Identification of Cochlear Hair Cell Injury after Treatment with Kanamycin and Furosemide in C57BL6 Mice

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1 Introduction

Systemic toxicity of new chemical entities is thoroughly investigated in preclinical testing. However, effects on otic structures have largely been ignored in standard screens. A number of drug classes, given to a diverse patient population, have already been shown to have the potential to cause ototoxicity. Such drugs include antibiotics, loop diuretics, platinum-based chemotherapy agents and several nonsteroidal anti-inflammatory drugs (NSAIDS). Damage to the otic system has the potential to significantly impact on the quality of life of patients, due to sensorineural hearing loss, dysequilibrium, or both. This has gradually resulted in increased interest in screening of new chemical entities during early drug development. In this study, an animal model of cochlear hair cell injury in the mouse was characterized, using two known ototoxic compounds, as a tool to screen for ototoxicity.

2 Methods

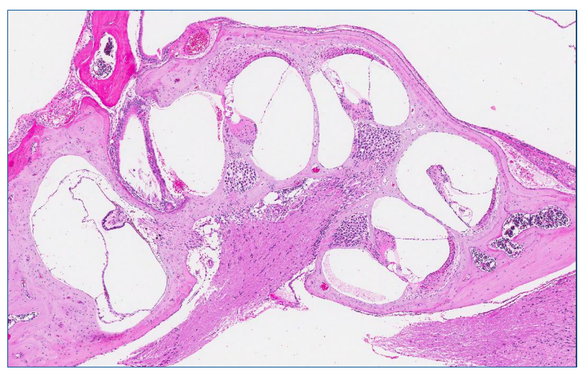
Four groups of 4 C57BL6 female mice/group (10 weeks old) were administered a single dose of kanamycin (K; 1 mg/g) by subcutaneous injection and furosemide (F; 0.08, 0.10, 0.25 or 0.40 mg/g) by intraperitoneal injection. Animals were monitored for reaction to treatment and changes in body weight and food consumption for 10 days. At necropsy, the tympanic bulla and inner ear were removed, and the tissue was fixed and decalcified using a solution of picric acid and formic acid (PFF). Once periodic x-ray monitoring indicated tissue was fully decalcified it was embedded in paraffin, step-sectioned at 75 µm intervals and stained with hematoxylin and eosin.

4 Conclusion

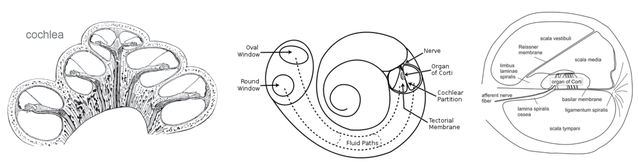
In conclusion, 10 days following systemic administration of kanamycin/furosemide to the C57BL6 mouse, ototocxicity was characterized by degeneration and loss of hair cells and supporting cells of the organ of Corti. This model demonstrates the role of preclinical screening and also represents a model that can be used as a comparative tool in the assessment of ototoxicity of new drugs.

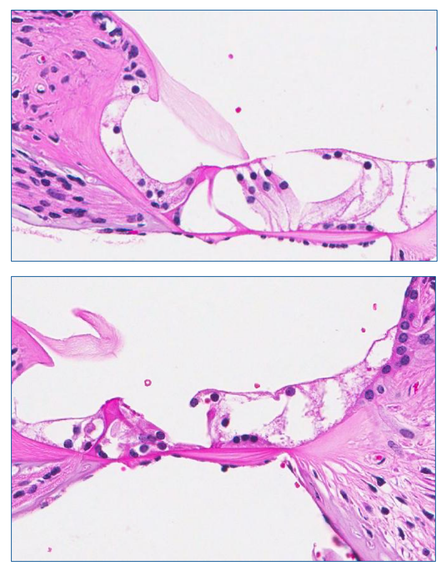
3 Results

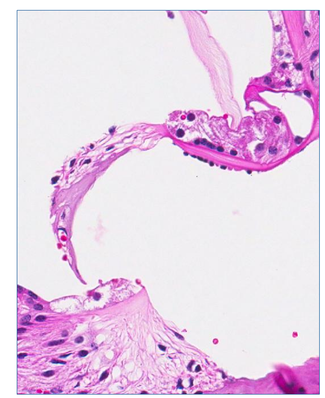
Following dose administration, reaction to treatment was observed at 0.25 and 0.40 mg/g F + 1 mg/g K. In general, transient decreased activity, partly closed eyes and decreased respiratory rate was observed on the day of dosing. In two animals (1/group at 0.25 and 0.40 mg/g F), a general deterioration of overall condition persisted up to 4 days after dosing and ultimately these animals were euthanized prior to schedule. At doses of ≤ 0.10 mg/g F + 1 mg/g K no clinical signs were observed. There were no changes in body weight or food consumption at any dose level.



Cochlea and vestibule of a control C57BL6 mouse. H&E stain (4X)







Top left: Organ of Corti showing normal hair and phalangeal cells (20X).
Bottom left: Organ of Corti with degeneration and loss of hair, phalangeal and pillar cells (26X).
Top right: Degeneration and necrosis of structures in the Organ of Corti (26X).

Microscopically, a generally dose-dependent increase in the incidence and severity of findings in the cochlea were observed in animals administered furosemide at ≥ 0.10 mg/g. Outer hair cells of the cochlea were the most susceptible to degenerative changes of kanamycin/furosemide, with slight to marked degeneration/loss 10 days after dosing. In a decreasing order of sensitivity, other affected structures were the inner hair cells (degeneration/loss), the supporting cells (i.e. pillar cells and/or phalangeal cells; degeneration) and the stria vascularis (degeneration/atrophy).