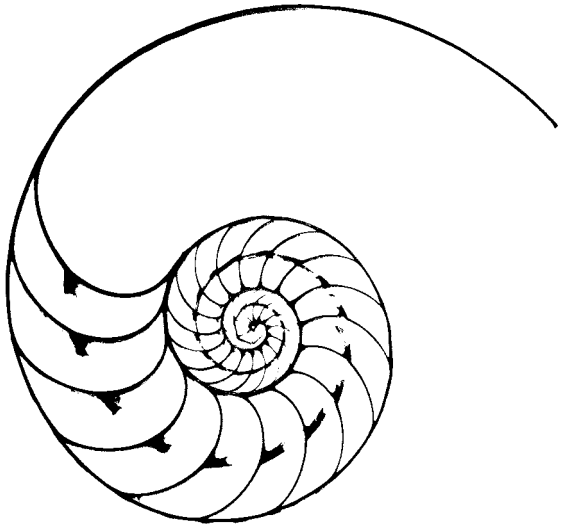
CISPLATIN-INDUCED OTOTOXICITY. PHARMACOKINETICS, PREDICTION AND PREVENTION.

ANDREAS EKBORN, MD



Stockholm 2003

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Silver is the moon

To my family

1 ABSTRACT

Cisplatin is an anticancer drug used against a number of malignancies e.g. testicular cancer, ovarian cancer and some paediatric malignancies. The main dose limiting side effects of cisplatin are oto- and neurotoxicity. Cisplatin is regarded as the most common cause of ototoxicity in Sweden today. Cisplatin ototoxicity manifests clinically as a hearing loss beginning in the high frequency region and involving successively lower frequencies. Tinnitus is another common complaint. There is a high interindividual variability in the ototoxic effect, a rough dose dependence and indications that the mode of administration affects the toxic effects. The aim of the thesis has been to study cisplatin ototoxicity especially as related to the pharmacokinetics of the drug and to prediction and prevention of ototoxicity.

The first hypothesis was that mode of administration affects the side effect profile of cisplatin. The pharmacokinetics of cisplatin and the monohydrated complex of cisplatin (MHC) a toxic biotransformation product of cisplatin were determined after a 15-second bolus injection or a one-hour infusion of cisplatin to guinea pigs. It was found that the interindividual variability in ototoxic effect was far greater than the variability in pharmacokinetics, suggesting that other factors are more important for the degree of hearing loss.

MHC has been suggested as the major cause of cisplatin side effects but has previously not been purified in sufficient amounts for animal studies of toxicity. This was effected by separation on a porous graphitic column. MHC caused an ABR thresholds shift, an increase in creatinine and a weight loss in the animals similar to those seen after administration of cisplatin in the double dose. Thus, MHC is approximately twice as toxic as cisplatin.

The antitumour effect of cisplatin is often assumed to be proportional to the systemic exposure to the drug. D-methionine has recently been advocated as a protectant against cisplatin toxicity. However, i.v. administration of D-methionine one hour before cisplatin caused a 30% decrease in the systemic exposure to cisplatin and an even greater reduction in MHC exposure. Our results indicate that the previously observed protection from cisplatin ototoxicity by systemic D-methionine can be explained by a lowered systemic exposure to cisplatin.

Local administration of a cytoprotective agent to the inner ear offers a possibility to prevent cisplatin-induced ototoxicity without risk of interference with the antitumour effect. Thiourea (TU) has unique properties that make it an interesting candidate for local protection against cisplatin ototoxicity. Ears administered TU by a direct intracochlear infusion demonstrated significantly lower OHC loss as compared to untreated ears but similar ABR. Thus, TU demonstrates partial protection against cisplatin-induced ototoxicity.

A trial was initiated to test the efficacy of high dose cisplatin treatment (125 mg/m^2) with amifostine (Ethyol®) protection in 15 patients with metastatic malignant melanoma. The most prominent side effects were ototoxic and in spite of amifostine treatment ototoxicity was unacceptable. After the second treatment all but one patient reported auditory symptoms and three patients ultimately required a hearing aid. No correlation was found on the individual level between subsequent hearing loss and plasma pharmacokinetics during the first course. An effect of amifostine on cisplatin pharmacokinetics was suggested as the analysis of platinum levels showed higher values than those obtained by selective analysis of cisplatin, corroborating the findings in the D-methionine study.

Keywords: cisplatin, ototoxicity, pharmacokinetics, monohydrated complex of cisplatin, protection

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LIST OF PUBLICATIONS

This thesis is based on the following original papers, which will be referred to in the text by their Roman numerals I-V.

Ekborn A, Laurell G, Andersson A, Wallin I, Eksborg S, Ehrsson H. Cisplatin-induced hearing loss: influence of the mode of drug administration in the guinea pig. *Hear Res* 2000;140(1-2):38-44.

Ekborn A, Laurell G, Johnström P, Wallin I, Eksborg S, Ehrsson H. D-Methionine and cisplatin ototoxicity in the guinea pig: D-methionine influences cisplatin pharmacokinetics. *Hear Res* 2002;165(1-2):53-61.

Ekborn A, Lindberg A, Laurell G, Wallin I, Eksborg S, Ehrsson H. Ototoxicity, nephrotoxicity and pharmacokinetics of cisplatin and its monohydrated complex in the guinea pig. *Cancer Chemother Pharmacol* 2003;51(1):36-42.

Ekborn A, Laurell G, Ehrsson H, Miller J. Intracochlear administration of thiourea protects against cisplatin-induced outer hair cell loss in the guinea pig. *Hear Res* 2003;181(1-2):109-115.

Ekborn, A. Hansson, J. Ehrsson, H. Eksborg, S. Wallin, I. Wagenius, G. Laurell, G. High dose cisplatin with amifostine: ototoxicity and pharmacokinetics.
Submitted for publication.

