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Published in:
Hearing Research

DOI:
10.1016/j.heares.2015.05.008

2015

Link to publication

Citation for published version (APA):
Degerman, E., in 't Zandt, R., Holmén-Pålbrink, A.-K., & Magnusson, M. (2015). Vasopressin induces endolymphatic hydrops in mouse inner ear, as evaluated with repeated 9.4 T MRI. *Hearing Research*, *330*(Part A), 119-124. https://doi.org/10.1016/j.heares.2015.05.008

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Vasopressin induces endolymphatic hydrops in mouse inner ear, as evaluated with repeated 9.4 T MRI

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Abbreviations: Gadolinium, Gd; endolymphatic hydrops, EH; magnetic resonance imaging, MRI

Abstract

From histopathological specimens, endolymphatic hydrops has been demonstrated in association with inner ear disorders. Recent studies have observed findings suggestive of hydrops using MRI in humans. Previous studies suggest that vasopressin may play a critical role in endolymph homeostasis and may be involved in the development of Ménière's disease. In this study we evaluate the effect of vasopressin administration *in vivo* in longitudinal studies using two mouse strains. High resolution MRI at 9.4 T in combination with intraperitoneally delivered Gadolinium contrast, was performed before and after chronic subcutaneous administration of vasopressin via mini-osmotic pumps in the same mouse. A development of endolymphatic hydrops over time could be demonstrated in C57BL6 mice (5 mice, 2 and 4 weeks of administration) as well as in CBA/J mice (4 mice, 2 weeks of administration; 6 mice, 3 and 4 weeks of administration). In most C57BL6 mice hydrops developed first after more than 2 weeks while CBA/J mice had an earlier response. These results may suggest an *in vivo* model for studying endolymphatic hydrops and corroborates the future use of MRI as a tool in the diagnosis and treatment of inner ear diseases, such as Ménière's disease. MRI may also be developed as a critical tool in evaluating inner ear homeostasis in genetically modified mice, to augment the understanding of human disease.

Keywords: magnetic resonance imaging, cochlea, endolymphatic hydrops, vasopressin, Ménière's disease

1. Introduction

Inner ear fluid homeostasis is critical to normal auditory and vestibular function. Disturbance of this fluid balance may result in excess accumulation of endolymph in the inner ear, endolymphatic hydrops (EH) [Semaan et al., 2005, Salt et al., 2010]. EH associated changes

in the inner ear are believed to be of significance in relation to the symptoms associated with for example Ménière's disease such as fluctuating hearing loss, tinnitus, vertigo and aural fullness [Semaan et al., 2011, Sajjadi et al., 2008]. Although the pathogenesis of EH is unclear, the underlying problem is believed to be either overproduction or malabsorption of endolymph. A number of methods have been used to develop animal models of EH [Salt et al., 2010] one of which involves administration of vasopressin to experimental animals using histological alterations post mortem of the inner ear as read-out [for example Takeda et al., 2000, Chihara et al., 2013, Katagiri et al., 2014]. One concern with histological methods is that the degree of hydrops may be influenced by the fixation and processing. Also, a histochemical approach does not allow for the analysis of one and the same individual before and after treatment, that is, demonstrating a development of hydrops in relation to intervention.

Magnetic resonance imaging (MRI) in combination with gadolinium (Gd) contrast agents can be used to differentiate the endo- and perilymphatic space of the inner ear *in vivo*. Thus, experimental and clinical MRI investigations of the inner ear have established that, when administered intravenously or intratympanically, the Gd complex readily penetrates the perilymphatic space, but not the endolymphatic space of the entire vestibulo-cochlea membranous labyrinth [Naganawa et al., 2012, Counter et al., 1999, Counter et al., 2000, Counter et al., 2003, Zou et al., 2003, Zou et al., 2010, Duan et al., 2004]. Indeed, MRI in combination with Gd contrast agents has been used to visualize EH in patients with Ménière's disease [Naganawa et al., 2014, Pyykkö et al., 2010, Zou et al., 2009, Baráth et al., 2014, Jerin et al., 2014] and in animal models developed to simulate human inner ear disease [Pyykkö et al., 2010, Zou et al., 2003, Niyazov et al., 2001].

In the present study we evaluate, in longitudinal studies using 9.4 T MRI in combination with intraperitoneal administration of Gd, an alternative to intravenously or intra tympanic administration, the development of endolymphatic hydrops after chronic administration of vasopressin to two mouse stains. We demonstrate, to our knowledge for the first time in mouse strains, the development of EH over time *in vivo* using MRI.

2. Materials and methods

2.1.Experimental Design

Five female C57BL6 mice and 4 and 6 female CBA/J mice of body weight 22-24 gram (8 weeks of age) were used in three different studies. All animal experiments were approved by the Ethical Committee, Lund/Malmö.

