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Review

Advances in research on labyrinth membranous barriers

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Abstract

Integrity of the membranous labyrinth barrier system is of critical importance, which promotes inner ear homeostasis and maintains its features. The membranous labyrinth barrier system is divided into several subsets of barriers which, although independent from each other, are interrelated. The same substance may demonstrate different permeability characteristics through different barriers and under different conditions, while different substances can have different permeability features even in the same barrier under the same condition. All parts of the membranous labyrinth barrier structure, including their morphology, enzymes and channel proteins, and theirs permeability characteristics under various physiological and pathological conditions are reviewed in this paper. Infections, noise exposure, ototoxicity may all increase permeability of the barriers and lead to disturbances in inner ear homeostasis.

Keywords: Membranous labyrinth; Barrier; Permeability; Inner ear homeostasis

Contents

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1. Introduction

Structures of biological barriers often control their permeability and restrict penetrance of substances across the barrier by various mechanisms to maintain stability in the barrier environment. Such barriers especially serve to protect the dynamic balance of inner ear fluids. Inner ear homeostasis depends on the dynamic equilibrium of inner ear fluid secretion and absorption involving inner ear blood supply, peri- and endolymph, ion transport system and integrity of the membranous labyrinth barrier system. The membranous labyrinth is divide into different chambers by a barrier system, which separates the endolymph, perilymph, cerebrospinal fluid (CSF) and serum. Previous studies have proposed that membranous labyrinth barriers are blood-labyrinth barriers (BLB), but failed to clearly explain permeability characteristics of each barrier (Hawkins, 1973; Juhn and Rybak, 1981; Ge, 1989; Yamasoba et al., 1996a; Liu et al., 2013; Hirose et al., 2014; Li et al., 2014; Wu et al., 2014; Zhang et al., 2015). In this review, we propose that the so called BLB (or intra-ear fluid barrier) consists of the blood–endolymph barrier, blood–perilymph barrier, cerebrospinal fluid–perilymph barrier, middle ear–labyrinth barrier and endolymph–perilymph barrier. All these barriers can be called membranous labyrinth barriers. Each barrier permits penetrance by different substances, which can be employed to administrate drugs into the inner ear.

