

**HYPERBILIRUBINEMIA AND AUDITORY NEUROPATHY***Zhao Lidong<sup>1</sup>, Wei Xiaoquan<sup>1,2</sup>, Cong Tao<sup>1</sup>, Guo Weiwei<sup>1</sup>, Lin Chang<sup>2</sup>, Yang Shiming<sup>1</sup>***Introduction**

Hyperbilirubinemia is common in the new born owing to their special metabolism characteristics. Physiologic bilirubin levels are protective because of their antioxidant effects<sup>[1]</sup>, while pathological jaundice is harmful to the health of the neonate. Previously, most pathological studies on the jaundice of neonates have concentrated on central nuclei in the auditory brainstem. Later, epidemiological investigation has demonstrated that the pathology is highly related with auditory neuropathy. That is to say, hyperbilirubinemia can also cause injury to peripheral hearing pathways. The present review mainly illustrates the neurotoxic effects on the hearing system by bilirubin and treatment aspects of auditory neuropathy.

**Metabolism of Bilirubin**

Bilirubin is a kind of pigment containing tetrapyrrole structure and the final decomposition product of proteins containing heme, such as hemoglobin, myoglobin and cytochrome. Average daily production of bilirubin is about 250–350 mg in adults. When the red blood cells are engulfed and digested by mononuclear macrophages or reticuloendothelial cells, heme is released from hemoglobin and turned into biliverdin under the action of various enzymes (heme oxygenase, NADPH, cytochrome c reductase) in the cytomicrosome. Biliverdin is then turned into unconjugated bilirubin (UCB) by biliverdin reductase. UCB is fat-soluble and is carried to the liver when bound with plasma albumin. In the smooth surfaced endoplasmic reticulum, UCB is turned into conjugated bilirubin under the catalysis by UDP-glucuronosyl transferase (UDPGT). Conjugated bilirubin is water-sol-

uble and can be filtrated from the glomerulus and then exhausted in the urine. Under normal circumstances, only a small part of unconjugated bilirubin is not combined with albumin which is not harmful to the nervous system.

**Hyperbilirubinemia and Injury of Bilirubin to the Hearing System**

Albumin can act as the carrier of UCB and prevent bilirubin from penetrating cell membrane when they combine together. The concentration of albumin is usually far higher than that of bilirubin. When they combine tightly at the ratio of 1:1, the concentration of free bilirubin does not rise. Under some situations, such as when red blood cells break in large scales as in the new born baby whose liver DUPGT is not mature with less than fully developed blood-brain-barrier, or as in pathological hemolysis or use of certain drugs (sulfa drugs, non-steroidal anti-inflammatory drugs, biliary contrast agent and free fatty acid) that compete for binding to plasma albumin, increase of plasma UCB can happen in the newborn baby's body. As UCB is not only lipid-soluble, but also able to pass through the blood-brain barrier and cell membrane, its' neurotoxic property can lead to neuronal damage.

Bilirubin is harmful at multiple sites in the auditory system, including central auditory nuclei such as dorsal and ventral cochlear nucleus, superior olivary nucleus, trapezoid body and lateral lemniscus<sup>[2]</sup>. Long exposure to bilirubin can result in extensive neuronal injury in the hearing system. Previous studies suggested that the injury was confined to the central auditory system and that the cochlea and the auditory nerve were not affected<sup>[3-5]</sup>,

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but latest epidemiologic study evidence revealed that hyperbilirubinemia was highly associated with auditory neuropathy and that the incidence is positively correlated with bilirubin concentration in blood<sup>[6]</sup>. Studies on the peripheral hearing system of Gunn rats with nuclear jaundice revealed that spiral ganglion neurons showed severe degeneration, especially those in large nerve fibers with myelin, while no morphological change was observed in hair cells<sup>[7]</sup>. Ye et al (2012) demonstrated that bilirubin could cause demyelination and nerve fibers decrease in the Habenula perforate. The synapses between hair cells and afferent nerve ends were also damaged without visible change in the inner or outer hair cells<sup>[8]</sup>. Although nobody has demonstrated that bilirubin affects the excitement of spiral ganglion neurons, injury on the SGN by bilirubin will inevitably cause neuronal dysfunction, leading to mild to profound hearing loss based upon exposure time and intensity<sup>[2]</sup>. The reason why SGN is particularly sensitive to the toxic effects of bilirubin is unclear.