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LIST OF ABBREVIATIONS

ABR	Auditory brainstem response
AUC	Area under the concentration time curve
Cisplatin	*cis*-diammine-dichloroplatinum
DDTC	Diethyldithiocarbamate
EEG	Electroencephalogram
EP	Endolymphatic potential
5-FU	5-Fluorouracil
LC	Liquid chromatography
MHC	Monohydrated complex of cisplatin
NSCL	Non small cell lung cancer
OHC	Outer hair cell
TI	Therapeutic index
$T_{1/2}$	Terminal elimination half-life
TU	Thiourea

2 THESIS SUMMARY

Foreword:

Knowingly or not, physicians are all aware of the role of pharmacokinetics and pharmacodynamics and, to some extent may consider these factors in our everyday practice. Moreover, treatment decisions are often more based on the side effects of treatment than on the effects as we often adjust the dose to avoid side effects. Intravenous morphine could be used as one example: the rate of injection affects both the effect and the side effects. Thus not only the dose but also the mode of administration matters. Hence, we should always consider the pharmacodynamics in relation to the pharmacokinetics for drugs to predict, avoid and prevent side effects.

2.1 INTRODUCTION

2.1.1 Cancer chemotherapy

Cancer chemotherapy was introduced in the 1940s when the chemical warfare agent nitrogen mustard was first used in clinical practice. Chemotherapy is currently first line curative treatment for a few neoplastic diseases, e.g. haematologic malignancies and has brought considerable prognostic improvement. For non-haematologic malignancies, chemotherapy is mostly utilised as complement to the other treatment modalities. All chemotherapeutic agents are burdened with severe dose dependent and dose limiting side effects. A chemotherapeutic agent is thus not characterised only by its effects but to a great extent also by its side effects. A basic principle in chemotherapy is combination treatment. Substances with different modes of action are combined to achieve additive or synergistic effects and to avoid additive or synergistic side effects.

2.1.2 Cisplatin

2.1.2.1 Cisplatin history

Cisplatin was first synthesised in 1845 by Peyrone (Peyrone 1845). It was to take another 120 years until the biological activity of the substance was discovered during an experiment designed to elucidate the effect of electrical fields on growing bacteria (Rosenberg et al., 1965). E-coli cells subjected to treatment formed long filaments, an observation which led to the conclusion that cell division was inhibited but cell growth was not markedly influenced. In a later experiment the cytotoxic effect was tied to cisplatin and a new class of antitumour agents was proclaimed (Rosenberg et al., 1969). The results from the first clinical trials were published in 1972 (Rossof et al., 1972).

Amazingly, a new drug destined to revolutionise cancer chemotherapy and medical oncology had reached the clinic from a chance discovery just 7 years before. Although two more platinum analogues have reached the market and clinical use, cisplatin might still be considered the most useful platinum compound on the basis of versatility, experience of use and documentation (Lokich 2001). Not surprisingly, carboplatin and oxaliplatin also have dose dependent and limiting side effects. Carboplatin is less ototoxic, making haematologic and neurotoxic side effects more prominent. Neurotoxicity is the most common dose limiting side effect of oxaliplatin (Gamelin et al., 2002). The cause of the observed differences in side effects is unknown so far.

2.1.2.2 Cisplatin today

Cisplatin is a mainstay in the treatment of testicular cancer, one of the early successes of modern chemotherapy for solid tumours (Einhorn 1997). It is also used in the treatment of paediatric malignancies such as medulloblastoma (Kortmann et al., 2000) and osteogenic sarcoma (Saeter et al., 1999). It is used in ovarian cancer (Berek et al., 1999). Cisplatin is cell cycle unspecific and is often used as a part in combination treatment. It has a toxic profile which is different from other important cytotoxic drugs. High doses cause nephrotoxicity, gastrointestinal toxicity, neurotoxicity and ototoxicity, where the two latter side effects can be dose limiting even with modern preventive measures.

2.1.2.3 Cisplatin Chemoradiotherapy

Due to its special properties cisplatin has been advocated as a radiosensitiser to achieve synergistic effects with radiation therapy. Lower doses are often used in multiday low dose schedules together with radiation therapy with rather limited systemic side effects (Marcu et al., 2003). A distinction should be made between concomitant and induction chemotherapy. Concomitant or concurrent chemoradiation with cisplatin has brought benefits in more advanced cases of cancer of the uterine cervix (Grigsby and Herzog 2001) and in lung cancer (Bartelink et al., 2002; Gandara et al., 2000). In non-small cell lung cancer (NSCL), even induction or neoadjuvant chemotherapy has improved survival. Adelstein et al. performed a randomised phase III trial in stage 3 and 4 head and neck cancer patients with resectable disease, adding concurrent chemotherapy with cisplatin and 5-FU to definitive radiation. They found increased disease clearance, increased disease free interval and increased primary site preservation at long-term follow up (Adelstein et al., 2000; Milas et al., 2003). The improved survival observed at early follow up was lost at the long-term follow up. This was attributed to the general state of health of the population with head and neck cancer, in which other causes of death are common. In a randomised and stratified phase III trial Al Sarraf et al. compared chemoradiation with cisplatin and 5-FU to radiation alone in advanced nasopharyngeal carcinoma. Their conclusion was that chemoradiation produced superior overall survival and progression free survival (Al-Sarraf et al., 1998).

2.1.3 Cisplatin side effects

2.1.3.1 Nephrotoxicity

Nephrotoxicity is a well-known side effect of cisplatin, described already in the early clinical trials. It is manifested biochemically by increasing creatinine values, decreased clearance and electrolyte wasting. It can manifest as acute renal failure or as a more chronic disease characterised by electrolyte wasting (Hutchison et al., 1988). Morphological studies show that mainly the proximal tubule is affected (Dobyan et al., 1980). This is a metabolically active region where most of the filtered NaCl is reabsorbed through active transport. Today nephrotoxicity is no longer a dose limiting side effect as it can effectively be ameliorated by prehydration and mannitol stimulated diuresis (Finley et al., 1985).

2.1.3.2 Gastrointestinal toxicity

Nausea, vomiting and anorexia are common side effects of cisplatin treatment. An antiemetic regimen is normally incorporated. Treatment has been improved by the addition of 5-HT-3 receptor antagonists, which now constitute a basis of every antiemetic regime together with steroids (Fauser et al., 1999). Administration of cisplatin minimising high peak concentrations might cause less gastrointestinal toxicity (Kurihara et al., 1996; Salem et al., 1984).