2. Morphological basis of membranous labyrinth barriers

2.1. Blood–endolymph barrier

The blood–endolymph barrier is seen in the stria vascularis. Hawkins first proposed the concept of blood-labyrinth barrier (BLB) in 1960 and re-emphasized the importance of this barrier when he researched mechanisms of aminoglycoside antibiotics induced ototoxicity in 1973 (Hawkins, 1973). He pointed out that functions of this barrier relied on the integrity of the stria vascularis and spiral ligament (as the stria vascularis, Reissner's membrane and spiral limbus secrete endolymph), and also on the integrity of endolymph spiral prominence, external sulcus, endolymphatic sac, which absorb endolymph. Hawkins believed that aminoglycosides induced pathological changes of the above mentioned structures, leading to disturbed endolymph secretion/absorption balance, followed by dysfunction of the membranous labyrinth and protein synthesis and compromised inner ear homeostasis, as the mechanisms of aminoglycoside ototoxicity. Juhn and Rybak (1981) proposed that substances being transported into the labyrinth may involve simple diffusion, ultrafiltration, osmosis, lipid solubility, special tissue affinity and metabolic activities of inner ear tissues. Sakagami et al. (1984) found that, in the stria vascularis, endolymph barrier structures constituted of tight junctions of marginal cells, and perilymph barriers of tight junctions of basal cells. The space between the two barriers is called the vascular space, which is further sealed off by tight junctions of spindle cells at the junction of the stria vascularis and vestibular membrane, as well as at the spiral prominence. Zhang et al. (2012), Neng et al. (2013a), Neng et al. (2013b) found large number of perivascular-resident macrophage-like melanocytes (PVM/Ms), perivascular cells and F4/80 + GST + melanocyte-like macrophages inside the barrier space. Epithelium derived factor (PEDF), a 50-kDa glycoprotein, is expressed in primary cultured PVM/Ms, and affects the expression of tight junction associated proteins, whereas PEDF receptor (PEDFR) is expressed in primary cultured endothelial cells (ECs). Studies implicate PEDF signaling between PVM/Ms and ECs as an important mediator of the effect PVM/Ms have on expression of tight- and adherens-junction proteins such as occludin, ZO-1 and ve-cadherin. Wu et al. (2014) indicated that a large number of tight junction proteins (TJs), including mainly Claudin-5 and Occludin, contributed to the integrity and permeability of BLB by connecting adherens proteins in pericytes and other TJs. Using rt-PCR and western blot, Neng et al. (2013a) found that signals secreted from either pericytes or PVM/Ms had an effect on the expression of TJs, directly linking pericytes and PVM/Ms with a mechanism that accounts for the changes in endothelial barrier permeability and increase of fluorescent antigen exudation from EC monolayer. Weber et al. (2001) found that Sodium-potassium-chloride cotransporter (NKCC) was present in the basolateral membrane of strial marginal cells as well as in type II, type V and limbal fibrocytes, which maintain K^+ and Na^+ homeostasis in the human cochlea. Deficiency of NKCC leads to compromised endolymph translation from marginal cells, reducing endolymph potential (EP) while increasing permeability of BLB which allows more water molecules and other substances into endolymph, resulting in endolymph hydrops. Yang et al. (2011) delineated that 625 proteins from isolated stria vascularis capillaries were identified in adult CBA/CaJ mouse cochlea. Na^+/K^+-ATPase a1 (ATP1A1) is the most abundant protein in the stria vascularis capillaries directly interacting with PKCg, an essential mediator of ATP1A1-initiated occludin phosphorylation, and is involved in the integrity of the BLB. The physiological and morphological basis of stria vascularis, called “sandwich-dissociation”, is comprised of a dense capillary network, indicating that endothelial cells, surrounding pericytes, PVM/Ms, TJs, PEDF/PEDFR, NKCC, ATP1A1 and PKCg kinase all participate in forming blood–endolymph barrier, which prevents some materials in blood from entering endolymph while allows others to pass. The permeability of this barrier, however, is very weak under physiological conditions.

2.2. Blood-perilymph barrier

By testing penetration of [3H] taurine (molecular weight 125) into the scala vestibule perilymph (PLV) at 1 and 2 h after intravenous infusion in nephrectomized animals, Angelini et al. (1998) found that blood–perilymph barrier was similar to blood–brain barrier. They concluded that there was a passive entry of taurine (as a tracer) into the perilymph

through the blood–perilymph barrier, as with urea (molecular weight 60) and mannitol (molecular weight 186) reported previously in rats. Laurell et al. (2008) evaluated function of blood–perilymph barrier and found no increased permeability for the used tracer mannitol, which is a low molecular-weight marker (182 Da) of paracellular transport and readily transported to the perilymphatic compartment, and no effect on the concentration of K^+ and Na^+ of the PLV by exposure to impulse noise of 160 db SPL_{peak}. But they did not describe the constituent of blood-perilymph barrier. The spiral ligament, spiral limbus, modiolus and osseous spiral lamina participate in forming the blood-perilymph barrier rather than a single semipermeable membrane (Hirose et al., 2014). Studies have found that this barrier allows penetration of taurine, urea, mannitol, cationic polyethyleneimine (PEI), tetramethylphenylammonium (TMPA), steroid hormones, ototoxic drugs and lipopolysaccharides (LPS) into the PLV (Zhang et al., 2015; Angelini et al., 1998; Laurell et al., 2008; Suzuki et al., 2002).

