Study on the Therapeutic Effect of a Novel Small Chemical Compound for Treating Acquired Hearing Loss

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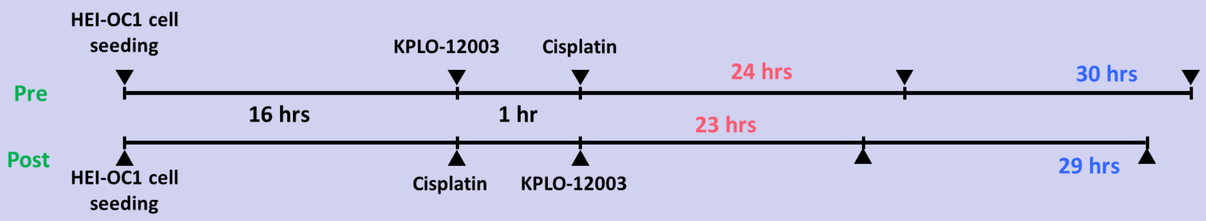
I. Introduction

- **Cisplatin is an effective anti-cancer drug widely used in the treatment of solid cancer, but side effects such as neurotoxicity, nephrotoxicity, and ototoxicity have been reported.**
- **In particular, in the case of ototoxicity, an increase in hearing threshold of 20 dB or more has been reported in more than two-thirds of administered patients, and it adversely affected communication ability, causing a problem in the patient's quality of life after treatment.**
- **However, until now, a drug capable of treating or alleviating ototoxicity caused by cisplatin has not been developed, so the need to discover a protective or therapeutic drug is emerging.**
- **In our previous study, we identified that the main function of ALA in the treatment and protection of ototoxicity induced by cisplatin is the removal of ROS by antioxidant effect.**
- **Through the massive drug screening to find a more effective drug for ototoxicity protection by cisplatin, I found Compound X, a powerful novel compound that recovered the mitochondrial function from cisplatin ototoxicity.**

II . Materials and methods

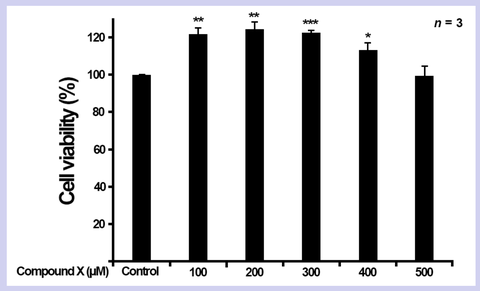
▪ **Cell line: House ear institute – organ of corti 1 (HEI-OC1)**

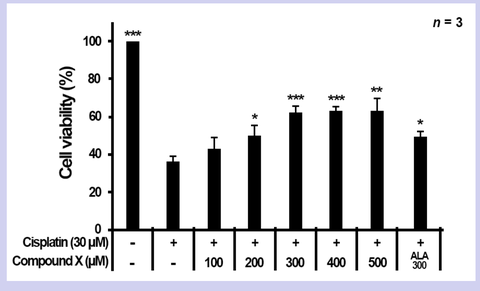


- ✓ **Cell culture and viability assay**
- ✓ **Apoptotic cell detection**
  - ▪ **Expression of caspase 3**
  - ▪ **TUNEL assay**
- ✓ **Western blot analysis**
  - ▪ **Expression of Bax/ Bcl-2**
- ✓ **Mitochondrial ROS analysis**
  - ▪ **Mitochondrial and cyctosolic ROS**
  - ▪ **Mitochondria membrane potential**
  - ▪ **Annexin V / PI**

III. Results

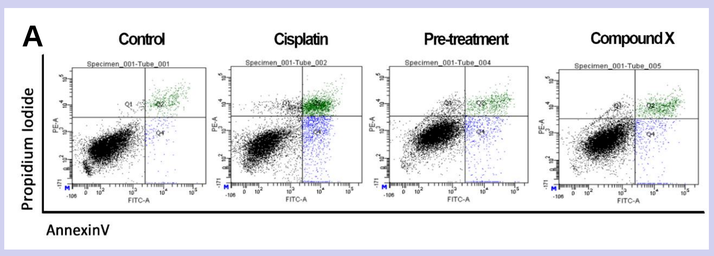
▪ **Drug screening with protective effect against cytotoxicity of cisplatin.**

