ANSWERING REVIEWERS

August 07, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 3769-Edited.docx).

Title: Noise-Induced Cochlear Inflammation

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Name of Journal: *World Journal of Otorhinolaryngology*

ESPS Manuscript NO: 3769

The manuscript has been improved according to the suggestions of reviewers:

1 Format has been updated

2 Revision has been made according to the suggestions of the reviewer

Reviewer 1 (00289614):

1. As requested by the reviewer, we have added several (8) new references from 2010 onwards. This review is the current state-of-the-art in this field of research, and reflects the fact that there have not been many recent studies investigating the noise-induced cochlear inflammation.
2. The following discussion on the association between NOX3 and cochlear inflammation has been included as suggested:
   "Similar to noise-induced hearing loss, oxidative stress and inflammation are major contributing factors to cisplatin-induced ototoxicity. Cisplatin has been shown to increase the expression of inflammatory mediators such as inducible nitric oxide synthase (iNOS), cyclo-oxygenase-2 (COX-2) and TNF-α, which are downstream targets of the transcription factor, signal transducer and activator of transcription-1 (STAT1) (Kaur et al., 2011). Cisplatin-induced activation of STAT1 is dependent on ROS generation through NOX3, a member of the NOX family of superoxide-generating nicotinamide adenine dinucleotide phosphate (NADPH) oxidases. NOX3 is expressed almost exclusively in the inner ear and serves as the primary source of ROS generation in the cochlea (Banfi et al., 2004). siRNA-mediated gene silencing of NOX3 mitigates cisplatin-induced hearing loss, demonstrating a key role of NOX3 in the development of cisplatin-mediated ototoxicity (Mukherjea et al., 2010). In contrast to these findings, recent data from our group show that exposure to noise results in a significant down-regulation of NOX3 in the cochlea (Vlajkovic et al., 2013). We propose that the reduction in NOX3 may represent an endogenous protective mechanism to reduce oxidative stress in the noise-exposed cochlea. These studies provide evidence that NOX3 is involved in the development of noise- and cisplatin-induced cochlear injury, albeit in a different way." (page 13-14)

Reviewer 2 (00503663):

1. Comparisons between the cochlea and retina have been made as suggested, and the following statements have been included in the revised manuscript:
   - Analogous to the central nervous system and the retina of the eye, the cochlea is separated from the systemic circulation by a blood-labyrinth barrier, which has similar physiological

characteristics as the blood-brain barrier and the blood-retinal barrier. (page 6)

- This recruitment of macrophages to the cochlea following excessive stimuli is similar to what occurs in other sensory organs, such as the retina of the eye. Thus, exposure to damaging light causes an infiltration of inflammatory cells to the light-damaged region of the retina (Rutar et al., 2010). (page 11)
- Similar to the cochlea, the retina of the eye contains perivascular macrophages, which also contribute to the maintenance of the blood-retinal barrier (Mendes-Jorge et al., 2009). (page 13)

2. The following statement on how the number of macrophages in the cochlea changes following acute noise exposure has been included:
"The study by Tornabene et al. (2006) showed that CD45-positive cells increased from an average of 0.3 cells/section in the non-exposed cochlea to a maximum of 88 cells/section at 2 and 4 days after noise exposure." (page 10-11)

3. The following paragraph on the blood-labyrinth barrier in the stria vascularis has been added, and how excessive noise affects the barrier:
"As mentioned earlier, a large population of PVMs exist in the stria vascularis, however, these cells are not found elsewhere in the cochlea, including the spiral ligament (Shi, 2010). The PVMs play an important role in regulating the integrity of the intrastrial fluid-blood barrier by modulating the expression of tight- and adherens-junction proteins between the endothelial cells via the secretion of pigment epithelium growth factor (PEDF) (Zhang, 2013; Neng, 2013). The integrity of the barrier is critical for establishing and maintaining the endocochlear potential and preventing the entry of toxic substances into the cochlea (Juhn et al., 2001). Exposure to excessive noise leads to breakdown and increased permeability of the blood-labyrinth barrier by causing PVMs to change morphology and detach from strial capillaries, and also by causing a significant downregulation of PEDF production and tight junction protein expression (Zhang et al., 2013). Recent evidence has demonstrated that bone marrow-derived cells (BMDCs) are recruited to the stria vascularis during the first week after acoustic injury to repair and restore the noise-damaged blood vessels (Dai et al., 2010). These cells promote angiogenesis and neovascularisation, differentiating into PVMs, pericytes and endothelial cells and integrating into the strial blood vessels by four weeks after noise exposure. This recruitment is mediated by an intrinsic iNOS-dependent stromal cell-derived factor-1α (SDF-1α) signalling pathway. Blocking the activity of iNOS or SDF-1α significantly reduced both the number of infiltrating BMDCs and the capillary density (vascular repair) in the stria vascularis of the noise-exposed cochlea." (page 13)

3 DOIs have been added to the reference list.

Thank you again for publishing our manuscript in the *World Journal of Otorhinolaryngology*.

Sincerely yours,

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