2.3. Cerebrospinal fluid–perilymph barrier

The cerebrospinal fluid–perilymph barrier consists of the cochlear aqueduct, which connects cerebrospinal fluid (CSF) of the subarachnoid space to perilymph of the scala tympani and provides the possibility of interrelation between the two fluid-containing compartments. Juhn et al. (1989) investigated distribution and metabolism of leukotriene C4 (TLC4), which can be metabolized into LTD4 and LTE4 in the CSF, and found that LTE4 was transported into blood for systemic circulation and uptake into the liver and kidneys. Conversion of LTC4 into LTD4 and LTE4 was lower in the perilymph as compared to the CSF, suggesting a rate limiting function of the cochlear aqueduct that can be defined as a cerebrospinal fluid–labyrinth barrier. Salt et al. (2015) studied reduction of dextran concentration caused by dilution from CSF entering the cochlea at a very low rate of flow (~30 nL/min) when the cochlea was in its normal, sealed state and confirmed that this is an important process that can have a major influence on the pharmacokinetics of some substances in the perilymph in the basal turn of scala tympanic. Transport of substances such as urea and hypertonic sodium chloride solution from CSF to perilymph are related to the pressure and osmolality on both sides of the barrier, which may have little influence on perilymph under physiological conditions.

2.4. Middle ear–perilymph barrier

The middle ear–perilymph barrier is a semipermeable membrane called round window membrane (RWM), which can be divided into three layers at a total thickness of about 0.7 um. The outer and the inner layers are made up of epithelial cells while the middle layer of connective tissue containing vascular and nerve fibers. Glycerol monooleate-based nanoparticles (GMO-based NPs) and cationic ferritin are found to pass quite easily through the normal RWM, but not anionic materials (Goycoolea and Lundman, 1997; Park and Moon, 2014). Nanoparticles as gene delivery vectors may be used to transport drugs to targeted inner ear cells in gene therapy for sensorineural deafness. Small molecular weight substances such as sterols, aminoglycoside antibiotics, 1 μ latex microspheres can pass through RWM under physiological conditions, but high molecular substances such as albumin (molecular weight 67,000) can only penetrate inflamed RWM (Goycoolea and Lundman, 1997; Park and Moon, 2014; Matsubara et al., 2014). Large amount of eosinophil infiltration in the scala tympani has been found 28 days after ovalbumin (VOA) is injected into the tympanic cavity. Leukotrienes (LTs) such as LTC4/LTB4 can penetrate RWM, leading to dilation of capillaries (Park and Moon, 2014; Matsubara et al., 2014). Sasa et al. (1989) found that pure oxygen, insufflated into the middle ear cavity, easily permeated the round window membrane and elevated perilymphatic oxygen tension by 319%. Salt and Ma (2001) studied substance penetration through the RWM to the scala tympani perilymph, and noticed a substance concentration gradient from the bottom to helicotrema of the cochlea. Ghossaini et al. (2013) used golimumab, a TNF-α blocker, as a local delivery into the inner ear to assess the RWM permeability in guinea pigs. They found that golimumab crosses the RWM and is detected in measurable concentrations in the inner ear fluid after 30 min of exposure to the membrane, but can't explain its pharmacokinetics and optimal concentration. The liposomes are considered to be most successful drug-carrier system to deliver drugs into inner ear (Bozzuto and Molinari, 2015; El et al., 2015). A variety of substances placed in the middle ear, including antibiotics, local anesthetics, tracers such as cationic ferritin, horseradish peroxidase, sterols, GMO-based NPs, golimumab, pure oxygen and 1 μ latex microspheres, can be detected across the RWM. Selective permeability of the RWM is determined by factors including the size, concentration, liposolubility and electrical charge of the substance, as well as the thickness of the membrane.