2.1.3.3 Neurotoxicity

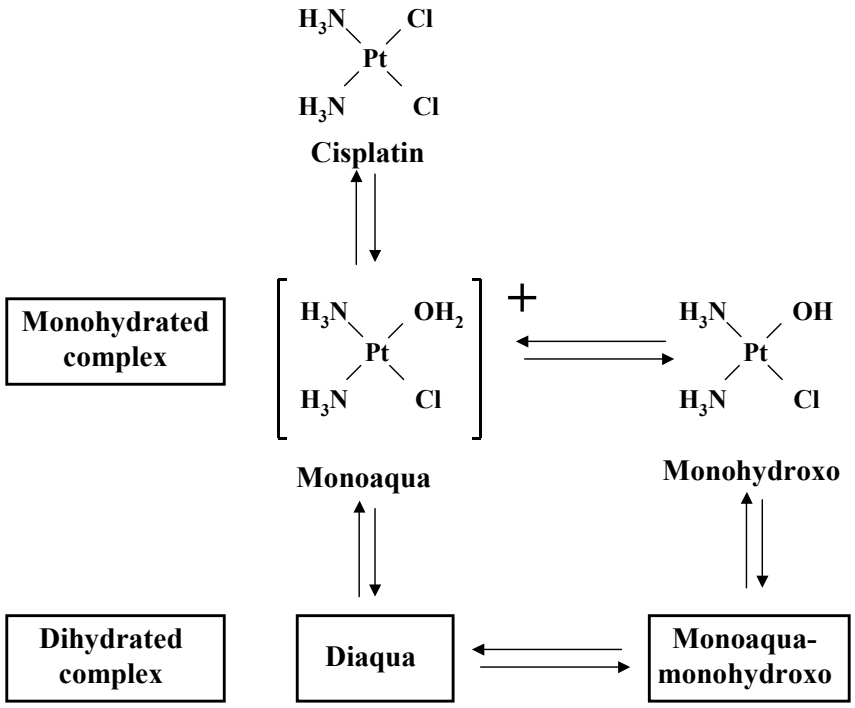
Neurotoxicity is another well-known dose limiting side effect of cisplatin treatment. It manifests commonly as distal sensory neuropathy, which may be distressing. Again there is a rough correlation to dose and dose intensity, with no known treatment options. Neurotoxicity is commonly irreversible (Alberts and Noel 1995; Quasthoff and Hartung 2002).

2.1.3.4 Ototoxicity

Ototoxicity is one dose limiting side effect of cisplatin treatment. It is normally manifested as a sensorineural hearing loss beginning in the high frequencies, successively progressing towards the speech frequency range (Laurell and Jungnelius 1990). It is often accompanied by transient or permanent tinnitus. Ototoxicity displays a rough dose dependence (Simon et al., 2002). Radiation to the ear may enhance ototoxicity (Miettinen et al., 1997). There is a high interindividual variability in ototoxicity, where some individuals may get a considerable hearing loss even after the first course (Laurell and Jungnelius 1990). The cause of the high interindividual variability is not known but possible explanations are pharmacokinetic differences, genetic factors and metabolic status. It is not yet possible to identify susceptible individuals before treatment. Early diagnosis may be aided by monitoring of high frequency audiometry (Fausti et al., 1999) or perhaps otoacoustic emissions (Ress et al., 1999).

2.1.4 Cisplatin chemistry

Cisplatin (*cis*-diammine-dichloroplatinum) is a highly reactive compound with electrophilic/oxidising properties. Cisplatin is hydrolysed in water losing a chloride ion and gaining a water molecule forming the monohydrated complex (MHC). MHC in its protonated form, monoaqua cisplatin, is positively charged and highly reactive with a pKa of 6.56 (Andersson et al., 1994), i.e. quite close to physiological pH. At physiological pH (7.4) 85% of the MHC will be in its deprotonated form, the much less reactive monohydroxo cisplatin. The important cisplatin equilibriums are affected by both pH and chloride concentrations in the solution of administration and the body fluids. In plasma, cisplatin is the dominant species but in the low chloride intracellular environment formation of MHC is believed to be promoted and MHC may be the species ultimately causing cytotoxicity (Hausheer et al., 1998). The positive charge of the MHC may cause it to be electrostatically attracted to the negatively charged DNA and thereby increasing the chance of a reaction. Indirect evidence suggests that formation of MHC in the circulation may significantly contribute to the side effects of cisplatin. Modification of the content of MHC in the solution of administration affects the toxicity of the treatment. Reconstitution in a hypertonic solution lowers the amount of MHC and cause less toxicity (Litterst 1981; Ozols et al., 1984). Hypotonic solutions of reconstitution, which increase the amount of MHC, have been used for local intra-arterial administrations (Ichinose et al., 1997). Cisplatin is a rather small uncharged molecule, properties which should facilitate diffusion. Cisplatin is believed to enter cells mainly through passive and partly through facilitated diffusion (Gately and Howell 1993). The high reactivity between cisplatin and nucleophilic sites on

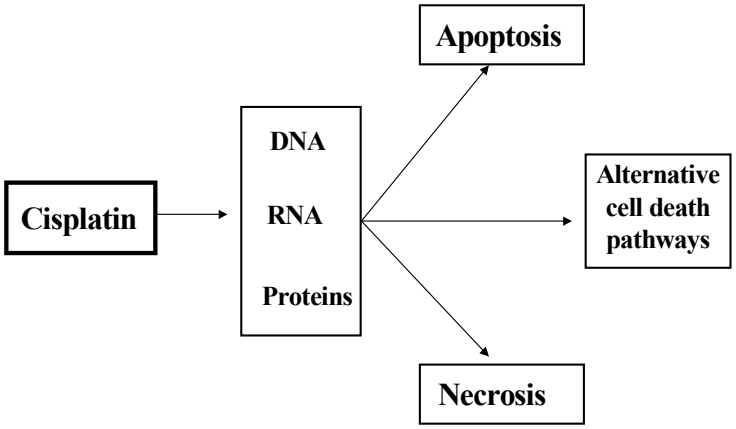


Cisplatin and its hydrolysis products

circulating endogenous substances makes covalent binding an important route of elimination. Cisplatin is bound to circulating high and low molecular complexes causing it to loose most of its cytotoxic properties (Takahashi et al., 1985) and is slowly excreted.