## **Molecular Mechanisms of Bilirubin Neurotoxicity**

### *Oxidative Stress*

The level of membrane bound hemoglobin in the blood, regarded as the symbol of oxidative stress, is higher in moderate jaundice infants compared with mild jaundice infants<sup>[9]</sup>. In vitro, UCB disrupts normal reduction-oxidation (REDOX) state in the mitochondria of neurons, which results in significantly higher levels of reactive oxygen species (ROS), protein oxidative products and lipid peroxidation products<sup>[10-11]</sup>. Besides, oxidative stress also leads to damage to synapses<sup>[12]</sup>, as well as DNA and therefore reduces cell proliferation<sup>[13]</sup>, which is related with the down regulation of glutathione (GSH) and the dynamic decline of DJ-1 protein. As a kind of low molecular weight mercapto compound, glutathione (GSH) is abundant in neurons and plays important roles in antioxidant. Bilirubin can decrease GSH concentration and its function and cause neurotoxicity<sup>[13]</sup>, which will in turn aggravate injury to the mitochondria and cause out of control oxidative stress<sup>[14]</sup>. What's more, GSH concentration in neuron is lower than in astrocyte, which results in more ROS, protein oxidative products and lipid peroxidation products than in astrocyte<sup>[15]</sup> and apoptosis or necrosis of affected neuron. The DJ-1 protein, a kind of atypical peroxidase body in the cell, is capable of removing H<sub>2</sub>O<sub>2</sub> and resisting ROS activity<sup>[16]</sup>, especially in the case of H<sub>2</sub>O<sub>2</sub> increase<sup>[17]</sup>. Moreover, DJ-1 is a sensor of ROS in cells and will over express in the case of oxidative stress and declined function of pro-

teasome. It protects neurons through oxidizing harmful cysteine residues. Neurons that don't express DJ-1 are apt to undergo apoptosis when they are exposed to UCB<sup>[17]</sup>, which suggests that DJ-1 is able to protect neurons under the oxidative stress induced by bilirubin. Long term culture with UCB down regulates DJ-1 expression, which may be part of the reason why neurotoxicity increases with time extension in jaundice<sup>[18]</sup>.

### *Neural Excitotoxicity*

Beside oxidative stress, neuronal apoptosis is also related with excitotoxicity. UCB may inhibit the uptake of excitatory neurotransmitter glutamate in the central nervous system<sup>[19]</sup>, which results in the accumulation of glutamate between the synapses and leads to over activation of glutamate receptors including N-methyl-D-aspartate (NMDA) receptors. Many studies have demonstrated that NMDA receptor inhibitors may relieve bilirubin induced neural excitotoxicity and neuron function decline<sup>[20-22]</sup>. In addition, bilirubin induces increased release of glutamate from presynaptic membrane and activates both NMDA and AMPA receptors, which in turn cause continuous firing of post synapses neurons and excessive Na<sup>+</sup> and Ca<sup>2+</sup> entering post synapses neurons through receptor-gated channels along with Cl<sup>-</sup> and H<sub>2</sub>O<sup>[23]</sup>. The overloaded Ca<sup>2+</sup> binds with calmodulin (CaM), activates many Ca<sup>2+</sup>-CaM dependent protein kinases and signaling pathways, results in wide phosphorylation of signaling molecular and activation of a number of enzymes, e.g. esterase (including arachidonic acid metabolites), phosphokinase A, phosphokinase C, etc., which then launch a series of gene expression and formation of oxygen free radicals<sup>[24]</sup>. When exposed to bilirubin, expression of Calbindin-D28k and parvabumin in the bilirubin sensitive cochlear nucleus and superior olivary nucleus complexes is down regulated<sup>[25]</sup>. As Calbindin-D28k can activate Ca<sup>2+</sup>/Mg<sup>2+</sup>-ATP and block excessive accumulation of Ca<sup>2+</sup> in the brain by buffering Ca<sup>2+</sup> in the neuron. Decrease of calmodulin aggravates the overloading of Ca<sup>2+</sup>. In addition, in the bilirubin exposed neuron, the activity of Calmodulin kinase II is inhibited<sup>[26]</sup>, which will inevitably lead to impairment of the central nervous system where the signaling transmission is mainly dependent on Calmodulin kinase II. Along with the accumulation of these ions and changes of gene expression, post synapses neurons become swollen, and the integrity of cellular organs and membranous structures are ruined. Cytoplasm contents are then released into the extracellular space and, finally, neurons damaged.