2.5. Endolymph–perilymph barrier

Epithelial cells of the Reissner membrane (RM) form the endolymph–perilymph barrier. The RM is located between the scala vestibule (SV) and scala tympani (ST) and attached to the osseous spiral lamina at the upper boundary of the stria vascularis. Epithelial cells facing the endolymph come from the ectoderm, have irregular shapes and a smooth surface, are arranged in a tight pattern, and show high levels of ATP-ase activities. Epithelial cells facing the perilymph come from the mesoderm, are flat in shape and contain oval nuclei. Scanning electron microscopy shows that on both sides of the membrane, there are microvilli on cell surfaces and pinocytosis vesicles in cytoplasm, indicating that RM participates in regulation of ionic equilibrium in the endolymph and perilymph. Using patch-clamp technique, Yeh et al. (1997) found three types of ion channels on the apical membrane of epithelial cells facing the endolymph in isolated Reissner's membranes from guinea pigs, namely, stretch-activated

nonselective cation channels, chloride channels and potassium channels. Immunolabeling shows that aquaporins (AQPs), i.e. AQP2, AQP7 and AQP9, are expressed in Reissner's membrane. They may work in concert to regulate endolymph electrochemical equilibrium and maintain homeostasis in the inner ear (Zhong and Liu, 2003; Huang et al., 2002).

3. Clinical significances of membranous labyrinth barriers

3.1. Noise exposure

Noise exposure (NE) is a major health hazard in modern time and can induce hearing impairment. Noise-induced ultrastructural changes of membranous labyrinth barriers lead to NE-dependent hearing impairment. In 1971, Hawkins (Hawkins, 1971) found that noise-induced hearing impairment was related with edema of stria vascularis and spiral ligament, vasoconstriction and aggregation of erythrocytes. By examining systemically administered cationic polyethyleneimine (PEI), Mitsuya et al. (Suzuki et al., 2002) showed that noise exposure increased macromolecular transport in the stria vascularis but not in the spiral ligament, spiral limbus or basilar membrane and that some macromolecules were readily transported through Reissner's membrane. They suggested that NE might increase transport of PEI particles through the basal lamina (BL) of Reissner's membrane. There are two possible routes of access to the BL of Reissner's membrane from the strial vessels: (1) through the epithelial cell surface of Reissner's membrane via the endolymph, and (2) through the superior portion of the stria vascularis at the point of attachment to Reissner's membrane. This finding suggests that an increase in strial vascularis permeability, induced by NE, may not result in increased accumulation of PEI particles in the endolymph. Wu et al. (2014) suggested that a dose-dependent NE caused a decrease of Claudin-5 and Occludin, significant outer hair cell (OHC) loss and increased permeability of membranous labyrinth barriers. Transport of cationic ototoxic drugs such as aminoglycoside and platinum from strial vascularis to cochlear tissues and OHCs is also accelerated (in both time and quantity), proportional to concentrations of the drug. But Laurell et al.'s findings (Laurell et al., 2008) through estimating paracellular transport of radioactive mannitol into scala vestibule perilymph (PLV) and electrolyte concentrations in perilymph contradicted the theory of increased permeability of the BLB as a result of extensive noise exposure. However, in most cases, noise exposure can induce hearing impairment of various degrees.

3.2. Infections

Lipopolysaccharide (LPS), an important component of bacterial endotoxin seen in Gram-negative bacterial infections, increases permeability of membranous labyrinth barriers because it can penetrate the RWM to appear in perilymph and cause nitric oxide synthase expression that leads to loss of TJs, disrupted continuity of ECs and infiltration of inflammatory cells and factors in the strial vascularis and spiral ligament. Zhang et al. (2015) showed that LPS not only induced middle ear infections, but also caused structural changes in the intra-strial fluid–blood barrier. Damage to this barrier has been linked to NE-dependent hearing impairment, autoimmune inner ear disease, presbycusis and gene-related inner ear diseases. Pathogenic mechanisms related to LPS may include: 1) effects on PCs and PVM/Ms in the barrier, causing PCs to migrate and release particles, as well as activation of PVM/Ms; and 2) significant down-regulation of ZO-1, occludin, and ve-cadherin expression, subsequently leading to barrier leakage. Virus infections can also lead to inner diseases. In mice infected with murine cytomegalovirus (MCMV) and showing hearing loss, distribution of tumor necrosis factor (TNF)-a, interleukin (IL)-6 and lymphocytes increase in the scala tympani and Reissner's membrane (Yuehua et al., 2012). Infection of MCMV results in hyperemia of strial vascularis and spiral ligament and hemorrhage of ST and SV (Yuehua et al., 2012; Li et al., 2014). Studies suggest that bacterial and viral infections not only lead to systemic inflammatory reactions but also inner ear diseases, especially hearing loss. These findings mandate use of medications that target the pathogen with effective penetration into the inner ear and low possibility of ototoxicity when treating infections.