2.1.5 Cytotoxic mechanisms

The exact mechanism of cisplatin cytotoxicity is not known. The main mode of action of cisplatin has traditionally been assumed to be analogous to that of the alkylating agents, implying that cisplatin cytotoxicity would mainly be caused by interactions with DNA, G-G intrastrand links being the most frequent formation (Kelman and Peresie 1979). However, a number of other possible mechanisms except interaction with nuclear DNA have been suggested such as interaction with RNA and mitochondrial DNA as well as with proteins involved in the antioxidant systems, energy production and cell signalling. This may result in either apoptosis (programmed cell death) or necrosis (disorderly cell death). Induction of apoptosis is believed to be important for the antitumour effect of cisplatin (Dive and Hickman 1991). Doses and dosing schedules may, apart from host factors, determine whether cell death occurs by apoptosis or necrosis. Effects of cisplatin on renal proximal tubular cells are believed to be exerted through apoptosis in lower doses and through necrosis at higher doses (Lieberthal et al., 1996). Nuclear DNA is one target and cisplatin causes dose dependent inhibition of DNA synthesis. Preferential binding to mitochondrial DNA has also been suggested (Daoud et al., 1995; Olivero et al., 1997) and mitochondrial damage is an early feature of cisplatin cytotoxicity (Brady et al., 1990). Mitochondrial transmembrane potential may be more decreased and mitochondrial toxicity more increased in cisplatin-induced necrosis than apoptosis (Troyano et al., 2001). Cytotoxicity may also be exerted by interactions with sulfhydryl groups, for which cisplatin has a high affinity, in proteins with crucial cellular functions. An important step in the cytotoxic action of cisplatin in the inner ear is depletion of intracellular antioxidants related to the glutathione system (Ravi et al., 1995). Mitochondrial injury in renal cells is associated with dysfunction in glutathione



peroxidase (Sugiyama et al., 1989). The glutathione system is one cellular system designed to prevent peroxidation of cellular constituents. Whether primary or secondary, the consumption of these components is associated with cellular death in the inner ear. The apical to base differences in levels of intracellular antioxidants in the cochlea have been suggested as a cause of the observed apical to base difference in outer hair cell (OHC) sensitivity to ototoxic substances such as cisplatin and aminoglycosides (Sha et al., 2001).

2.1.6 Analytical aspects

Several methods have been used for determination of cisplatin in biological samples. These methods differ in pre-treatment of the sample, separation and detection.

Analysis of platinum in plasma
An early used method was detection of platinum in plasma by flameless atomic absorption spectrophotometry. This is a simple method where all active and inactive biotransformation products will be codetermined with the active drug. Using this method in patients a biphasic decay of platinum in plasma was found with elimination half-lifes of 23 minutes and 67 hours, respectively (Gormley et al., 1979). Indeed, ultra sensitive methods of detection have now revealed that blood platinum levels may be increased above baseline even 20 years after treatment (Gelevert et al., 2001).

Analysis of platinum in ultrafiltrate
Selectivity may be increased by using ultrafiltration of blood or plasma to remove all high molecular weight cisplatin complexes before platinum determination. Inactive low molecular weight complexes between cisplatin and, for example, amino acids will still be co-determined. The result is often termed free platinum.

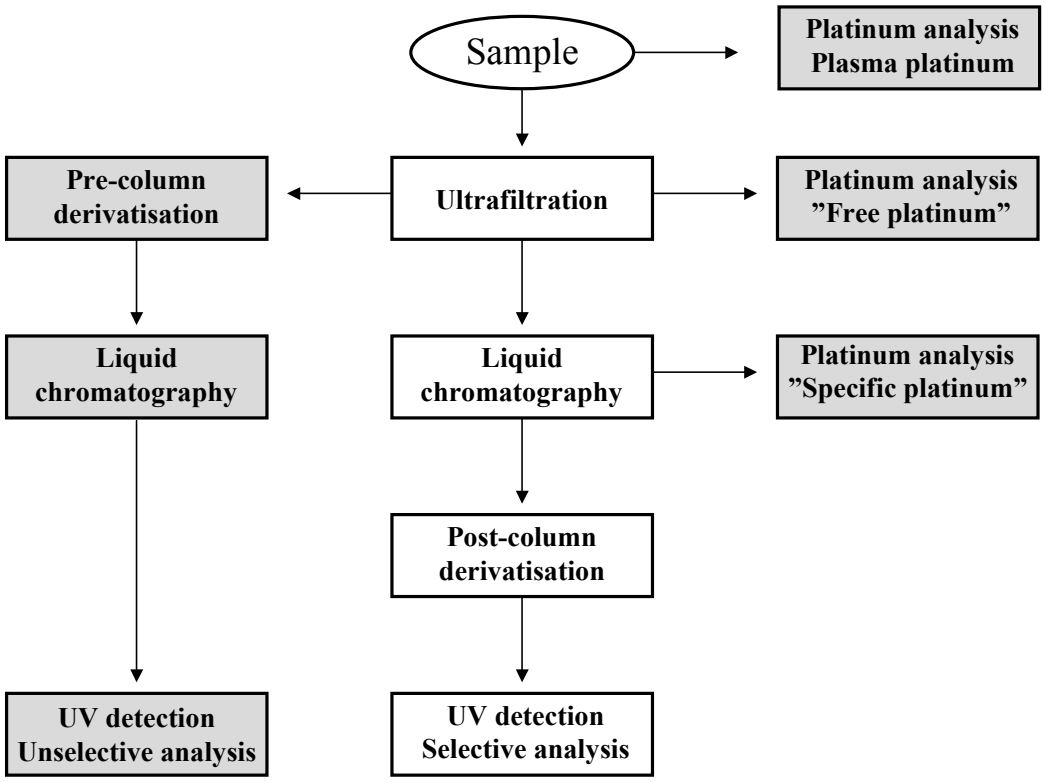
Separation of ultrafiltrate before analysis
Separation of substances in the ultrafiltrate by liquid chromatography on ion exchanger columns will make highly selective separation of cisplatin, cisplatin biotransformation products and cisplatin low molecular weight complexes possible.

Detection methods
After separation platinum may, for example, be detected by inductively-coupled plasma atomic emission spectrometry (Dominici et al., 1986) or atomic absorption spectrophotometry (Bannister et al., 1978). UV detection is a rather simple non-selective method commonly used with LC. To further increase selectivity and sensitivity to make the detection of low levels in small samples possible with UV detection, a derivatisation between cisplatin or MHC with diethyldithiocarbamate (DDTC) can be performed giving a complex with higher molar absorbance. Detection

may be performed "on line" when the detector is directly coupled to the separation system or "off line" where aliquots obtained after separation are injected separately into the detection system. On line analysis is often preferred but demands a detection system which is directly compatible with the separation method, such as UV detection.