UCB is able to swiftly increase tumor necrosis factor receptors (TNFR1) on the surface of astrocytes and oli-

godentoglia and induce inflammatory reactions, as well as apoptosis<sup>[27-28]</sup>. UCB may also activate nuclear factor  $\kappa$ B (NF- $\kappa$ B) and/or mitogen-activated protein kinase (MAPK) signaling pathway. NF- $\kappa$ B, as a transcription factor, is decisive in UCB induced astrocyte activation and/or cytotoxicity, and is able to induce production of some cellular factors such as TNF- $\alpha$ , IL-1 $\beta$ , IL-6, etc. MAPK is a regulator in inflammatory reaction by astrocytes<sup>[29]</sup>, involved in the expression of many cell factors<sup>[30]</sup>. Excessive production and releasing of NF- $\kappa$ B and MAPK may injure glial cells and neurons during the process of UCB neurotoxicity<sup>[28]</sup>. Besides, the microglia is also an important inflammation induction cell in the central nervous system<sup>[31]</sup> that reacts to stimulation by UCB and involved in the disintegration of neurons by releasing glutamate and NO. Therefore, there are multiple mechanisms involved in the neurotoxicity of bilirubin. We propose that they may work at different sites of a reaction cascade or via different signaling pathways triggered by bilirubin. These pathways may promote and influence each other and in the end lead to the degeneration, apoptosis or even necrosis of the delicate tender neurons.

### **Prevention and Treatment of the Auditory Neuropathy Induced by Hyperbilirubinemia**

It is a consensus nowadays that hyperbilirubinemia is harmful to the auditory system. There are usually two or more risk factors to the hearing that are simultaneously present in a newborn baby, e.g. premature birth, sepsis, and Rh hemolysis. In this case, even slight increase of bilirubin level may lead to acute ABR abnormality, with mild jaundice and neural function impairment<sup>[32]</sup>. All these risk factors have to be taken into consideration in order to prevent the occurrence of auditory neuropathy. Shapiro et al. (2003) stated that the prediction accuracy may be higher if one takes into account of the level of free bilirubin, UCB, total bilirubin, albumin and pH simultaneously<sup>[33]</sup>. With a more sensitive method to detect free bilirubin and a reliable risk threshold, the sensitive and specificity of utility of blood indices may be improved, which will be useful for the prevention of bilirubin toxicity<sup>[34]</sup>.

The Neonatal behavioral neurological assessment (NBNA), an index for observing behavioral ability with high sensitivity and specificity, is able to detect malfunction of the newborn brain. The Auditory brainstem response (ABR), commonly used in otological clinical work up is very sensitive in finding nerve impairment in many pathologic conditions such as brain injury, metabolic disorders, demyelination and bilirubin exposure. It

is capable of finding the effects of bilirubin on the neural system at an early stage, which usually demonstrates as wave interval elongation and decline of wave amplitude. The change of ABR will recover if the baby undergoes hemodialysis<sup>[35-36]</sup>. The maximum length sequence brainstem auditory evoked response (MLS BAER) is also used for monitoring CNS injury in neonates, which is more sensitive in detecting auditory pathway lesions caused by hyperbilirubinemia and helps decrease false negative rate than brainstem auditory evoked response (BEAR)<sup>[37]</sup>.

