EFFECTS OF OTOTOXIC DRUGS ON A NEW *IN VITRO* MODEL OF STRIA VASCULARIS

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Background: Strial pericytes are an essential component of stria vascularis (Blood Labyrinth Barrier) for maintaining and regulating homeostatic conditions in the inner ear. Strial pericytes were shown impaired in different pathological conditions and/or by a variety of pharmacological treatments, including antibacterial aminoglycoside antibiotics, anticancer agents, and loop diuretics.

Material and methods: We previously set Bovine Cochlear Pericytes (BCPs) as an in vitro model of stria vascularis. Here we evaluated the sensitivity of BCPs to ototoxic drugs, by challenging with furosemide, cisplatin and gentamicin (10-250 μM) for 24h and 48h. In some conditions, cells were also co-treated with ototoxic drug (250 mM) and dexamethasone (1-100 nM) for 48h to evaluate its potential protective effect. Cell viability was evaluated by MTT assay and LDH release. Reactive oxygen species (ROS) production was estimated by using 2',7'-dichlorofluoresceindiacetate (DCFDA). The percentage of apoptotic cells was assessed through cytofluorimetric analysis (Annexin V).

Results: We observed a significant reduction of cell viability (about 15%) in BCP treated with gentamicin (250 μM) for 48h, while furosemide, at all tested concentration, did not decrease BCPs viability. Treatment with cisplatin reduced cell viability by about 15% and 50% after 24h with 50 μM and 250 μM respectively ($p<0.05$) and by about 15%, 35% and 65% after 48h of treatment with 10 μM, 50 μM and 250 μM respectively ($p<0.05$) compared to control. Dexamethasone (100 nM) improved cell viability ($p<0.05$) of cells exposed to gentamicin or cisplatin. The release of LDH by BCPs increased in a dose dependent manner ($p<0.05$) after a 48h treatment with furosemide, cisplatin, or gentamicin. Co-treatment with dexamethasone (100 nM) significantly decreased LDH release with all ototoxic drugs. Furosemide, gentamicin and cisplatin significantly increased ROS production by BCPs, especially at concentration of 250 μM after 48h, while co-treatment with dexamethasone (100 nM) significantly ($p<0.05$) decreased ROS production. Worthy of note, dexamethasone decreased furosemide-induced ROS production already at 1nM. Finally, dexamethasone 100 nM decreased the percentage of apoptotic BCPs treated with cisplatin 250 μM.

Discussion and conclusions: We validated a new in-vitro model of stria vascularis by assessing the effects of known ototoxic drugs on different endpoints. The results indicate that this in vitro model of stria vascularis may serve as a tool in pharmacologic and toxicodynamic preclinical studies, to screen ototoxic effects of new chemical entities and/or drugs endowed with putative protective action to prevent hearing loss.