Pre vs. postcolumn derivatisation

The derivatisation may be performed before separation, precolumn derivatisation, or after the separation, postcolumn derivatisation. Precolumn derivatisation is simpler to perform but selectivity is lost as some of the biotransformation products will generate the same complex as cisplatin itself and hence be codetermined. Moreover, it has been shown that DDTC can reverse the formation of even rather stable cisplatin-DNA adducts (Treskes et al., 1992). Similar reactions taking place before separation could contribute to an overestimation of drug levels. Postcolumn derivatisation is technically more difficult as the reaction has to be optimised to avoid unnecessary broadening of the peak while maintaining a high yield. If properly performed liquid chromatography of blood or plasma ultrafiltrate with postcolumn derivatisation is a highly sensitive and selective method (Andersson and Ehrsson 1994). It is only through the use of separation before detection that the systemic interaction between cisplatin and low molecular weight protectors with a chemical propensity to form complexes with cisplatin and its biotransformation products can be studied.



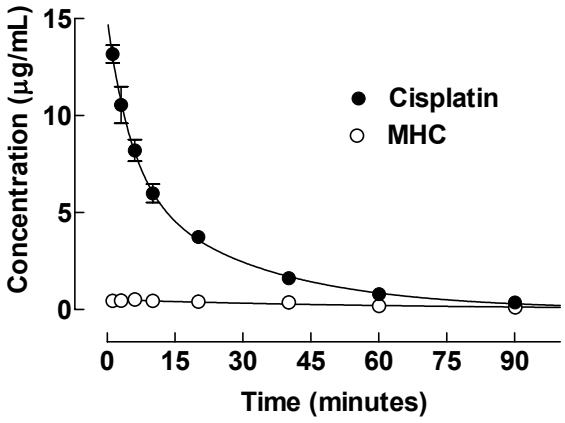
Schematic illustration of different systems used for cisplatin analysis

2.1.7 Pharmacokinetics and pharmacodynamics

Pharmacokinetics is the study of delivery and removal of a drug to tissues and fluids in the body. To complete the analysis of the dose response relationship we also need to study the pharmacological response in the body as a consequence of the drug, i.e. pharmacodynamics.

2.1.8 Pharmacokinetics

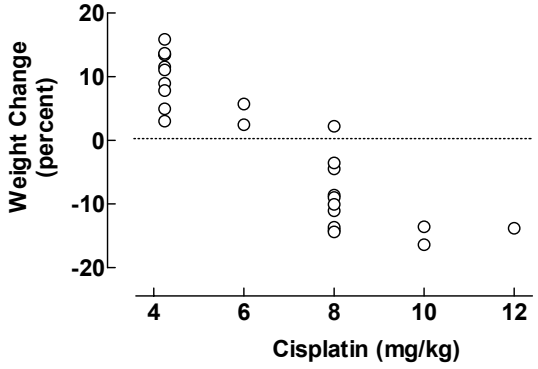
The effect of a drug exerted at the cellular level is believed to be partly proportional to the concentration of the drug in the fluid bathing the cells and, particularly for drugs which react irreversibly, the time during which it exerts its effect. The concentration at the cellular level might be difficult to measure. However, for rapidly diffusing drugs the concentrations in the extracellular fluid are often assumed to be proportional and close to those in plasma ultrafiltrate. If, however, measurements of drug concentration at different time points are made after drug administration an approximation of time concentration relation ships can be obtained. Using statistical and mathematical methods including pharmacokinetic modelling, series of individual concentrations may be used to estimate key pharmacokinetic parameters with great precision. Differences in drug exposure and pharmacokinetics are confounding factors, which should be controlled when comparing doses, drug effects and side effects. After drug administration, concentration measurements and model approximation several pharmacokinetic parameters are often presented. Area under the concentration time curve (AUC) and peak concentration are generally of major importance. High peak concentrations may for example favour passive diffusion to secluded compartments. In the case of substances that react covalently, such as cisplatin, the reaction may be determined by both the drug concentration and the time of exposure and thus more correlated to the AUC.



Cisplatin and MHC concentrations after bolus injection of cisplatin, mean±SEM

2.1.9 Pharmacodynamics

A basic concept in cancer chemotherapy and radiotherapy is that the dose given is related to the therapeutic response. Increasing the dose is supposed to be connected to an increased response among tumour cells. This appears to be the case also for cisplatin (Ozols 1989). The dose response relationship is rarely, if ever, linear. There is a threshold dose for response, an increasing phase and a plateau phase. For many cytotoxic drugs, different cell types show different dose response relationships. Effects on tumour cells are generally thought to be beneficial to the host whereas effects on host cells cause side effects. The relationship between therapeutic effects and side effects is described by the therapeutic index (TI).



Dose-response scattergram

2.1.10 Therapeutic index

Therapeutic index for a drug is the quotient Maximum non-toxic dose/ Minimum effective dose (Rang and Dale 1986). In clinical chemotherapy that would correspond to the dose almost causing troublesome side effects/minimum effective antitumoural dose. A high TI makes effect without troublesome side effects more likely. It is difficult or impossible to actually calculate a TI as the sensitivity of the normal tissue cells or the sensitivity of the tumour cells of the individuals are unknown, but the concept as such is useful when optimising treatments. Successful treatments are based on the presumption that the tumour cells are more sensitive than the normal cells of the host or that the normal tissue can somehow be saved or replaced, for example through bone marrow transplant. In clinical practice treatment is often delivered until critical toxicity is reached and the treatment is then modified (in case of a tumour response) or discontinued. There is often a differential response among different types of normal host cells, causing the particular profile of side effects of each antineoplastic drug. A side effect which cause the treatment to be modified or discontinued is termed dose limiting. The term therapeutic window describes the case where tumour cells of the individual are killed by the treatment while the side effects are somehow manageable.
Thus, the success of a treatment depends on several factors: The systemic exposure to the drug, the sensitivity of the tumour cells to the drug and the individual sensitivity to side effects of the drug.

2.1.11 Optimising chemotherapy and increasing therapeutic index

Three ways are currently used to optimise specific treatments:

1) Selection of suitable patients,
2) Optimising the mode of administration
3) Administration of chemoprotectors/antidotes to rescue host cells.