Early diagnosis and treatment is very important for language and intelligence development in babies with impaired hearing. Hearing screening in neonatal babies has been used in the clinic, which usually includes transient evoked otoacoustic emissions (TEOAEs) and auto auditory brain-stem responses (AABRs). Studies show that babies with abnormal results of any one or both of these two screening methods correlate to the same occurrence of hearing loss and warrant follow-up monitoring. Although AABR is easy to implement, it does not effectively assess low frequency hearing ability. Sometimes, it is necessary to conduct joint screening with ABR and 40Hz auditory event-related potential (40Hz-AERP).

Treatment studies on Gunn rats showed that minocycline could protect neurons from acute neurotoxicity induced by bilirubin. The earlier the treatment, the better recovery of brainstem auditory evoked potential<sup>[38]</sup>. Glycoursodeoxycholic acid (GUDCA) and taurine were proved to be able to inhibit the inflammatory injury of UCB to the neuron<sup>[11, 39-40]</sup>. Thus monocycline, GUDCA and taurine may be useful and potentially supplemental therapy in treating hyperbilirubinemia in neonates when blue light is not sufficient.

Traditional hearing aids are not effective in treating hearing loss in auditory neuropathy patients as it is good in amplifying exogenous sound but the main problem in patients with auditory neuropathy is poor speech discrimination with relatively less severe hearing threshold deterioration. On the other hand, hearing aids may further injure patients' hearing because the amplified sound may be harmful to hair cells in the inner ear. Cochlear implantation, as a substitution therapy to severe or profound hearing loss, may be helpful in some patients with auditory neuropathy caused by hyperbilirubinemia, especially in premature infants with profound hearing loss caused by nuclear jaundice or in those with OTOF gene mutation. Although cochlear implant (CI) is helpful in some AN patients, it is expensive and requires sophisticated surgical operation. What's more, the sound perceived by the patients via CI is often distorted and un-natural. Thus the CI is not perfect for treating AN patients.

Presently, stem cells are a good candidate material in treating these patients, because they are able to differentiate into various tissues and cells in proper circumstances. Before treatment study, AN animal models have to be established. Schmiedt et al. (2002) have successfully set up a gerbil model with AN by applying ouabain to the round window membrane<sup>[41]</sup>. Further studies by Lang et al. showed that ouabain induced AN by selectively killing type I spiral ganglions but not type II neurons<sup>[42]</sup>. Subsequently, they transplanted mouse ESCs into the inner ear of the AN gerbil model and demonstrated that grafted stem cells could survive in the Roshenthal's canal and scala tympani and differentiate into neurons or glial cells<sup>[43]</sup>. One interesting research is translating B-5 mouse ESCs into the scala tympani of guinea pig cochleae. The cells survived and differentiated into glutamatergic neurons, indicating that stem cells may restore tissue structures and function of the auditory nerve as the afferent neurotransmitter of type I SGNs is glutamate<sup>[44]</sup>. Since then, inspiring results have been acquired by other groups. Cho et al. (2011) transplanted neural differentiated human mesenchymal stem cells into the cochlea of an AN guinea pig model and found that the number of SGNs increased and the hearing of the animals recovered<sup>[45]</sup>. Chen et al. (2012) transplanted human embryonic cell-derived otic progenitors to the inner ear of AN models which significantly improved animals' hearing<sup>[46]</sup>. These results suggest that stem cell transplantation is promising in treating AN. But extensive basic research has to be done to resolve many problems in this area, such as selecting the best routine to transplant stem cells into the inner ear and inducing directional differentiation and directional migration of stem cells. On the other hand, whether grafted stem cells would form tumor, and whether they would induce immunoreactions, as well as how to improve the efficiency and to lengthen the survive time of the grafted stem cells so that the treatment effects can last a long time, are also tough problems that confront scientists in this field.