3.3. Drugs

Ototoxic drugs can enter the inner ear and cause damage to membranous labyrinth barriers, leading to hearing impairment and vestibular dysfunction as a result of toxicity to hair cells of the cochlea and vestibular organs. Tatsuya Yamasoba et al. (1996b) suggested that chronically administered kanamycin (cationic tracer) could selectively, progressively and irreversibly affect anionic sites in the stria vascularis and basilar membrane near the spiral limbus, resulting in disruption of barrier function in the cochlea. Keiko Hirose et al. (2014) found that LPS-pretreated mice showed greater long term auditory threshold shifts and more extensive outer hair cell loss following combined kanamycin-furosemide treatment when compared with animals receiving ototoxic agents alone. Changes in vascular permeability within the inner ear may play a critical role in LPS-induced exacerbation of ototoxicity and further studies are necessary to explore this possibility. Takehisa Saito et al. (1997) reported P-glycoprotein (P-gp), a multidrug resistance (*mdr*) gene product found in multidrug-resistant tumor cells, expressing in inner ear capillary endothelial cells that might play an important role in the blood–inner ear barrier by acting as an extrusion pump that prevents many substances such as aminoglycoside and antitumor drugs from entering the inner ear. Expression of P-gp is low in the *mdr1a* (yry) mouse and the absence of P-gp results in elevated drugs (adriamycin and vincristine) levels in many tissues leading to endolymphatic sac dysfunction and impaired endolymphatic absorption, and eventually endolymphatic hydrops. Liu et al. (2013) investigated the impact of lead (Pb^{2+}) on the auditory system including ABR thresholds

and its molecular mechanisms. Down-regulation of occludin, ZO-1 and claudin-5 in the stria vascularis suggested that increased permeability of the blood–endolymph barrier could attribute to Pb^{2+}-induced decline of TJPs expression. But Wu et al. (2011) found that lead primarily damages cochlear nerve fibers and SGN rather than hair cells. Di et al. (2011) studied that styrene ototoxicity in animals mainly disrupt cochlear cells (outer hair cells). Further studies about whether if styrene damage inner ear barrier or not, it will be investigated. Long time accumulation and impaired expulsion of ototoxic drugs in the inner ear can cause damage to inner ear membranous labyrinth barriers via a variety of mechanisms. Understanding of these mechanisms may guide clinical practice, so prolonged use of combination of potentially ototoxic drugs can be avoided, ototoxic side effects of drug treatment monitored, and appropriate measures taken promptly when indicated.