The mice were subjected to continuous administration of vasopressin ([Arg]8-VP, Sigma Chemical, St Louis, MO) via mini-osmotic pumps (model 2002; Alzet Corp., Palo Alto, CA) at a rate of 50 μg/100g/day for 14-30 days as indicated in the results section. The pumps were implanted surgically in the subcutaneous tissues on the back between the scapulae (Takeda et al., 2000, Marshall et al., 2010). Gd contrast agent (Dotarem) (gadoteric acid) (279.3 mg/ml, 0.5 mmol/ml) was administered intraperitoneally (100μl/20g) in the left abdominal quadrant. Sixty minutes after Gd administration, MR images were obtained at day zero (before vasopressin) and after initiation of vasopressin administration.

2.2.Magnetic Resonance Imaging

The animals were anesthetized with 3.5% isoflurane in mixture of 200 ml/min oxygen and 200 ml/min nitrous oxide and maintained at 1.5-2% isoflurane inside the magnet. Inside the magnet, the respiratory rate of the animal was monitored and the body temperature was maintained using warm air (SA Instruments Inc, New York, USA).

MR imaging was performed with a 9.4 T MR scanner (Agilent Inc., Palo Alto, USA) equipped with a 6 cm inner diameter gradient system having a maximum gradient strength of 1000 mT/m. The animals were fixated inside the scanner using a home-built animal holder in which a 15x11 mm^2 transmit/receive surface coil (MRCoils BV, Drunen, the Netherlands) was mounted on the mouse head. T1-weighted 3D images were acquired with a gradient echo 3D sequence; repetition time TR: 10 ms, echo time TE: 3.67 ms, number of averages: 4, data matrix size 256x128x256 pixels, field of view 20 x 10 x 20 mm^3. Images were reconstructed by filtering the data using a 3 dB Gaussian filter and by zero filling to increase the apparent resolution of the image to a matrix size of 512x256x512 pixels.

2.3.Quantitative assessment of gadolinium in endolymphatic relative perilymphatic space

Image J 1.48v as well as Adobe Photoshop CS5 were used for post-production processing of images for the quantification of signal intensity in regions of interest and for labeling and demonstration of perilymph in the scala tympani, scala vestibuli and vestibulum and of endolymph in the scala media and vestibulum.

In the basal turn of the cochlea, the cross-sectional areas of the the scala media and the scala media plus scala vestibuli were measured and the ratio of the area of the scala media to that of scala media plus scala vestibuli was evaluated in images parallel to the modiolus of the cochlea (see Fig.1) (Naganawa et al., 2014, Baráth et al., 2014). The ratio of areas was subsequently converted to percentage as shown in Fig 2-4.

2.4. Statistical analysis

For statistical analysis, endolymph/perilymph ratios (see section 2.3), performed before and after vasopressin treatment in the same mouse, were compared using Student's paired t-test with $P < 0.05$ accepted as an indication of statistical significance.

3. Results

Vasopressin induced development of endolymphatic hydrops in mice as measured by 9.4 T MRI

Vasopressin was continuously administered to female C57BL6 and CBA/J mice using mini-osmotic pumps implanted surgically in the subcutaneous tissues on the back between the scapulae. At specific time-points MRI in combination with intraperitoneal Gd contrast was used to estimate the relative size of the endolymphatic compartment in the basal turn of the cochlea as described in material and methods. Fig 1 shows examples of images from before (B, D) and after (A, C) vasopressin administration. To demonstrate the difference of Gd uptake in other parts of the inner ear as well, an image showing the cochlea as well as the saccule and utricle is presented (Fig 1E).

Vasopressin administration to five C57BL6 mice for 27 days induced 1.4 fold increase of the endolymphatic space as compared to the pre-vasopressin situation (before vasopressin=22.3%, after vasopressin=31.0%, n=10, P=0.0025) whereas no significant increase of the space was detected after 14 days (Fig 2A). Pre and post vasopressin values are indicated for each ear in Fig 2B and Fig 2C. At 14 days of treatment most ears showed no increase in the endolymphatic space whereas at 27 days of treatment 8 out of 10 ears showed at least 1.2 fold increase in the endolymphatic space.

The ability of vasopressin to induce endolymphatic hydrops was also tested in CBA/J mice in two separate studies with four and six mice, respectively. As shown in Fig 3A, in as study consisting of four mice, vasopressin administration for 14 days increased 2 fold the endolymphatic space (before vasopressin=12.5%, after vasopressin=24.5%, n=8, P=0.0022). In a separate study with six CBA/J mice, longer exposure times to vasopressin were used. As

seen in Fig 4A, 19 and 30 days of exposure resulted in 1.4 fold increase in the endolymphatic space (before vasopressin=20%, 19 days after vasopressin=27.8% and 30 days after vasopressin=28.4%, n=12, P=0.00068 and 0.00027). Pre and post vasopressin values are indicated for each ear in the CBA/J groups (Fig 3B and Fig 4B and Fig 4C). In summary, of the 32 ears measured, 26 ears showed at least 1.2 fold increase in endolymphatic space.