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## References

- [1] Mireles, L.C., M.A. Lum, and P.A. Dennery, Antioxidant and cytotoxic effects of bilirubin on neonatal erythrocytes. *Pediatr Res*, 1999. 45(3): p. 355-62.
- [2] Shapiro, S.M. and G.R. Popelka, Auditory impairment in infants at risk for bilirubin-induced neurologic dysfunction. *Semin Perinatol*, 2011. 35(3): p. 162-70.
- [3] Gerrard, J., Nuclear jaundice and deafness. *J Laryngol Otol*, 1952. 66(1): p. 39-46.
- [4] Kelemen, G., Erythroblastosis fetalis. Pathologic report on the hearing organs of a newborn infant. *AMA Arch Otolaryngol* 1956 (63): p. 392-398.
- [5] Shapiro, S.M., Chronic bilirubin encephalopathy: diagnosis and outcome. *Semin Fetal Neonatal Med*, 2010. 15(3): p. 157-63.
- [6] Nickisch, A., C. Massinger, B. Ertl-Wagner, and H. von Voss, Pedaudiologic findings after severe neonatal hyperbilirubinemia. *Eur Arch Otorhinolaryngol*, 2009. 266(2): p. 207-12.
- [7] Shaia, W.T., S.M. Shapiro, and R.F. Spencer, The jaundiced guinea pig model of auditory neuropathy/dyssynchrony. *Laryngoscope*, 2005. 115(12): p. 2167-73.
- [8] Ye, H.B., H.B. Shi, J. Wang, D.L. Ding, D.Z. Yu, Z.N. Chen, C.Y. Li, W.T. Zhang, and S.K. Yin, Bilirubin induces auditory neuropathy in neonatal guinea pigs via auditory nerve fiber damage. *J Neurosci Res*, 2012. 90(11): p. 2201-13.
- [9] Brito, M., R. Silva, and D. Brites, Cell response to hyperbilirubinemia: a journey along key molecular events. In: Chen FJ, editor. *New trends in brain research*. NY: Nova Science Publishers, Inc, 2006a: p. 1-38.
- [10] Rodrigues, C.M., S. Sola, M.A. Brito, D. Brites, and J.J. Moura, Bilirubin directly disrupts membrane lipid polarity and fluidity, protein order, and redox status in rat mitochondria. *J Hepatol*, 2002. 36(3): p. 335-41.
- [11] Brito, M.A., S. Lima, A. Fernandes, A.S. Falcao, R.F. Silva, D.A. Butterfield, and D. Brites, Bilirubin injury to neurons: contribution of oxidative stress and rescue by glycoconjugated bilirubin. *Neurotoxicology*, 2008. 29(2): p. 259-69.
- [12] Brito, M.A., D. Brites, and D.A. Butterfield, A link between hyperbilirubinemia, oxidative stress and injury to neocortical synaptosomes. *Brain Res*, 2004. 1026(1): p. 33-43.
- [13] Deganuto, M., L. Cesaratto, C. Bellarosa, R. Calligaris, S. Vilotto, G. Renzone, R. Foti, A. Scaloni, S. Gustincich, F. Quadrifoglio, C. Tiribelli, and G. Tell, A proteomic approach to the bilirubin-induced toxicity in neuronal cells reveals a protective function of DJ-1 protein. *Proteomics*, 2010. 10(8): p. 1645-57.
- [14] Kang, Y., V. Viswanath, N. Jha, X. Qiao, J.Q. Mo, and J.K. Andersen, Brain gamma-glutamyl cysteine synthetase (GCS) mRNA expression patterns correlate with regional-specific enzyme activities and glutathione levels. *J Neurosci Res*, 1999. 58(3): p. 436-41.
- [15] Brito, M.A., A.I. Rosa, A.S. Falcao, A. Fernandes, R.F. Silva, D.A. Butterfield, and D. Brites, Unconjugated bilirubin differentially affects the redox status of neuronal and astroglial cells. *Neurobiol Dis*, 2008. 29(1): p. 30-40.
- [16] Kahle, P.J., J. Waak, and T. Gasser, DJ-1 and prevention of oxidative stress in Parkinson's disease and other age-related disorders. *Free Radic Biol Med*, 2009. 47(10): p. 1354-61.
- [17] Duan, X., S.G. Kelsen, and S. Merali, Proteomic analysis of oxidative stress-responsive proteins in human pneumocytes: insight into the regulation of DJ-1 expression. *J Proteome Res*, 2008. 7(11): p. 4955-61.
- [18] Vaz, A.R., S.L. Silva, A. Barateiro, A.S. Falcao, A. Fernandes, M.A. Brito, and D. Brites, Selective vulnerability of rat brain regions to unconjugated bilirubin. *Mol Cell Neurosci*, 2011. 48(1): p. 82-93.
- [19] Silva, R., L.R. Mata, S. Gulbenkian, M.A. Brito, C. Tiribelli, and D. Brites, Inhibition of glutamate uptake by unconjugated bilirubin in cultured cortical rat astrocytes: role of concentration