Selection of suitable patients

Selection of suitable patients is a strategy for risk/benefit assessment which is effectively used in every patient. The first step is the selection of patients with a drug-sensitive tumour and an acceptable performance status. Moreover, the patient should not have any failures in organs which most often present critical toxicities e.g. kidneys, heart/lung/liver or haematologic system, which could predispose the patient to severe side effects. The second step is to monitor the ongoing treatment on the individual, i.e. the tumour response and the side effects.
Another approach that could theoretically be used to predict treatment is the measurement of some key biological effect. One example is the intrapatient dose escalation of cisplatin after assessment of DNA platinum adduct levels in peripheral blood cells which, according to one study, correlates with cisplatin pharmacokinetics and tumour response (Schellens et al., 1996).

Optimising the mode of administration

The mode of administration is believed to be important for dose response relationship and side effects, both for cytotoxic drugs and for radiation therapy. In radiation oncology this is effected through different fractionation schedules. Possible alterations to the mode of administration of cytotoxic drugs that can be considered include the rate of drug administration, the timing of one drug as related to the administration of other drugs or treatments and the interval between treatment courses. In this context one has theoretically also to consider the specific cytotoxic effect of the antineoplastic drugs, e.g. some drugs have effect only during part of the cell cycle whereas others are cell cycle unspecific. If the drug is cell cycle specific administration should be tailored in such a way that the drug reach the tumour cells in sufficient amounts when they are in the vulnerable stage. If the drug is non cell cycle specific, considerations regarding the importance of the mode of administration might be that high concentrations will facilitate passive diffusion of the drug to the tumour cells and cause levels high enough to overcome drug resistance systems. Common speculations pertaining to the role of mode of administration for cisplatin is for example that intermittent bolus injection may cause more gastrointestinal toxicity than repeated infusions (Salem et al., 1984) whereas a slow infusion might be more myelotoxic (Forastiere et al., 1988). The AUC has been suggested to be more important than the peak for the nephrotoxic effect (Nagai and Ogata 1997). Conflicting results have been obtained in cell cultures concerning the cytotoxic effects of cisplatin as related to the extra-cellular

concentration time curve. Possible explanations might be differences in intracellular kinetics of uptake and binding (El-Kareh and Secomb 2003) or perhaps by the kinetics of reparative mechanisms. Hence, pharmacokinetics may affect cisplatin effects and side effects in many different and unpredictable ways.

2.1.12 Administration of chemoprotectors

Administration of specific antidotes

In some cases protector treatment is already an integral part of the treatment, for example in high dose methotrexate treatment where the antidote leucovorin is administered after a certain time to avoid serious/fatal toxicity. As cisplatin has no specific and well-defined mode of action no specific antidotes are available.

Administration of a substance which specifically supports surviving host cells

Administration of the growth factor GM-CSF (granulocyte macrophage colony stimulating factor) is commonly used with regimens causing bone-marrow toxicity. Administration is supposed not to stimulate the tumour. *In vivo* Neurotrophine 3 signalling prevents cisplatin-induced spiral ganglion cell toxicity (Bowers et al., 2002). It could be speculated that a similar treatment may acquire a role in the prevention or treatment of cisplatin ototoxicity in the future.

Administration of substances with a more unspecific mode of action

Some protective substances used in chemotherapy, including many of those tried against cisplatin toxicity, may interact chemically directly with the cytotoxic drug. For some putative protectors the effect may be explained either by such a systemic chemical interaction or by the specific support of drug resistance systems in normal tissues. The radio- and chemoprotector amifostine (WR 2721) is presumably an example of a substance with a selective mode of action. The active metabolite of amifostine, WR1065, contains a sulfhydryl group with antioxidant properties. However, since chemical activation of WR 2721 is effected by tissue bound alkaline phosphatase which supposedly is present to a higher degree in the capillary walls of normal tissues a preferential protection of host tissues can be hypothesised. Apart from amifostine a great number of other compounds, many of which contain sulfhydryl/thiol groups, have been evaluated for promising nephro- and/or oto-protective effects in cisplatin treatment (Bokemeyer et al., 1996; Hamers et al., 1994; Jones and Basinger 1989; Kaltenbach et al., 1997; Lautermann et al., 1995; Rybak et al., 1997, 1999; Schweitzer et al., 1986). Among tested substances D- and L-methionine have attracted a fair amount of attention as putative protectors (Campbell et al., 1996, 1999 and Reser et al., 1999). Its effect has been attributed to support of the antioxidant systems in the cochlea (Kopke et al., 1997). Trials have even been made with animal tumour models with methionine to ensure a similar

antineoplastic activity (Cloven et al., 2000; Reser et al., 1999). Reser et al. (1999) showed only a partial retention of the antineoplastic activity of cisplatin after the administration of D- and L- methionine suggesting some negative effect on the antitumour activity of cisplatin from methionine. Furthermore, another study concluded that the sulphur-containing protector sodiumthiosulphate failed to increase the therapeutic index of cisplatin (Aamdal et al., 1988).

2.1.13 Different modes of protector treatment

Systemic treatment and systemic protection

For chemoprotector treatment during cisplatin therapy three principally different strategies have been employed. The first is systemic treatment with cisplatin and systemic protection. This has been tried experimentally (Kaltenbach et al., 1997) as well as clinically (Gandara et al., 1990, 1995) with a variety of substances and by a large number of investigators. The protector has been administered either before or after cisplatin. There is always a theoretical risk of systemic chemical interaction between cisplatin and the protector as well as unwanted selective protective effects on tumour cells.

Local treatment and systemic protection

This treatment modality is also used clinically. Cisplatin is for example administered in a high dose intraperitoneally (Guastalla et al., 1994) or intra-arterially (Samant et al., 1999) to the tumour and thiosulphate is administered intravenously. The timing of the chemoprotector administration may be assumed to affect the pharmacokinetics of cisplatin but has not been systematically studied. Whether the treatment results in an improved therapeutic index or merely an amelioration of side effects is not known.