4. Discussion

Vasopressin is believed to play an important role in inner ear pathophysiology in humans as well as animals [Takeda et al., 2009, 2010]. The effects of vasopressin on inner ear fluid homeostasis is mediated by the vasopressin 2 receptor which, via effects on cAMP/Protein kinase A, lead to translocation of aquaporin 2 to the plasma membrane [Sawada et al., 2002, Takeda et al., 2003]. In this study we demonstrate using 9.4 T field strength MRI in combination with intraperitoneally administered Gd contrast agent the development of EH in two mouse strains, C57BL6 and CBA/J mice, induced by chronic administration of vasopressin and comparing the same ear before and after treatment. Hydrops seemed to develop at an earlier time point in CBA/J mice as compared to C57BL6 mice which could be related to strain differences as discussed in [Katagiri et al., 2014].

Endolymphatic hydrops in experimental models has to a large extent been demonstrated using histological analysis which requires several manipulations; fixation, decalcification, embedding, and sectioning. One concern with histological methods is thus that the degree of hydrops may be influenced by the fixation and processing [Brunschwig et al., 1997]. MRI studies on guinea pigs support however the induction of EH induced surgically or induced by other treatments [Zou et al., 2003]. The demonstration of vasopressin induced EH in two mouse strains as demonstrated in this study further support the usefulness of MRI as an *in*

vivo read-out for EH in a living animal. The possibility to study the mouse inner ear *in vivo* is of particular interest due to the availability of relevant genetically modified mice in the inner ear field mimicking human inner ear dysfunction [Kikkawa et al., 2012]. Furthermore, the latency in the development of hydrops and the time course of possible effect of future interventions may need *in vivo* approaches to be followed over time.

We used the basal turn of the cochlea as a measurement region (Naganawa et al., 2014). This semi quantitative method used is time consuming and susceptible to operator bias. It was considered that poorly aligned scans could have influenced the volume ratio estimation. However, calculations of areas of endolymphatic and perilymphatic space were done in our 3D MRI volume after alignment of the center axis of the modiolus in a sagittal plane. This would exclude any larger deviation in observation angle for the measurements done between recordings. Therefore, it is less likely that any deviation between the planes of images could have contributed to a misleading result. Furthermore, in the present study the data from the images were corroborated by a second independent assessment.

We used high concentrations of vasopressin, 50 μg/100g/day, a concentration used recently to induce EH in mice [Katagiri et al., 2014]. The authors demonstrated vasopressin induced mild to severe hydrops as judged by histological post mortem analysis after daily subcutaneous injection between 5 days and 8 weeks. In a study on guinea pigs [Takeda et al., 2000], continuous administration of vasopressin via mini-osmotic pumps at approximately 100-fold lower dose per gram per day resulted in EH as judged by histological changes in the inner ear after one week. Pilot experiments using the lower vasopressin concentration used for guinea pigs did not induce EH in our experimental set-up (data not shown).

In this study we used 9.4 T MRI which permitted acquisition of high resolution images of non-hydropic and hydropic mouse inner ears *in vivo*. A 9.4 T MRI study on normal mouse inner ear was recently published [Counter et al., 2013] demonstrating high resolution images of anatomical and physiological features *in vivo*. Also, this study established that the intravenously administration of Gd complex did not permeate the scala media and the blood-endolymph barrier of the endolymphatic membranous space confirming earlier experimental MRI observations at 4.7 T of an impermeable blood-endolymph barrier of the scala media throughout the membranous inner ear labyrinth of mammals [Naganawa et al., 2012, Counter et al., 2000, Counter et al., 2003, Zou et al., 2003, Duan et al., 2004].

Intraperitoneal administration of Gd contrast agent was used in this study. Compared to intravenous injections, intraperitoneal administration of Gd contrast agent in mice is less traumatic with practically no risk for emboli or hypervolemia. To our knowledge, intraperitoneal administration of Gd, has not been utilized in combination with MRI to visualize inner ears of experimental animals. Rather, intravenous or transtympanic Gd administration has been the method of choice. Transtympanic administration can cause damage to the tympanic membrane and/or middle ear complications and intravenous administration in mice is not trivial to perform. Thus, especially in serial investigations, intraperitoneal administration of contrast might be an advantage.

Techniques to directly detect structural alterations associated with EH *in vivo* are not yet available, however, ultra-high imaging techniques for the use on small animals and pathological specimens have been developed including micro CT (Chihara et al., 2013, Lane et al., 2004), MR microscopy (Lane et al., 2005, Pettit et al., 2002) and optical coherence tomography (OCT) (Kakigiet al., 2013, Cho et al., 2015). These approaches avoid tissue destruction inherent to histological preparation using standard light microscopy.

In conclusion, EH was induced, to our knowledge for the first time, by chronic administration of vasopressin via mini-osmotic pumps in two mouse strains using 9.4 T MRI in combination with Gd contrast agent intraperitoneally as read-out. We could also for the first time demonstrate that endolymphatic hydrops can be developed in an individual animal and that vasopressin actually can instigate such a development.