- and pH. *Biochem Biophys Res Commun*, 1999. 265(1): p. 67-72.
- [20] Grojean, S., V. Koziel, P. Vert, and J.L. Daval, Bilirubin induces apoptosis via activation of NMDA receptors in developing rat brain neurons. *Exp Neurol*, 2000. 166(2): p. 334-41.
- [21] Grojean, S., V. Lievre, V. Koziel, P. Vert, and J.L. Daval, Bilirubin exerts additional toxic effects in hypoxic cultured neurons from the developing rat brain by the recruitment of glutamate neurotoxicity. *Pediatr Res*, 2001. 49(4): p. 507-13.
- [22] Hanko, E., T.W. Hansen, R. Almaas, R. Paulsen, and T. Rootwelt, Synergistic protection of a general caspase inhibitor and MK-801 in bilirubin-induced cell death in human NT2-N neurons. *Pediatr Res*, 2006. 59(1): p. 72-7.
- [23] Amit, Y., W. Cashore, and D. Schiff, Studies of bilirubin toxicity at the synaptosome and cellular levels. *Semin Perinatol*, 1992. 16(3): p. 186-90.
- [24] Clark, G.D., Role of excitatory amino acids in brain injury caused by hypoxia-ischemia, status epilepticus, and hypoglycemia. *Clin Perinatol*, 1989. 16(2): p. 459-74.
- [25] Spencer, R.F., W.T. Shaia, A.T. Gleason, A. Sismanis, and S.M. Shapiro, Changes in calcium-binding protein expression in the auditory brainstem nuclei of the jaundiced Gunn rat. *Hear Res*, 2002. 171(1-2): p. 129-141.
- [26] Churn, S.B., Multifunctional calcium and calmodulin-dependent kinase II in neuronal function and disease. *Adv Neuroimmunol*, 1995. 5(3): p. 241-59.
- [27] Fernandes, A., A.S. Falcao, R.F. Silva, A.C. Gordo, M.J. Gama, M.A. Brito, and D. Brites, Inflammatory signalling pathways involved in astroglial activation by unconjugated bilirubin. *J Neurochem*, 2006. 96(6): p. 1667-79.
- [28] Gupta, S., A decision between life and death during TNF-alpha-induced signaling. *J Clin Immunol.*, 2002 Jul. 22(4): p. 185-94.
- [29] Kyriakis, J.M. and J. Avruch, Mammalian mitogen-activated protein kinase signal transduction pathways activated by stress and inflammation. *Physiol Rev*, 2001. 81(2): p. 807-69.
- [30] Saklatvala, J., J. Dean, and A. Clark, Control of the expression of inflammatory response genes. *Biochem Soc Symp*, 2003 (70): p. 95-106.
- [31] Silva, S.L., A.R. Vaz, M.J. Diogenes, N. van Rooijen, A.M. Sebastiao, A. Fernandes, R.F. Silva, and D. Brites, Neuritic growth impairment and cell death by unconjugated bilirubin is mediated by NO and glutamate, modulated by microglia, and prevented by glycothiocholic acid and interleukin-10. *Neuropharmacology*, 2012. 62(7): p. 2398-408.
- [32] Newman, T.B., P. Liljestrand, and G.J. Escobar, Infants with bilirubin levels of 30 mg/dL or more in a large managed care organization. *Pediatrics*, 2003. 111(6 Pt 1): p. 1303-11.
- [33] Shapiro, S.M., Bilirubin toxicity in the developing nervous system. *Pediatr Neurol*, 2003. 29(5): p. 410-21.