Systemic treatment and local protection

This modality has only been evaluated experimentally but the theoretical advantages are obvious. The possibility of systemic interaction is avoided by local administration and a greater freedom exists in the choice of protective substances and in the timing of administration. Animal experiments suggest that middle ear administration of an antidote may be used for the protection against cisplatin-induced ototoxicity (Li et al., 2001). A major problem is to find a suitable substance, which penetrates into the intact human inner ear after middle ear administration. Assuming that the protective effect is exerted either through the support of the antioxidant systems or through direct interaction with cisplatin the ideal substance would be a small molecule with antioxidant properties and a high reactivity towards cisplatin such as thiourea (TU). TU is known to penetrate from the middle to the inner ear (Laurell et al., 2002) and it is highly reactive towards cisplatin (Riley et al., 1982). Direct administration to the inner ear is not yet available in humans.

2.1.14 The Ear

2.1.14.1 The sense of hearing

Trying to imagine a deaf life is difficult as the use of the ear is more instinctive than the use of the eye. We constantly scan the environment for sound all around us, around corners, in darkness and through walls. Losing your hearing you lose the ability to hear a car approaching or a child playing or crying just outside your field of vision. As hard of hearing you do not only lose the ability to hear other persons but you also lose the ability to control your own voice to make it understandable. Often, perception of meaningful sounds is not replaced by a peaceful silence but instead by tinnitus which distracts thoughts and ruins sleep. Although hearing impairment from chemotherapy rarely results in all the severe consequences mentioned above, it is of clinical importance to decrease, avoid and treat cisplatin-induced ototoxicity.

2.1.14.2 Sound and the ear

The ear is our high frequency mechanoreceptor organ. The human ear is sensitive to sound with frequencies roughly between 20 Hz and 20 kHz. The dynamic range of reception is very large, spanning 10^7 times, from what is barely audible to what is painful. The corresponding sound pressures range from $2x10^{-5}$ to 200 Pa. To have a more easily manageable unit, dB SPL is normally used. The sound pressure in dB SPL is 20*log P/P0, where P0 is the reference pressure 20 μPa. The reference pressure roughly represents the average hearing threshold in the population at frequencies between 1-5 kHz. Pure-tone audiometry is the most common measure of hearing sensitivity. As the sensitivity is frequency dependent and non-linear, audiograms are graded in dB hearing level (HL) where 0 dB HL is normalised to the mean hearing level for the reference population at that frequency. A sound pressure increase of 6 dB denotes a doubling of the relative sound pressure.

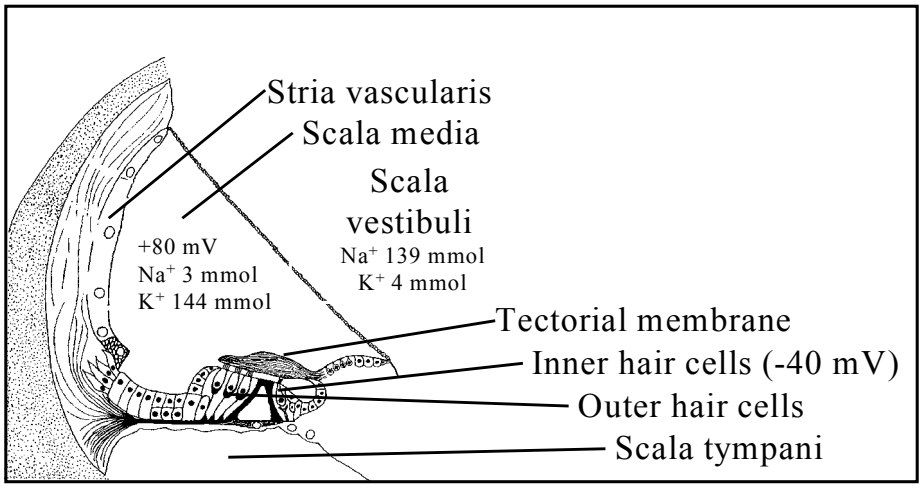
2.1.14.3 From sound to sensation

Sound can be conducted to the sensory cells in the inner ear through the outer and the middle ear or directly through bone. Air conducted sound enters the external ear and travels through the ear canal to the tympanic membrane. The pressure difference across the tympanic membrane causes it to vibrate and these vibrations are conducted through the bones of the ossicular chain (malleus, incus and stapes) to the fluids of the cochlea. Big movements of the tympanic membrane are transformed to small movements in the inner ear fluid, in effect an impedance matching. In all, these systems improve transmission considerably, especially in the frequency region important to speech perception in noisy environments, 2-5 kHz. The movements of the tympanic membrane and ossicles and, hence, transmission is controlled by reflexes to the tensor tympani and stapedius muscles. In the inner ear movements of the fluid are transmitted through the tectorial and basilar membranes to the primary sensory cells—the inner hair cells (IHC). The cochlea acts as a mechanical demodulator, transforming sound frequency to

a physical location along the basilar membrane. In addition to the IHCs there are also outer hair cells (OHC). These are believed to have dynamic micromechanical properties contributing to the high sensitivity, high frequency selectivity and wide dynamic range of the human ear (Brownell et al., 1985). Movement of the hair-bundles on the IHCs causes depolarisation of the cell and neurotransmitter release, which stimulate the associated nerve endings. The neuronal signals initiated by the IHCs are conducted through afferent nerves with connecting stations in the cochlear ganglion, cochlear nucleus, superior olivary complex, the inferior colliculus, the medial geniculate body and the auditory cortex. At each of these stations neuronal processing of the auditory stimulus takes place, but it is as the neuronal signal reaches the primary auditory cortex that it causes a sensation of sound.

2.1.14.4 Microanatomy and physiology

The membranous labyrinth is divided into three compartments, the scala vestibuli, the scala tympani and the scala media. The first two scalae are in continuity and are filled with perilymph, a fluid with a composition similar to extracellular fluid, low in potassium and high in sodium (approximately 4 and 139 mM, respectively). The composition of the endolymph, on the other hand, resembles that of the intracellular fluid with a high potassium content, approximately 144 mM and a low sodium content (approximately 2-4 mM) (Sterkers et al., 1984). Chloride is the principal anion with a concentration of approximately 130 mM (Sterkers et al., 1984), similar to that in plasma, thus theoretically not promoting the biotransformation from cisplatin to MHC. Bicarbonate is the other major anion with a concentration of approximately 20 mM (Isaacs Jr et al., 1989). With regard to the perilymph and the surrounding tissues there are potential differences between the different cells and compartments. The interior of the HCs and the stria vascularis carry a negative potential, which is bigger than in most normal cells whereas the endolymph itself has a positive potential, the EP. This



Radial section through the scala media

potential is believed to be at least partially generated in the stria vascularis by an electrogenic Na^+ K^+ ATPase. According to the Davis resistance theory, the potential across the hair cell membrane helps depolarise the hair cell when the resistance across the hair cell top membrane is changed through movement of the hair-bundles. When the EP is lowered, for example from asphyxia, the micromechanical properties of the basilar membrane are changed and the frequency selectivity and sensitivity is lowered. Thus in some respects the stria vascularis seemingly act as a power source for the sensory cells through the EP.