Figure legends

Fig.1. **MRI of mouse cochlear and vestibular structures.** Imaging was performed 60 minutes after intraperitoneal administration of gadolinium contrast**. Fig 1A-B** is an example of imaging and quantification of an inner ear from a C57BL mouse (filled circles, Fig 2C) and **Fig 1C-D** is an example from a CBA/J mouse (open circles, Fig 4C). **Upper panels** show images parallel to the modiolus of the cochleas of vasopressin treated mice (**A, C**) and control mice (**B, D**). SV=scala vestibuli, ST=scala tympani, SM=scala media, 1st=first turn, 2nd=second turn, Arrow=osseous spiral lamina, LW=lateral wall. **Lower panels** show the same images but now indicating the endolymphatic and perilymphatic compartments used for the estimation of EH. To evaluate the effect of vasopressin on the size of the endolymphatic fluid compartment, the relative area of scala media in the basal turn of the cochlea was estimated by calculating the ratio between scala media (dotted area, endolymph, non contrast enhanced) and scala vestibuli (non-dotted area, perilymph, contrast enhanced) plus scala media. To demonstrate the difference of gadolinium uptake in other parts of the inner ear as well, a section obtained in a plane through the vestibulum, showing the saccule and utricle is presented (**E**) (section from mouse in Fig 2C, open squares). SV=scala vestibuli, ST=scala tympani, SM=scala media U=utricle, S=saccule, V=vestibuli

Fig.2. Vasopressin induced EH in C57 BL6 mice. Five C57BL6 mice (10 ears) were treated chronically with vasopressin and MRI was performed after 14 and 27 days. Quantification of endolymphatic and perilymphatic cochlear compartments before (PreVP) and after vasopressin (VP) administration was performed as described in Material and methods and in Fig.1. Data are presented as mean+/- SD (A) and as individual values for each ear before and after vasopressin (B, C, in the two graphs a specific ear is indicated with the same symbol).

Fig.3. Vasopressin induced EH in CBA/J mice. Four CBA/J mice (8 ears) were treated chronically with vasopressin for 14 days and thereafter analyzed using MRI. Quantification of endolymphatic and perilymphatic cochlear compartments before (PreVP) and after vasopressin (VP) administration was performed as described in in Material and methods and in Fig.1. Data are presented as mean+/- SD (A) and as individual values for each ear before and after vasopressin (B).

Fig.4. Vasopressin induced EH in CBA/J mice. Six CBA/J mice (12 ears) were treated chronically with vasopressin and MRI was performed after 19 and 30 days. Quantification of endolymphatic and perilymphatic cochlear compartments was performed before (PreVP) and after vasopressin (VP) administration as described in Material and methods and in Fig. 1. Data are presented as mean +/- SD (A) and as individual values for each ear before and after vasopressin (B, C, in the two graphs a specific ear is indicated with the same symbol).

Acknowledgements

We want to thank Anki Knutsson at the Department of Experimental Medical Sciences, the Medical Faculty, Lund University for expert advice and support regarding the mini-osmotic pumps and Adnan Bibic at Lund University Bioimaging Center (LBIC) for advice and support

on post processing of images. LBIC, Lund University, is gratefully acknowledged for providing experimental resources. The work was supported by grants from the Swedish Diabetes Association Foundation and from the Crafoord Foundation.

References

Baráth K, Schuknecht B, Naldi AM, Schrepfer T, Bockisch CJ, Hegemann SC. Detection and grading of endolymphatic hydrops in Menière disease using MR imaging. AJNR Am J Neuroradiol. 2014 Jul;35(7):1387-92.

Brunschwig AS, Salt AN. Fixation-induced shrinkage of Reissner's membrane and its potential influence on the assessment of endolymph volume. Hear Res. 1997 Dec;114(1-2):62-8.

Chihara Y, Wong C, Curthoys IS, Brown DJ. The effect of systemic administration of desmopressin on cochlear function in guinea pigs. Acta Otolaryngol. 2013 Jul;133(7):676-84.

Cho NH, Lee JW, Cho JH, Kim J, Jang JH, Jung W. Evaluation of the usefulness of three-dimensional optical coherence tomography in a guinea pig model of endolymphatic hydrops induced by surgical obliteration of the endolymphatic duct. J Biomed Opt. 2015 Mar 1;20(3):36009.

Counter SA, Bjelke B, Klason T, Chen Z, Borg E. Magnetic resonance imaging of the cochlea, spiral ganglia and eighth nerve of the guinea pig. Neuroreport 1999; 10: 473-9.

Counter SA, Bjelke B, Borg E, Klason T, Chen Z, Duan ML. Magnetic resonance imaging of the membranous labyrinth during in vivo gadolinium (Gd-DTPA-BMA) uptake in the normal and lesioned cochlea. Neuroreport 2000; 11: 3979-83.

Counter SA, Zou J, Bjelke B, Klason T. 3D MRI of the in vivo vestibulo-cochlea labyrinth during Gd-DTPA-BMA uptake. Neuroreport 2003; 14:1707-12.

