ANALYSIS OF C-JUN-N-TERMINAL KINASE (JNK) DOWNSTREAM TARGETS IN GENTAMICIN INDUCED HAIR CELL DEATH.

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Introduction

Sensorineural hearing loss (SNHL) is characterized by damage of cochlear hair cells (HC) and can result from a variety of causes, e.g. genetic disorders, aging, certain drug exposure, infectious disease and intense sound overexposure. Intracellular events that mediate aspects of aminoglycosides caused damage to hair cells have been partially unravelled. Several independent research groups have demonstrated crucial role of MAPK (mitogen activated protein kinase) signalling in aminoglycosides induced otoxicity1.

Fig.1. Mammalian MAP kinase signal transduction pathways

MAPKs are important mediators of signal transduction from the cell surface to the nucleus. JNK kinases are in cell culture conditions strongly activated by stress-inducing stimuli, including UV, translational inhibitors, heat shock and tumor necrosis factor; therefore they are also referred as stress-activated protein kinases (SAPKs).

Recently it has been shown that JNK pathway is activated in HCs in response to aminoglycosides. Activation of JNK leads to phosphorylation and thereby activation of transcription factors and consequently to altered gene expression. There are many nuclear substrates of JNK including c-Jun, ATF2, Elk-1.

There are many studies which revealed AP-1 transcription factor as a promising target for development of new otoprotective strategies.

Results

Protein Binding Activity to TRE after Different Time Points of Gentamicin Treatment

Fig.4. Nuclear extracts were prepared from OCs treated with 80 µM gentamicin for indicated time points. In control untreated explants instead of gentamicin treatment medium change was performed and these explants were kept in culture for indicated time periods. 3 µg of nuclear extracts were incubated with 30 fmol32P labeled TRE oligonucleotides and analyzed by EMSA.

The arrow points to the AP-1 specific protein-DNA complex. In each experiment a positive control reaction with nuclear extracts from serum stimulated NIH3T3 fibroblasts was performed. All experiments were performed in triplicates.

Specificity of TRE Binding Activity and Composition of AP1 Complexes Formed after Gentamicin Treatment

Fig.5A. Unlabelled competitor TRE oligonucleotide was preincubated at the indicated ratio with nuclear extracts from Corti organ prior to the addition of the labeled TRE oligonucleotide. Corti organ explants were treated with 80 µM gentamicin for 24 h. The arrow points to the AP-1 specific protein-DNA complex.

Fig.5B. Effects of anti AP-1 antibodies on gel migration of TRE oligonucleotide-nuclear protein complexes. EMSA was performed with TRE oligonucleotide with or without preincubation of nuclear extracts with indicated anti AP-1 antibodies.

Nuclear extracts were prepared from Corti explants treated for 24 h with 80 µM gentamicin. The arrow points to the AP-1 specific protein-DNA complex. The supershifted bands are indicated by double arrow.

Immunohistochemical Analysis of Corti Explants

Fig.6. Basal turns of rat cochleae immunostained with phalloidin, or with antibody directed against the c-Fos protein. In first line the untreated control group kept in culture for 24 hours is shown, in second line Corti explants treated for 24 hours with gentamicin. First row shows the phalloidin staining, second row the c-Fos immunostaining, last row is the overlay of phalloidin and c-Fos staining.

Conclusion

In this study we have demonstrated gentamicin mediated induction of the transcription factor AP-1, 24 hours after gentamicin treatment there is a strong activation of AP-1, which displays a transient character, 48 hours after initiation of gentamicin treatment the AP-1 levels are down regulated to basal level. Furthermore, we have shown that the major component of the activated AP-1 dimers is the c-Fos protein. On cellular level the AP-1 induction occurs in hair cells.

These data provide insight into the molecular mechanisms underlying gentamicin induced otoxicity, thereby opening new pathways for therapeutic interventions in future. This study revealed AP-1 transcription factor as a promising target for development of new otoprotective strategies.

References


Material and Methods

Corti explants were microdissected according to standard protocols 1, 2. Nuclear extracts were prepared according to Schreiber et al. with minor modifications 3. Molecular analysis of induced proteins was performed with electrophoretic shift assay.

Fig.3. Schematic cartoon of electrophoretic shift assay (EMSA).

Panel A: Unbound DNA (lane 1 and lane 2); Panel B: shift: Unbound DNA (lane 1) and DNA-protein complex (lane 2); Panel C: super-shift: Unbound DNA (lane 1) and DNA-protein-antibody complex (lane 3).

Fig.2. AP-1 (activating protein-1) Transcription Factor

AP-1 is a dimeric complex composed of members of the Fos and Jun proteins. Varieties of different stimuli are known to induce AP-1 activity. The induction of AP-1 is thought to play a central role in reprogramming gene expression in response to external stimuli. JNK signalling pathway was shown to be essential for gentamicin induced otoxicity.

Aim

In the presented study we addressed the JNK downstream targets in gentamicin induced hair cell death. Involvement, composition and induction time course of AP-1 transcription factor was analyzed in detail.

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References