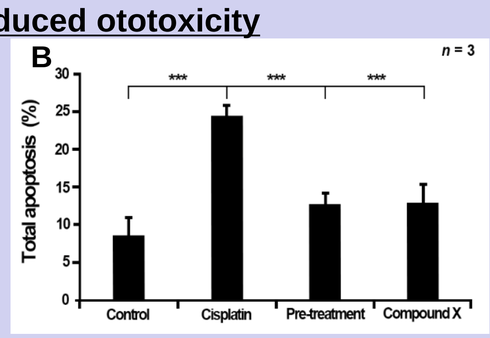


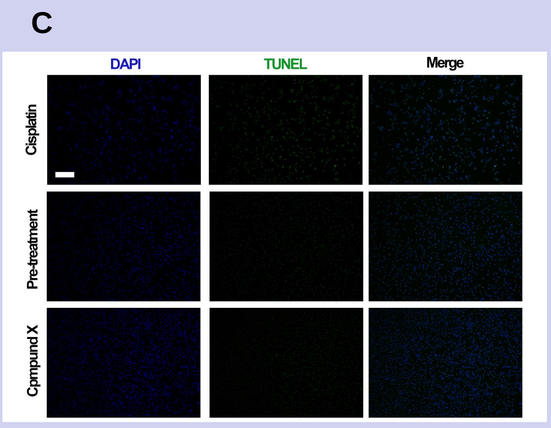


- ✓ **(A) HEI-OC1 cells were treated with 100-500 μM of Compound X for 30 h.**
- ✓ **(B) HEI-OC1 cells were treated with 100-500 μM of Compound X for 1 h, before treatment of 30 μM cisplatin for 30 h. Cell viability was measured using the CCK-8 assay. The data represent the means ± SE of three separate experiments. *p < 0.05, compared with cisplatin group.**

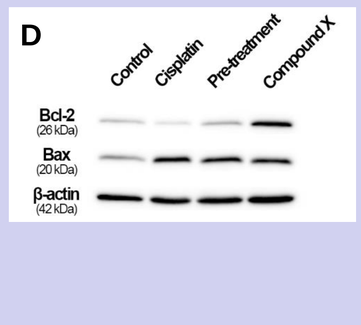
▪ **Effects of Compound X against cisplatin-induced ototoxicity**

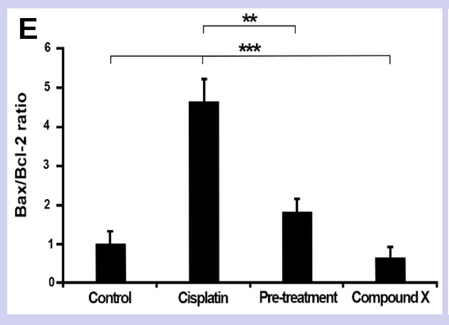


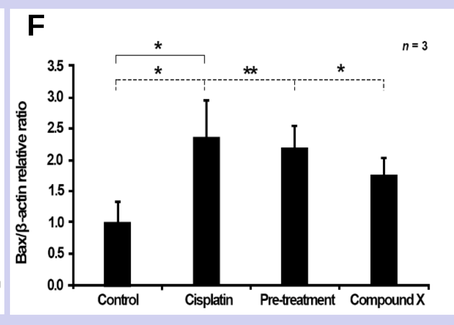


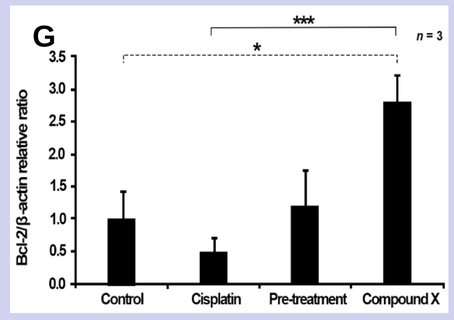


- ✓ **(A) Apoptotic cells were detected by using Annexin V/PI staining. Live and dead cells (black), Early apoptotic cells (blue), Late apoptotic cells (green) were stained and analyzed under a FACS.**
- ✓ **(B) Percentage of cells stained for Annexin V/PI among total cells in each group.**
- ✓ **(C) TUNEL assay to detect DNA fragmented cells among treatment groups. Fragmentation of nucleic DNA (green) and the nuclei (blue) were stained and observed under a fluorescence microscope. Cells not treated with TdT Enzyme Solution were presented as a negative control, and cells treated with DNase were presented as a positive control. The scale bar represents 100 μm.**