Counter SA, Nikkhou S, Brené S, Damberg P, Sierakowiak A, Klason T, Berglin CE, Laurell G. RI evidence of endolymphatic impermeability to the gadolinium molecule in the in vivo mouse inner ear at 9.4 tesla. Open Neuroimag J. 2013 Jun 28;7:27-31.

Duan M, Bjelke B, Fridberger A, Counter SA, Klason T, Skjönsberg A, Herrlin P, Borg E, Laurell G. Imaging of the guinea pig cochlea following round window gadolinium application. Neuroreport 2004; 15: 1927-30.

Egami N, Kakigi A, Sakamoto T, Takeda T, Hyodo M, Yamasoba T. Morphological and functional changes in a new animal model of Ménière's disease. Lab Invest. 2013 Sep;93(9):1001-11.

Jerin C, Krause E, Ertl-Wagner B, Gürkov R. Longitudinal assessment of endolymphatic hydrops with contrast-enhanced magnetic resonance imaging of the labyrinth. Otol Neurotol. 2014 Jun;35(5):880-3.

Kakigi A[1], Takubo Y, Egami N, Kashio A, Ushio M, Sakamoto T, Yamashita S, Yamasoba T. Evaluation of the internal structure of normal and pathological Guinea pig cochleae using optical coherence tomography. Audiol Neurootol. 2013;18(5):335-43. doi: 10.1159/000354620.

Katagiri Y, Takumida M, Hirakawa K, Anniko M. Long-term administration of vasopressin can cause Ménière's disease in mice. Acta Otolaryngol. 2014 Oct;134(10):990-1004.

Kikkawa Y, Seki Y, Okumura K, Ohshiba Y, Miyasaka Y, Suzuki S, Ozaki M, Matsuoka K, Noguchi Y, Yonekawa H. Advantages of a mouse model for human hearing impairment. Exp Anim. 2012;61(2):85-98. Review.

Lane JI, Witte RJ, Henson OW, Driscoll CL, Camp J, Robb RA. Imaging microscopy of the middle and inner ear: Part II: MR microscopy. Otolaryngol Head Neck Surg. Clin Anat. 2005 Sep;18(6):409-15.

Lane JI, Witte RJ, Driscoll CL, Camp JJ, Robb RA. Imaging microscopy of the middle and inner ear: Part I: CT microscopy. Clin Anat. 2004 Nov;17(8):607-12.

Marshall AF[1], Jewells VL, Kranz P, Lee YZ, Lin W, Zdanski CJ. Magnetic resonance imaging of guinea pig cochlea after vasopressin-induced or surgically induced endolymphatic hydrops. 2010 Feb;142(2):260-5. doi: 10.1016/j.otohns.2009.10.006.

Naganawa S, Yamazaki M, Kawai H, Bokura K, Sone M, Nakashima T. Imaging of endolymphatic and perilymphatic fluid after intravenous administration of single-dose gadodiamide. Magn Reson Med Sci. 2012; 11(2): 145-50.

Naganawa S, Nakashima T. Visualization of endolymphatic hydrops with MR imaging in patients with Ménière's disease and related pathologies: current status of its methods and clinical significance. Jpn J Radiol. 2014 Apr;32(4):191-204.

Niyazov DM, Andrews JC, Strelioff D, Sinha S, Lufkin R. Diagnosis of endolymphatic hydrops in vivo with magnetic resonance imaging. Otol Neurotol. 2001 Nov;22(6):813-7.

Pettit K[1], Henson MM, Henson OW, Gewalt SL, Salt AN. Quantitative anatomy of the guinea pig endolymphatic sac. Hear Res. 2002 Dec;174(1-2):1-8.

Pyykkö I, Zou J, Poe D, Nakashima T, Naganawa S Magnetic resonance imaging of the inner ear in Meniere's disease. Otolaryngol Clin North Am. 2010 Oct;43(5):1059-80. Review.

Sajjadi H, Paparella MM. Meniere's disease. Lancet. 2008 Aug 2;372(9636):406-14. Review.

Salt AN, Plontke SK. Endolymphatic hydrops: pathophysiology and experimental models. Otolaryngol Clin North Am. 2010 Oct;43(5):971-83. doi: 10.1016/j.otc.2010.05.007. Review.

Sawada S, Takeda T, Kitano H, Takeuchi S, Kakigi A, Azuma H. Aquaporin-2 regulation by vasopressin in the rat inner ear. Neuroreport. 2002 Jul 2;13(9):1127-9.

Semaan MT, Alagramam KN, Megerian CA. The basic science of Meniere's disease and endolymphatic hydrops. Curr Opin Otolaryngol Head Neck Surg. 2005 Oct;13(5):301-7. Review.

Semaan MT, Megerian CA. Ménière's disease: a challenging and relentless disorder. Otolaryngol Clin North Am. 2011 Apr;44(2):383-403. Review.

Takumida M, Akagi N, Anniko M. A new animal model for Ménière's disease. Acta Otolaryngol. 2008 Mar;128(3):263-71.

