Background

The pattern of hearing loss post cochlear electrode implant insertion trauma (EIT) is described as an acute loss of hearing followed by the gradual loss of residual hearing on the days following the implantation.(1) This inner ear trauma initiates multiple molecular mechanisms such as oxidative stress, JNK activation and caspase-3 activation in hair cells (HCs) or support cells (SCs) resulting in initiation of programmed cell death within the damaged tissues of the cochlea which leads to loss of residual hearing.(2) In earlier studies L-N-acetylcysteine (L-NAC) (an antioxidant), Mannitol (osmotic and diuretic effects) and dexamethasone (Dex) (a steroid) have been shown independently to protect the HCs loss against different types of inner ear trauma.(3-7) These 3 molecules have different types of ototrophic properties that might have synergistic effects.

Objectives

To test the otoprotective effects of the tri-therapy (L-NAC + Mannitol + Dex) in an in vitro model of inner ear trauma (e.g. cochlear implantation trauma).

Materials and Methods

Cochlea explants were dissected from P-3 rats and placed in serum-free media. Explants were divided into multiple groups:

1. Control groups (6 explants per group; a total of 12 explants):
   1. Control (no trauma, no drug)
   2. EIT (implant trauma, no drug)
2. Ten experimental groups (6 explants per group; a total of 60 explants):
   1. EIT + L-NAC (5, 2 or 1 mM)
   2. EIT + Mannitol (100, 50 or 10 mM)
   3. EIT + Dex (20, 10 or 5 µg/mL)
   4. EIT + L-NAC + Mannitol + Dex

In the EIT groups, a 0.28-mm diameter monofilament fishing line was introduced through the small cochleostomy located next to the round window area, allowing for an insertion of between 110 and 150 degrees. Oxidative stress was studied in all explants post this EIT.

After EIT was caused, explants were cultured in media containing L-NAC alone, Mannitol alone or Dex alone at decreasing concentrations.

Concentrations of L-NAC, Mannitol and Dex that showed 50 percent protection of hair cell loss individually were used as a combination in the experimental group 4.

Molecular mechanisms involved in EIT

For EIT in the 3-day-old (P-3) rat cochlea, a 0.28-mm diameter monofilament fishing line was introduced through the small cochleostomy located next to the round window area, allowing for an insertion of between 110 and 150 degrees.(3) After EIT was caused, explants were cultured in media containing the single therapeutic compound (L-NAC, Mannitol, and Dex) at varying concentrations as well as the cocktail containing all three.

Result: Immunostaining for ROS (CellROX)

CellROX™ labeling is seen in the middle and basal turns of the EIT exposed specimens in both HCs and SCs, but is absent in the control and EIT + L-NAC + Mannitol + Dex specimens. The graph shows the mean signal intensity of CellROX™ labeling (n = 9 samples) in the HCs and SCs for each group (control, EIT, and EIT + L-NAC + Dex + Mannitol).

Result: Immunostaining for membrane damage (HNE)

Surface preparations of HCs and SCs stained with anti-HNE (red) and DAPI (blue) of the middle and basal turns from each group (control, EIT, and EIT + L-NAC + Mannitol + Dex) are represented. Anti-HNE labeling is seen in the basal turn of the EIT exposed specimens but is absent in the control and EIT + L-NAC + Mannitol + Dex specimens. The graphs show the mean signal intensity of anti-HNE labeling (n=9 samples) in the HCs and SCs for each group (control, EIT, and EIT + L-NAC + Mannitol + Dex).

Results: FITC-phalloidin staining of HCs & HC counts

The organ of Corti from control explants shows three well organized rows of outer hair cells (OHCs) and a single row of inner hair cells (IHCs), while the organ of Corti having EIT shows areas of damaged OHC and IHCs with missing hair cells or damaged stereocilia. The explants exposed to EIT and treated with L-NAC (5mM) + Mannitol (100mM) + Dex (20 µg/mL) shows the same pattern of preservation of OHC and IHCs organization as seen in control explants. The graph show the percentage of living OHCs in each group.

Results: FITC-phalloidin staining of HCs

While the lower concentrations of L-NAC (2mM), Mannitol (10mM) and Dex (5µg/ml) showed only partial protection against HCs loss, the cocktail containing those same concentrations combined showed a total protection against HCs loss.

Conclusion

1. EIT involves oxidative stress and lipid peroxidation early on after the implantation.
2. L-NAC, Mannitol and Dex are effective alone in protecting the sensory cells in vitro at high doses.
3. A cocktail containing L-NAC, Mannitol and Dex at much lower doses of each compound, is effective in protecting sensory cells.
4. The three compounds can be combined with a synergistic effect allowing a decrease in the potential side effects of each of the compound.

References