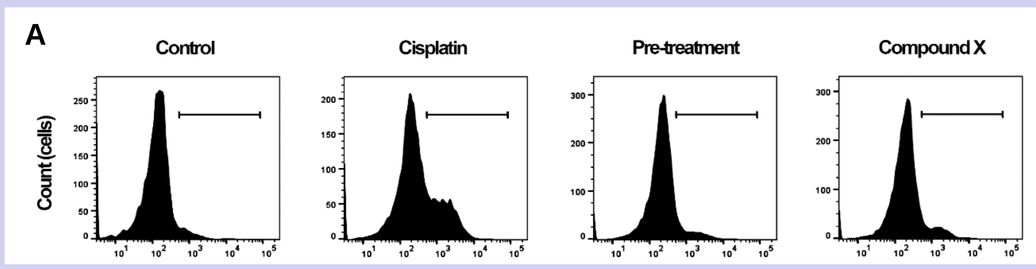


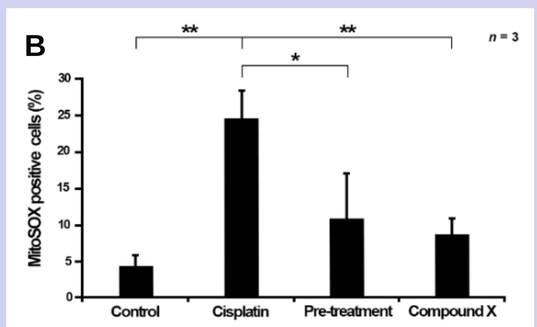




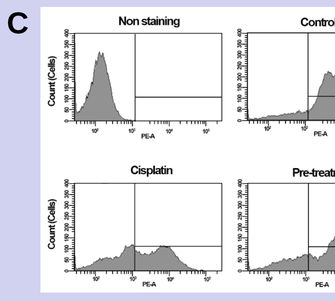
- ✓ **(D) Western blot analysis of Bax and Bcl-2 expression, compared with β-actin as the loading control group (n = 3 per lane).**
- ✓ **(E) Relative expression ratio of Bax and β-actin measured by densitometry.**
- ✓ **(F) Relative expression ratio of Bcl-2 and β-actin measured by densitometry.**
- ✓ **(G) Bax/Bcl-2 expression ratio was calculated. Compound X restored the balance between Bax and Bcl-2 protein.**
- ✓ **The data are shown as the means ± SD of three separate experiments. *p < 0.05,**

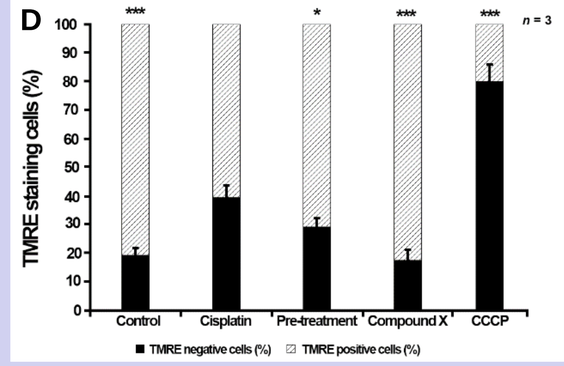
▪ **Effects of Compound X on cisplatin-induced intracellular ROS**



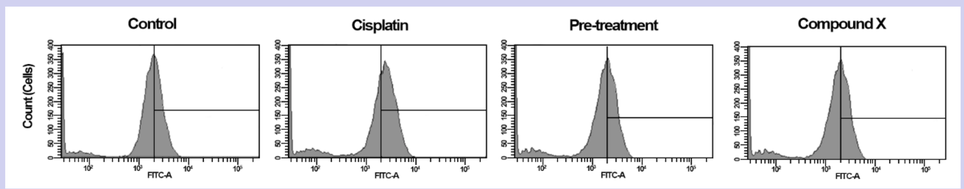


- ✓ **(A) Measurement of mitochondrial ROS levels using the MitoSOX-Red probe. Fluorescence intensity was measured at 10,000 events using flow cytometry.**
- ✓ **(B) Percentage of cells positive for the MitoSOX-Red probe among total cells in each group.**

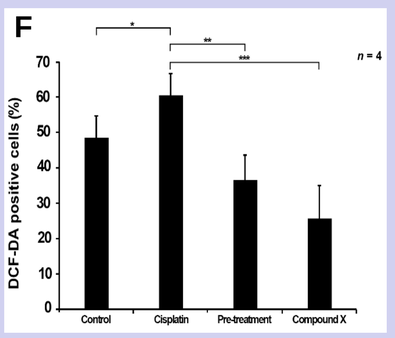




- ✓ **(C) Mitochondrial membrane potential were analyzed using the TMRE probe. Fluorescence intensity was measured at 10,000 events using flow cytometry. As a baseline for cell staining, up to the maximum fluorescence value measured in unstained cells without TMRE staining was defined as TMRE negative cells. CCCP, which directly dysfunction mitochondria membrane potential, was presented as a positive control of the experiment.**
- ✓ **(D) Percentage of positive and negative cells for the TMRE probe among total cells in each group were analyzed. Cells with a dysfunctional mitochondrial membrane potential that are TMRE negative are represented by a black bar graph, and cells with a normal mitochondrial membrane potential that are TMRE positive are represented by a hatched bar graph.**



- ✓ **(E) Intracellular ROS were measured using the DCF-DNA probe. Fluorescence intensity was measured at 10,000 events using flow cytometry.**
- ✓ **(F) Percentage of DCF-DA probe positive cells among total cells in each group were measured. The data represent the means ± SD. *p < 0.05, **p < 0.01, ***p < 0.005 compared with cisplatin group. Cells were treated with 300 μM Compound X and 30 μM cisplatin for 1 and 24 h, respectively.**



IV. Discussion and Conclusion

- **Cisplatin increases mitochondrial ROS and causes mitochondrial dysfunction in auditory cells.**
- **CompoundX helps the role of glutathione reductase to increase the antioxidant effect of glutathione, effectively removing the ROS accumulated by cisplatin.**
- **This study indicates that CompoundX shows a protective and therapeutic effect from apoptosis caused by oxidative stress against cisplatin-induced ototoxicity in vitro, suggesting the potential as a novel antioxidant in humans.**