Takeda T, Takeda S, Kakigi A, Okada T, Nishioka R, Taguchi D, Nishimura M, Nakatani H (2010). Hormonal aspects of Ménière's disease on the basis of clinical and experimental studies. ORL J Otorhinolaryngol Relat Spec. 71 Suppl 1:1-9.

Takeda T, Takeda S, Kitano H, Okada T, Kakigi A. Endolymphatic hydrops induced by chronic administration of vasopressin. Hear Res. 2000 Feb;140(1-2):1-6.

Takeda T, Taguchi D. Aquaporins as potential drug targets for Meniere's disease and its related diseases. Handb Exp Pharmacol. 2009;(190):171-84. Review.

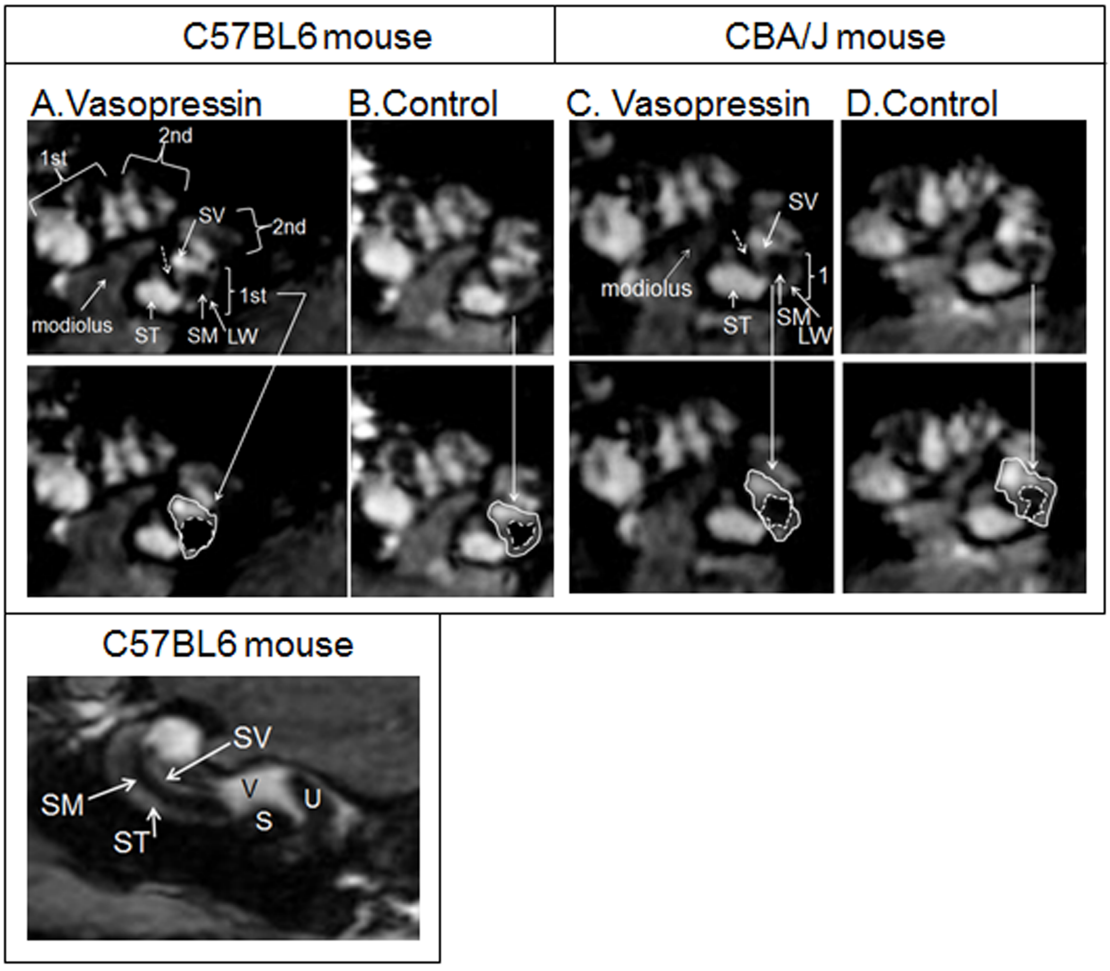
Takeda T, Sawada S, Takeda S, Kitano H, Suzuki M, Kakigi A, Takeuchi S. The effects of V2 antagonist (OPC-31260) on endolymphatic hydrops. Hear Res. 2003 Aug;182(1-2):9-18.

Zou J, Poe D, Bjelke B, Pyykko I. Visualization of inner ear disorders with MRI in vivo: from animal models to human application. Acta Otolaryngol Suppl. 2009 Feb;(560):22-31.

Zou J, Zhang W, Poe D, Zhang Y, Ramadan UA, Pyykkö I. Differential passage of gadolinium through the mouse inner ear barriers evaluated with 4.7T MRI. Hear Res. 2010; 259: 36-43.

Zou J, Pyykkö I, Bretlau P, Klason T, Bjelke B. In vivo visualization of endolymphatic hydrops in guinea pigs: magnetic resonance imaging evaluation at 4.7 tesla. Ann Otol Rhinol Laryngol. 2003 Dec;112(12):1059-65.

