Karolinska Institutet

**Institutionen för klinisk vetenskap, intervention och teknik, Enheten för öron- näs- och halssjukdomar**

# "Pharmacokinetics and inner ear transport of cisplatin"

AKADEMISK AVHANDLING
som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Rolf Luft auditorium (Hus L1), Karolinska Universitetssjukhuset, Solna.

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# Abstract

**Background**
Cisplatin is a commonly used platinum anti-cancer drug. Regrettably cisplatin has dose-limiting ototoxic side effects, e.g. the drug can induce an irreversible hearing loss. The ototoxic mechanisms of cisplatin have not been elucidated in the human ear and no clinically useful oto-protectors are yet available. Cisplatin is a necessary part of many treatment regimes. Its beneficial therapeutic effects might be reduced if cisplatin was excluded from the treatment in order to protect the hearing function. In this work the ototoxic effects of cisplatin are studied with the aim to better understand the mechanisms behind the irreversible hearing loss induced by this drug. Oxaliplatin is a second generation platinum-derivative anti-cancer drug, free from ototoxic side effects in clinical practice. The effects of oxaliplatin on the inner ear have been studied in this work and the results are compared with cisplatin treatment. The two drugs differ regarding both anti-cancer effects and side effects, which could be attributed to differences in pharmacokinetic factors, cellular uptake and apoptotic mechanisms. The thioredoxin redox system with the enzyme thioredoxin reductase (TrxR) was studied in cochleae due to a suggested DNA-independent apoptotic mechanism of the hair cells. The cochlear pharmacokinetics of cisplatin was assessed and the transport protein organic cation transporter 2 (OCT2) was studied in relation to the ototoxic effect of cisplatin.

**Material and methods**
Cultured human colon carcinoma cells and cell cultures of rat organ of Corti were used for apoptosis studies *in vitro* following exposure to cisplatin and oxaliplatin. Cisplatin and oxaliplatin were administered i.v. to guinea pigs, followed by *in vivo* sampling of blood, cerebrospinal fluid (CSF) and scala tympani (ST) perilymph. Liquid chromatography with post-column derivatization was used to determine the concentration of parent drug in the samples. Electrophysiological hearing thresholds and the loss of hair cells were assessed to evaluate their ototoxic effects. Phenformin, a potential blocker of OCT2 was administered and the ototoxic side effect of cisplatin was evaluated. For immunohistochemical studies, cochlea from rat, guinea pig and pig were used, where TrxR and OCT2 were evaluated in the cochlea. TrxR-assays were used to measure the TrxR activity in cochlear tissue, both *in vivo* and *in vitro*.

**Results**
The results from the *in vitro* studies showed that addition of either cisplatin or oxaliplatin to the culture medium in organ of Corti cell cultures caused a similar amount of outer hair cell loss and inhibition of TrxR activity. Cisplatin exposure to cultured human colon carcinoma cells also reduced the activity of TrxR. The results from the *in vivo* studies showed that a considerable concentration of cisplatin was present in ST perilymph as compared with weak concentrations of oxaliplatin after high dose oxaliplatin i.v. Ten minutes after cisplatin administration, its concentration in ST perilymph was 4-fold higher in the basal turn of the cochlea as compared to the apex. Cisplatin could be analysed in ST perilymph for up to 120 min. Phenformin i.v. did not reduce the ototoxic side-effect of cisplatin. Positive immunoreactivity to TrxR was evident in both hair cells and spiral ganglion cells. Futhermore, OCT2 was expressed in the supporting cells of organ of Corti and in the spiral ganglion cells.

**Conclusion**
The transport of cisplatin to the vulnerable cells of hearing seems to be of major importance for the ototoxic effects. An early high concentration of cisplatin in the base of the cochlea and delayed elimination of cisplatin from ST perilymph may be related to the cisplatin-induced loss of outer hair cells in the basal turn of the cochlea. Cisplatin and oxaliplatin both cause similar ototoxic effects when the organ of Corti is directly exposed *in vitro*. The thioredoxin redox system with the TrxR enzyme may well play a critical role in cisplatin-induced ototoxicity. The presence of OCT2 in the supporting cells indicates that this transport protein is primarily not involved in the uptake of cisplatin from the systemic circulation but rather from the deeper compartments of the cochlea. The knowledge elicited in this work will hopefully suggest objectives for further studies in order to develop oto-protective treatments to preserve the hearing of cisplatin treated patients.


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