- [34] Wennberg, R.P., C.E. Ahlfors, V.K. Bhutani, L.H. Johnson, and S.M. Shapiro, Toward understanding kernicterus: a challenge to improve the management of jaundiced newborns. *Pediatrics*, 2006. 117(2): p. 474-85.
- [35] Perlman, M., P. Fainmesser, H. Sohmer, H. Tamari, Y. Wax, and B. Pevsmer, Auditory nerve-brainstem evoked responses in hyperbilirubinemic neonates. *Pediatrics*, 1983. 72(5): p. 658-64.
- [36] Nwaesei, C.G., J. Van Aerde, M. Boyden, and M. Perlman, Changes in auditory brainstem responses in hyperbilirubinemic infants before and after exchange transfusion. *Pediatrics*, 1984. 74 (5): p. 800-3.
- [37] Jiang, Z.D. and A.R. Wilkinson, Impaired function of the auditory brainstem in term neonates with hyperbilirubinemia. *Brain Dev*, 2013.
- [38] Rice, A.C., V.L. Chiou, S.B. Zuckoff, and S.M. Shapiro, Profile of minocycline neuroprotection in bilirubin-induced auditory system dysfunction. *Brain Res*, 2011. 1368: p. 290-8.
- [39] Ye, H.B., J. Wang, W.T. Zhang, H.B. Shi, and S.K. Yin, Taurine attenuates bilirubin-induced neurotoxicity in the auditory system in neonatal guinea pigs. *Int J Pediatr Otorhinolaryngol*, 2013. 77(5): p. 647-54.
- [40] Zhang, B., X. Yang, and X. Gao, Taurine protects against bilirubin-induced neurotoxicity in vitro. *Brain Res*, 2010. 1320: p. 159-67.
- [41] Schmiedt, R.A., H.O. Okamura, H. Lang, and B.A. Schulte, Ouabain application to the round window of the gerbil cochlea: a model of auditory neuropathy and apoptosis. *J Assoc Res Otolaryngol*, 2002. 3(3): p. 223-33.
- [42] Lang, H., B.A. Schulte, and R.A. Schmiedt, Ouabain induces apoptotic cell death in type I spiral ganglion neurons, but not type II neurons. *J Assoc Res Otolaryngol*, 2005. 6(1): p. 63-74.
- [43] Lang, H., B.A. Schulte, J.C. Goddard, M. Hedrick, J.B. Schulte, L. Wei, and R.A. Schmiedt, Transplantation of mouse embryonic stem cells into the cochlea of an auditory-neuropathy animal model: effects of timing after injury. *J Assoc Res Otolaryngol*, 2008. 9(2): p. 225-40.
- [44] Altschuler, R.A., K.S. O'Shea, and J.M. Miller, Stem cell transplantation for auditory nerve replacement. *Hear Res*, 2008. 242(1-2): p. 110-6.
- [45] Cho, Y.B., H.H. Cho, S. Jang, H.S. Jeong, and J.S. Park, Transplantation of neural differentiated human mesenchymal stem cells into the cochlea of an auditory-neuropathy guinea pig model. *J Korean Med Sci*, 2011. 26(4): p. 492-8.
- [46] Chen, W., N. Jongkamonwiwat, L. Abbas, S.J. Eshtan, S.L. Johnson, S. Kuhn, M. Milo, J.K. Thurlow, P.W. Andrews, W. Marcotti, H.D. Moore, and M.N. Rivolta, Restoration of auditory evoked responses by human ES-cell-derived otic progenitors. *Nature*, 2012. 490(7419): p. 278-82.

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