Zou J, Pyykkö I, Counter SA, Klason T, Bretlau P, Bjelke B. *In vivo* observation of dynamic perilymph formation using 4.7 T MRI with Gadolinium as a Tracer. Acta Otolaryngol 2003; 123: 910-5.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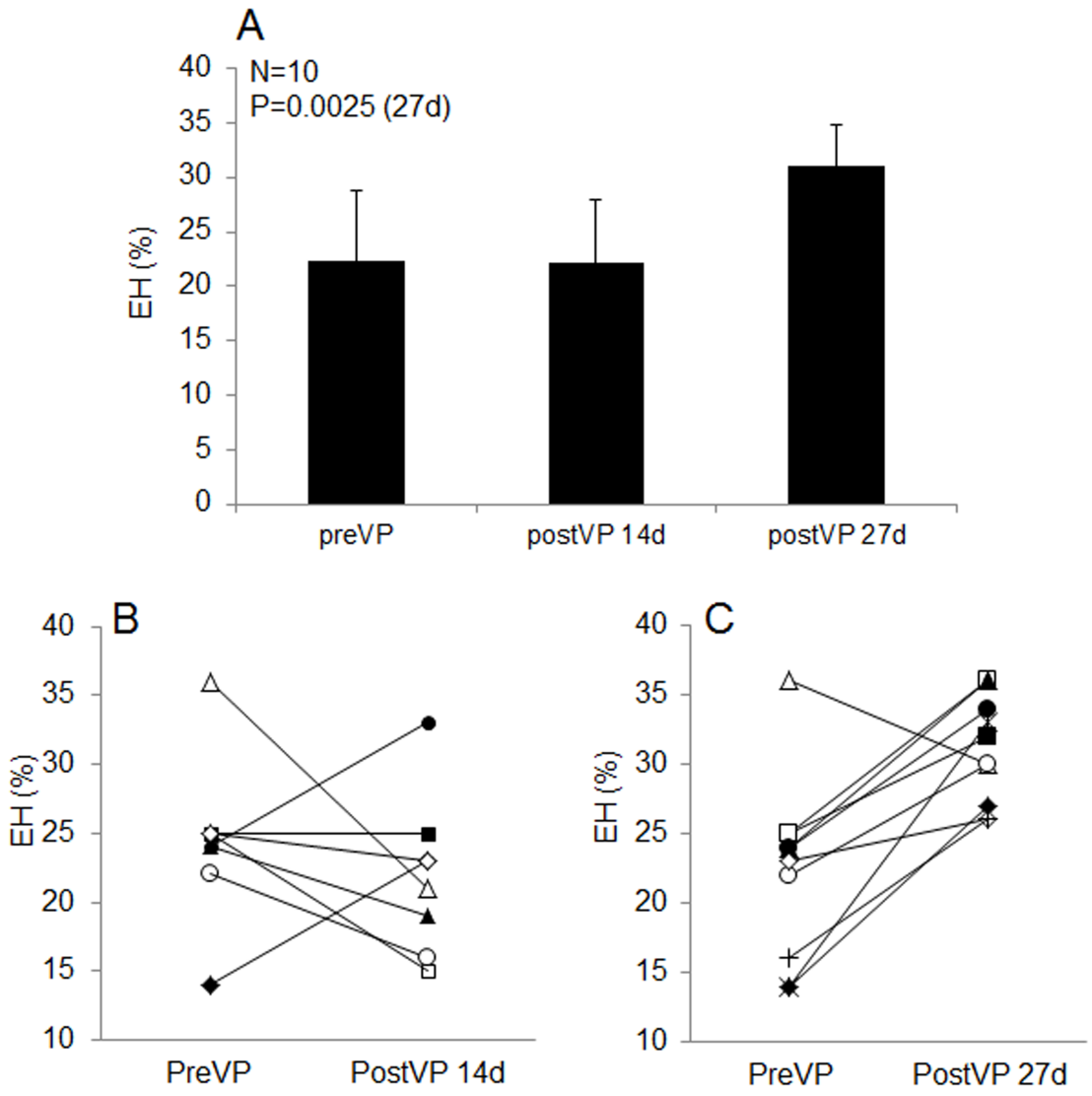
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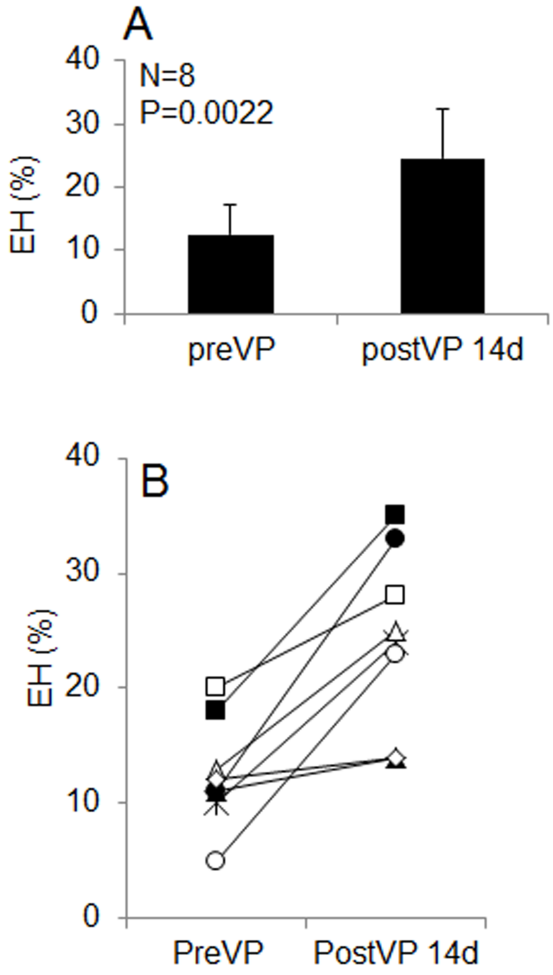