Osmotic diuretics can enter the inner ear by various mechanisms and have different effects on the inner ear. Juhn and Rybak (1981) in an animal study found that injected osmotic agents, glycerol and urea, appeared in the perilymph to a considerable degree. Using ferritin as a tracer, Ge (1989) showed that glycerol could serve as an opener of blood-labyrinth barriers. However, mannitol injection resulted in unexpected findings, i.e. perilymph osmolality remained higher than that of the serum, suggesting that mannitol did not have any effect of drawing water from the labyrinth into the blood. Also, the magnitude and duration of the partial oxygen pressure increase in perilymph have been found to be larger after administration of glycerol than after that of mannitol (Yoshida and Uemura, 1991). These results correspond well with the clinical experience that glycerol is more effective than mannitol in improving hearing in Meniere's disease (MD). The concentration of furosemide, an ototoxic diuretic, measured by high pressure liquid chromatography, is fairly constant at the time of full recovery of EP after doses of *50–200* mg/kg (Juhn and Rybak, 1981). Kim et al. (2014) found that isosorbide, small in size (0.76 nm in diameter) and light in molecular weight (146.14 g/mol), rapidly passed through the RWM after round window perfusion (RWP) in guinea pigs. Perfusion of 30 min is considered to be appropriate and, over a 6-h period, delivers the agent at 7 times higher concentrations than those achieved with oral administration. Therefore, local application of isosorbide, which can deliver high dose without systemic adverse effects, is considered to be promising for the treatment of MD. Osmotic diuretics can pass through the blood–endolymph barrier and/or middle ear–perilymph barrier to perilymph but not endolymph, as shown by some studies. More investigations should analyze their base-to-apex concentration gradients through the entire perilymph and the time course and durations of their actions. Further studies are also needed to study their concentrations not only in perilymph, but also in endolymph and serum.

Steroids can reduce inflammation, immune over-reaction and edema and improve microcirculation in the inner ear. They are widely used in the treatment of inner ear diseases. However, long-term oral steroids are known to carry systemic side effects. Local application of drugs may increase their concentrations in the inner ear and greatly reduce their systemic side effects. Bachmann et al. (2001) demonstrated that high levels of prednisolone-21-hydrogen succinate in perilymph were achievable by local application of a single dose into the round window niche than by intraperitoneal injection. Applying steroids to treat cochleovestibular disorders, such as sudden hearing loss, is a common clinical practice, with various clinical efficacy and side-effect profiles. Novel drug delivery techniques such as the Silverstein MicroWick, round window microcatheter, biodegradable hydrogels, biopolymers, nanoparticles, cochlear implant arrays, osmotic mini/micro pumps, and reciprocating perfusion systems hold significant promise. These sustained delivery systems provide more effective inner ear pharmacokinetics than systemic administration by animal data.

4. Conclusions

Each part of the membranous labyrinth barriers has different functional and morphological bases, and demonstrates variable permeability to different substances under various physiological and pathological conditions. Under physiological conditions, permeability of the blood-endolymph barrier is low and allows passage of only a small amount of water molecules, Na^+, K^+, Cl^-, and ototoxic drugs. Water molecules, steroids, osmotic diuretics (such as glycerin, mannitol and isosorbide) and some ototoxic drugs (such as aminoglycoside antibiotics, platinum antitumor drugs and furosemide) can pass through the blood–perilymph barrier, although its permeability is less than that of the middle ear–perilymph barrier which also allows passage of horseradish peroxidase, cationic ferritin, and 1 μm latex microspheres. Substances in the CSF, including certain ions, proteins and leukotrienes, can pass through the CSF-perilymph barrier. The endolymph-perilymph barrier allows penetration by water molecules and steroids but not ototoxic drugs or osmotic diuretics (such as glycerin, mannitol and isosorbide). Under pathological conditions, the permeability of most of these barriers increases, although the RWM can be thickened by chronic inflammation resulting in decreased permeability. Combination of some drugs may increase their penetration through membranous labyrinth barriers, leading to potentially enhanced passage of therapeutic drugs into the inner ear but also increased ototoxicity. Inner ear barriers deserve further assessment of their structures and functions for potential clinical significances.

In this review, we have proposed the concept of distinct labyrinth membranous barriers and described characteristics of their permeability, which can influence functional homeostasis and drug distributions in the inner ear. Pharmacokinetics of various drugs in the inner ear in relation to continuous delivery systems need to be studied in animal models for potential clinical applications that may provide improved treatment efficacy and reduced adverse effects. Models of drug delivery systems for inner ear diseases such as sensorineural hearing loss, tinnitus and vestibular disorders are forthcoming and will guide practice and research into a brand new age for otology.

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