a very small fragment of endolymphatic sac tissue remained attached to the sigmoid sinus, without connection to the dissected part.

Group 2: The interconnections were cauterized with crystallized silver nitrate, no piece of latex or absorbable hemostatic sponge (Spongostan®, Ferrosan, Danmark) was used.

Fixation procedure

After three weeks all animals were killed using sublethal administration of sodium pentobarbital (60 mg/kg i.p.), and intracardiac perfusion was performed with tri-aldehyde fixative (3% glutaraldehyde, 2% formaldehyde, 1% acrolein and 2.5% DMSO in 0.08 mol/l sodium cacodylate buffer; pH 7.4). After temporal bone dissection was performed, the cochleas were decalcified, postfixed and subsequently imbedded in Spurr’s low-viscosity resin. The part of the temporal bone with the endolymphatic sac and duct was imbedded in glycolmethacrylate.

The cochleas were cut in a midmodiolar plane. Semi-thin sections from the cochleas as well as the endolymphatic sac and duct were obtained. These were stained with toluidin blue and basic fuchsin, and examined using light microscopy.

Results

Degree of hydrops

The hydrops was rated according to Sperling et al.\textsuperscript{9} as:

1. Slight hydrops: bulging of Reissner’s membrane without contact with the bony wall of the scala vestibuli.
2. Moderate hydrops: displacement of Reissner’s membrane with contact with the bony wall but with an angle of less than 90 degrees with the osseous spiral lamina.
3. Severe hydrops: displacement of Reissner’s membrane with bony contact, with an angle greater than 90 degrees with the spiral lamina.
The first (basal), second (middle), and third (apical) cochlear turns of each cochlea were separately examined for their degree of hydrops. An overall degree of hydrops for the cochlea was determined from these results. Table 1 demonstrates the overall degree of hydrops in the dissected and cauterized ears.

### Table 1. Overall degree of hydrops in dissected or cauterized ears.

<table>
<thead>
<tr>
<th>Treatment Modality</th>
<th>Degree of Hydrops</th>
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<tbody>
<tr>
<td></td>
<td>None</td>
</tr>
<tr>
<td>Dissection (n=12)</td>
<td>3</td>
</tr>
<tr>
<td>Cauterization (n=5)</td>
<td>3</td>
</tr>
</tbody>
</table>

**Dissection**

This group represents the ears in which the sac was dissected. An interanimal variation in the degree of hydrops was measured. Most cochleas turned out to have a moderate to severe degree of hydrops (Figure 1, Table 1). Intracocharlear variation was present. In cochleas with moderate to severe degrees of hydrops, a gradient was observed in which the most severe degrees of hydrops were found in the apical turns, and lesser degrees in the basal turns.

**Cauterization**

This group represents the ears in which the sac was cauterized. Although the use of crystallized silver nitrate in our study appeared to provide a better controlled procedure than using a solution of silver nitrate as was used in an earlier study, it was very difficult to establish a standardized cauterization of the sac. In some cases, the silver nitrate rapidly spread out following a short and slight touch to the endolymphatic sac, sometimes even to the intraosseous portion of the endolymphatic sac. Furthermore, intercochlear and intracocharlear variation was measured in this group. None of the animals showed moderate to severe degrees of hydrops (Table 1). The degree of hydrops did not correlate to the degree of cauterization. Hydrops was mainly found in the apical windings.

**Control**

The contralateral ears in which the sac was not dissected or cauterized served as a control. No hydrops was detected in the turns of these cochleas.
Endolymphatic sac
The normal, dissected, and cauterized endolymphatic sacs were investigated by light-microscopical techniques. The observations were concentrated on the histopathological changes of the distal and intermediate part of the sac after dissection or cauterization, in correlation with the degree of hydrops.

In contralateral, normal ears these different parts were recognized and served as a control (Figure 2a and 3a). The intraosseous and extraosseous intermediate part of the endolymphatic sac was easily identified by its high cylindrical cells which protrude into the lumen in irregular papillae. In these cylindrical epithelial cells, many cellular inclusions can be found. The lumen of the endolymphatic sac is normally provided with a population of free floating cells, predominantly rounded macrophages with large vacuoles. The epithelial lining of the distal portion of the endolymphatic sac is cuboidal except at the extreme end where it is squamous. The loose connective tissue is richly vascularized.

**Distal portion**

1 Dissection
Despite careful dissection, a very thin layer of endolymphatic sac tissue may remain attached to the sigmoid sinus (Figure 2b), as sometimes experienced during surgery and confirmed by histological evaluation. The loose connective tissue was replaced by fibrous tissue which was arranged in fibrils along the sigmoid sinus. This fibrous tissue seemed to separate the distal part of the extraosseous dissected endolymphatic sac from the sigmoid sinus, and may act as a barrier for endolymphatic outflow.

2 Cauterization
Cauterization of the endolymphatic sac resulted in an abundance of fibrous tissue in and around the extraosseous endolymphatic sac. Bands of fibrous tissue were observed in the endolymphatic sac. No lumen was detected in the distal part. Silver particles were observed in macrophages in the tissue between the sac and the sigmoid sinus where the sac was cauterized. Although fibrous tissue was present between the sac and the sigmoid sinus, these two structures were, in contrast to the dissection procedure, not separated by the process of cauterization.