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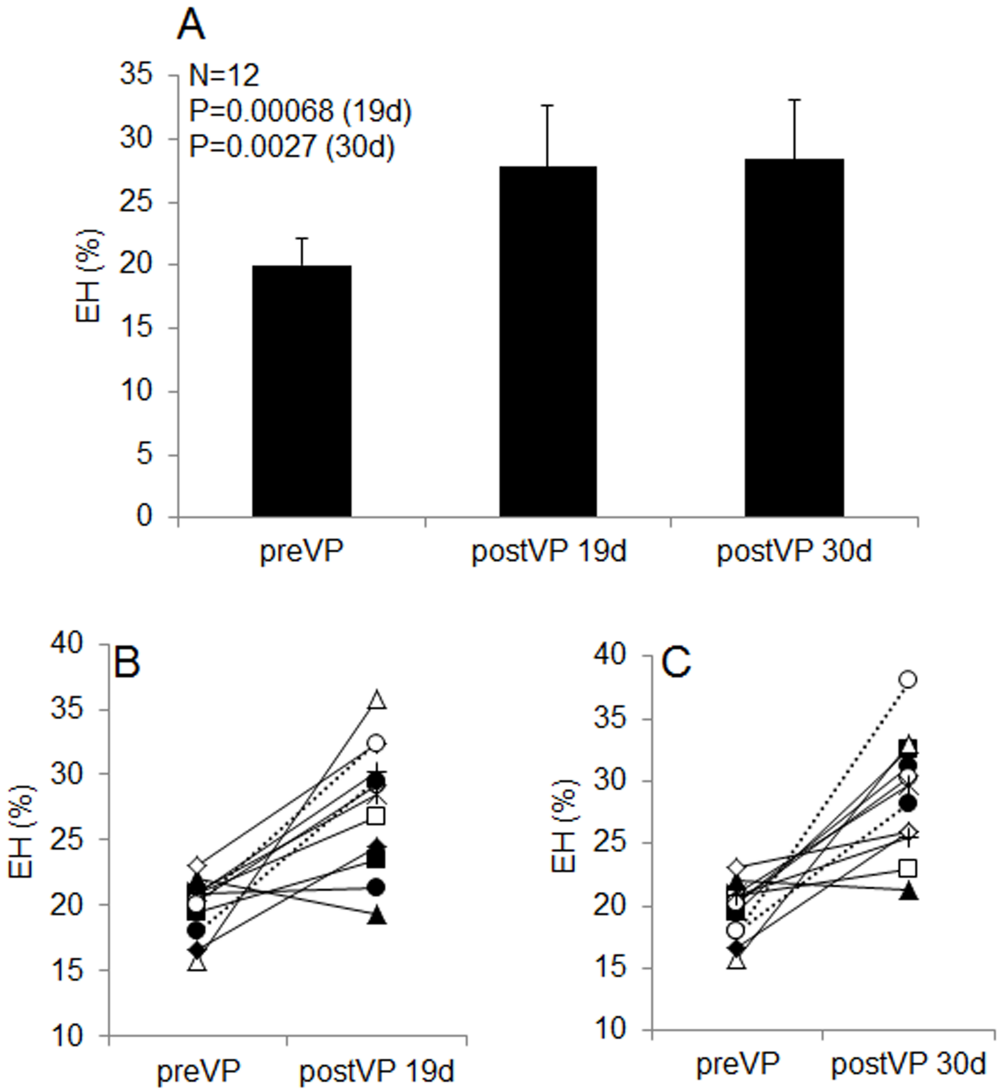
40
30
20
10
0
PreVP
PostVP 14d


A
N=12
P=0.00068 (19d)
P=0.0027 (30d)
EH (%)
35
30
25
20
15
10
5
0
preVP
postVP 19d
postVP 30d
B
EH (%)
40
35
30
25
20
15
10
preVP
postVP 19d
C
EH (%)
40
35
30
25
20
15
10
preVP
postVP 30d