June 20, 2013

Dear Editor,

Please find enclosed the edited manuscript in Word format (file name: 3731-review.doc).

Title: Adenosine Amine Congener Ameliorates Cisplatin-Induced Hearing Loss

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

1 Format has been updated

2 Revision has been made

Reviewer (1)

1. Introduction, page 5, line 20: the sentence has been changed to "Cross-linking of DNA by cisplatin may lead to p53-mediated apoptosis of the outer hair cells and the lateral wall tissues, the spiral ligament and stria vascularis" and the new references included as suggested.
2. The last sentence in paragraph 2 on Page 5 has been amended according to the reviewer's suggestion: "A reduction in the endocochlear potential, probably resulting from a dysfunctional stria vascularis, often precedes outer hair cell loss in the acute model of cisplatin ototoxicity."
3. This sentence has been changed accordingly: "Cisplatin reacts with the outer hair cells to form the highly reactive monohydrate complexes".
4. This has been changed to "Hydroxyl radicals and peroxynitrite" as suggested.
5. Ketamine and xylazine are now written in lowercase.
6. "Grass" has been capitalized.
7. It has been changed to stainless steel electrodes
8. ABR responses were inconsistent at high frequencies with our current sound source. However, the frequency of 24 kHz used in our study is located in the upper basal turn in rats (Viberg and Canlon, Hear. Res. 197:1-10, 2004), and thus represents the high frequency spectrum.
9. ABR waveforms are now given in lower case roman numerals (i-v) as suggested.
10. We have amended the text accordingly and used only "PBS" after defining it initially.
11. We have used Citifluor AF1 antifading mounting medium (Agar Scientific, London, UK).
12. The acronym TdT has been defined as suggested.
13. The specimen were decalcified in 5% EDTA. This sentence has been amended, and now reads: "After fixation with 4% PFA, decalcification in 5% EDTA solution for 7 days and overnight

cryoprotection in 30% sucrose, the cochleae were embedded in Tissue-Tek optimal cutting temperature compound (O.C.T., Miles Laboratories, Elkhart, IN, USA), snap-frozen in isopentane, and stored at -80°C."

14. Only the abbreviation "PBS" is shown on page 9 of the revised manuscript.
15. The acronym TdT (terminal deoxynucleotidyl transferase) is now defined at the first appearance on Page 9 of the revised manuscript.
16. This has been clarified in response to question # 11.
17. The construct "Group 1" has been deleted on Page 9.
18. Statistics: This has been amended as suggested.
19. Legend for Figure 3 has been amended accordingly.
20. Figure 3 legend now states that the images represent the middle turn organ of Corti.
21. The number of animals has been included in the Figure 3 legend. We have already stated the number of animals in the text (Page 9, Statistical Analysis).
22. $P < 0.05$ for the middle turn and $P < 0.001$ for the basal turn; this has been included on Page 10 of the revised manuscript.
23. Unfortunately we don't have high quality images of the basal turn suitable for inclusion in this publication.
24. The following statement has been included on page 11 of the revised manuscript: "As expected, the number of apoptotic cells was the highest in the basal turn, slightly lower in the middle turn and minimal in the apical turn. In the basal and middle turns, we mostly observed 1 out of 3 or 2 out of 3 TUNEL-positive outer hair cells, whilst in the apical turn TUNEL-positive cells were observed only occasionally. In all turns, however, there were more advanced stage outer hair cells than inner hair cells, and in extreme cases complete disintegration of the outer hair cells was observed. Supporting cells were mostly unaffected except in the basal turn, where some TUNEL-positive Deiters' cells were observed."
25. A series of 6 optical sections were collected for each sample, and each image in Figure 4 represents a single optical section obtained from the centre of the stack. The nucleus in the background of row 3 ohc (panel 4c) is likely coming from a neighbouring ohc or supporting Hensen's cell. The smaller size of the ohc nucleus in panel 4d is likely due to the angle of cryosectioning.
26. The following statement has been included in Legend of Figure 4: Images are single optical sections of the middle turn.
27. This has been amended, and the revised sentence reads: As a qualitative outcome, ADAC treatment reduced apoptosis of the outer hair cells and marginal cells in the stria vascularis.

Reviewer (2)

A discussion on inner ear inflammation has been omitted from the manuscript as suggested.

Reviewer (3)

Positive feedback from the reviewer is greatly appreciated.

3 References and typesetting were corrected

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

Sincerely yours,

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