**Intermediate portion**

1 Dissection
In the intermediate portion of the dissected endolymphatic sac resulting in endolymphatic hydrops, the amount of cytoplasm of the cylindrical cells was increased as compared to the control specimens (Figure 3b). Furthermore, these cells contained more vacuoles in their cytoplasm and on their surface. These vacuoles seemed to be excreted from the cylindrical cells. The cylindrical cells were not replaced by cuboidal or flat epithelial cells. No fibrosis of the subepithelial tissue was noticed.

2 Cauterization
Epithelial cells and subepithelial tissue of the intermediate portion of the endolymphatic sac in cauterized specimens did not demonstrate epithelial changes as demonstrated in the dissected group.

**Discussion**

In our study, both dissection and cauterization resulted in a variety of hydrops, mostly concentrated in the apical windings. The degree of hydrops of the endolymphatic sac and the condition may be determined by the extent of damage due to the dissection or cauteri-
Figure 3. (top) Luminal epithelium of the intermediate part of the normal endolymphatic sac. High cylindrical cells (IC) protrude into the endolymph in irregular papillae. On the surface of these cells vacuoles can be found (arrow). The lumen of the endolymphatic sac is normally provided with a population of free floating cells, predominantly rounded macrophages with large vacuoles. Bar=20 µm.

(bottom) Intermediate part of a dissected endolymphatic sac of a guinea pig which developed severe degrees of endolymphatic hydrops. The cylindrical cells are still present, but appear to be more swollen. Many more vacuoles (arrows) can be observed in and on these cylindrical cells as compared to the non-operated specimen. The free-floating macrophages seem to contain more vacuoles. Bar=20 µm.
zation. Severe damage may compromise the function of the different parts of the endolymphatic sac. In our study, the dissection procedure was relatively standardized. Despite this standardization, the degree of hydrops showed interanimal variation. In most cases, total dissection resulted in moderate to severe degrees of hydrops.

Dissection

The relative severe degrees of hydrops which developed as a result of the dissection procedure, may be a consequence of simultaneous dysfunction of different parts of the endolymphatic sac. The dissection may result in total obstruction of venous outflow. This may furthermore compromise endolymph outflow in more proximal, intraosseous parts of the endolymphatic sac. In general, we may ascribe our findings, which are mainly based on the dissection procedure, to several causes: (a) The dense fibrous tissue between the endolymphatic sac and the sigmoid sinus (Figure 2b) which may act as a barrier to the outflow of endolymph in the distal portion. (b) The damage to the distal portion or the resulting fibrous tissue may compromise the venous outflow of the entire endolymphatic sac, which may disturb endolymph homeostasis of the intermediate portion of the endolymphatic sac.

Cauterization

Cauterization of the endolymphatic sac was, although the sac was carefully touched by crystallized silver nitrate, difficult to standardize. In some cases, mild cauterization resulted in hydrops, while severe cauterization did not. The absence of extensive hydrops may indicate that the outflow is still sufficient for prevention of the development of hydrops. This may be ascribed to the fact that, in contrast to the dissection procedure, cauterization did not separate the endolymphatic sac from the sigmoid sinus.

Implications

One of the interesting findings in our study is that an extraosseous dissection of the most distal part of the endolymphatic sac from the sigmoid sinus, is already destructive enough to produce endolymphatic hydrops. Although this procedure was less damaging than the classical method of destroying the endolymphatic sac as well as the endolymphatic duct, it produced a large amount of hydrops. This indicates that the distal portion of the endolymphatic sac may play a more important role in the regulation of endolymph homeostasis, than has been postulated before.

Furthermore, it was not our aim to produce the highest degrees of hydrops, but to refine the animal model of endolymphatic hydrops to a situation in which the condition of the endolymphatic sac correlates better to the condition of the endolymphatic sac as seen in Menière’s disease. In our study we attempted to induce a relatively mild standardized
damage to the sac resulting mainly in a partial dysfunction, in contrary to the total destruction and dysfunction of the endolymphatic sac as seen in the classical methods of experimental endolymphatic hydrops. The degree of hydrops is in fact not important, but merely a sign of the compliance of the endolymphatic sac of that specific guinea pig in this study.

The high incidence of endolymphatic hydrops due to the dissection procedure in this study invites a question. If such relatively mild damage to the extraosseous part of the endolymphatic sac can have such obvious effects, what is the effect of manipulations of the endolymphatic sac in human surgery? Biopsies or endolymphatic sac surgical procedures, such as drainage procedures for Menière’s disease, have been considered to be diagnostical or therapeutical, but have never been considered as harmful to the function of the endolymph homeostasis. Our results indicate that any damage to the endolymphatic sac compromises its function, and may contribute to the development of hydrops, either asymptomatic or symptomatic. This may explain the difference between the acute positive effects of sac surgery and the poor or negative outcome of the long-term results, which was found in some studies.

In conclusion, the extraosseous portion of the endolymphatic sac has proven to be a delicate structure which may play an important role in the regulation of endolymph homeostasis. In this study a more refined guinea pig model of endolymphatic hydrops was developed, which may better simulate the histopathological findings of the endolymphatic sac in patients with Menière’s disease, and may represent a more realistic approach to the understanding of Menière’s disease. Although considered to be of diagnostic or therapeutical value, surgical manipulations of the endolymphatic sac in patients with Menière’s disease have to be reconsidered.

References

3 Kimura RS, Schuknecht HF. Membranous hydrops in the inner ear of the guinea pig after obliteration of the endolymphatic sac. Pract Otorhinolaryngol 1965;27:343-354.