2.1.14.5 Types of hearing loss

We discern two main types, conductive and sensorineural hearing loss. Conductive hearing loss is caused by abnormalities of the outer and middle ear and can in many cases be treated surgically or with a hearing aid. Sensorineural hearing loss is caused by abnormalities in the inner ear (cochlear hearing loss) or in the nerve conducting the neural signal to the brainstem (retrocochlear hearing loss). Hearing impairment of cochlear origin is the most common form of hearing loss and affects roughly 10% of the adult Swedish population. A hearing aid is prescribed when found appropriate. In rare cases hearing loss of cochlear origin may be treated with a cochlear implant, which electrically stimulates the afferent nerves. Central auditory impairment is sometimes encountered when the central auditory pathways are damaged. Regardless of the cause of hearing impairment, all patients with a hearing loss of certain severity can be offered general rehabilitative measurements.

2.1.15 Ototoxicity

Various drugs and toxins present in the circulation or the middle ear may reach the inner ear and cause a loss of auditory sensitivity of the sensorineural type. The clinically most important forms of ototoxicity today are either caused by cisplatin or antibiotic substances (aminoglycosides or rarely vancomycin). However, in the developed countries monitoring schemes for plasma concentrations of aminoglycosides and the use of other antibiotics have decreased the incidence of aminoglycoside-induced hearing losses. The changing resistance patterns among pathogens may necessitate an increased use of aminoglycosides in the future. The ototoxic damage from aminoglycosides is characterised by loss of OHCs and IHCs, mainly in the basal turn of the cochlea and also by damage to the vestibular apparatus, as well as degeneration of the associated cochlear neurones (Sone et al., 1998). Nephrotoxicity is another known side effect of aminoglycosides, as well as a known side effect of cisplatin.

2.1.15.1 Cisplatin ototoxicity

The most common cause of drug-induced ototoxicity in Sweden today is cisplatin. Cisplatin ototoxicity has several characteristics. In man it is mainly evident in the basal

turn of the cochlea as degeneration of the OHCs and to some extent the IHCs and associated nerves (Hinojosa et al., 1995; Wright and Schaefer 1982). It has been shown that the toxic effect of cisplatin may result in a degeneration of the vestibular organs as well (Schaefer et al., 1985) although it is rarely diagnosed. Under experimental conditions, toxicity is normally manifested among the outer hair cells (Laurell and Bagger-Sjoback 1991) and in the stria vascularis (Meech et al., 1998). Histological alterations have also been observed among the spiral ganglion cells in the guinea pig (Cardinaal et al., 2000). Apoptosis is implicated in cisplatin-induced spiral ganglion ototoxicity (Bowers et al., 2002). A high dose of cisplatin is associated with an acute lowering of the endolymphatic potential. It is reported to cause a rather acute loss of auditory sensitivity, which became stable after about three days (Laurell and Engstrom 1989). Multiple low dose administration may cause a more specific OHC loss, which appears more slowly. In man cisplatin ototoxicity is mostly irreversible. However, in the guinea pig there is a reversible component after treatment with cisplatin in multiple low doses, which has been attributed to recovery of the function of the stria vascularis (Klis et al., 2000, 2002).

2.1.15.2 Pharmacokinetics and ototoxicity

Very little is known about cisplatin pharmacokinetics as related to specific side effects. A few studies have been performed but either unselective or less selective methods have been used for cisplatin analysis. Although transport of drugs to most parts of the body is effected through the systemic circulation the final transfer from capillaries to cells is normally effected through diffusion. Some parts of the inner ear are further from the circulation as compared to other more well vascularised tissues in the body, most likely making diffusion a limiting factor for local drug delivery. A number of factors influence diffusion and thus the amount of a given drug that can reach its target. A large concentration difference favours rapid diffusion and thereby high concentrations in the target organ. The presence of membranes such as capillary walls may limit diffusion. Capillaries in the central nervous system form very tight junctions, the so-called blood-brain barrier, allowing only certain substances to pass. This also holds true for the capillaries in the inner ear forming what has been termed the blood-perilymph barrier (Juhn et al., 1982; Sterkers et al., 1982). Indeed, for cisplatin Laurell et al. have shown that concentrations rise more slowly in perilymph than in plasma or CSF (Laurell et al., 1995). It is conceivable that the rather protected position of the inner ear as related to the systemic circulation may be one cause of the relative differences in ototoxicity between the different platinum compounds, as the less ototoxic compounds are larger molecules. In one recent study intracochlear administration of cisplatin caused less interanimal variability in ototoxicity, one interpretation could be that local pharmacokinetic parameters are important for the degree of the ototoxic effect (Wolters et al., 2003). Moreover, the protected position is a factor worth considering when searching for putative protectors.

2.2 AIMS OF THE PRESENT STUDY

This study was performed:

I. To determine whether the high interindividual variability for the ototoxic side effect of cisplatin can be explained by individual variations in pharmacokinetics.

II. To determine whether the thiol-containing protector D-methionine can be used systemically without chemical interactions with cisplatin.

III. To determine the toxicity of the cisplatin biotransformation product MHC, as MHC is most probably important for toxicity and its turnover is strongly affected by certain treatment alterations such as administration of nucleophilic protectors.

IV. To test the feasibility of protection from ototoxicity by a local administration of a chemoprotective substance.

V. To test whether the chemo- and radioprotector amifostine offers significant auditory protection and whether cisplatin pharmacokinetic parameters can be used for prediction of ototoxic effects on the individual level.

VI. To determine if there is a direct correlation between cisplatin nephrotoxicity and ototoxicity.

2.3 MATERIALS AND METHODS

2.3.1 Subjects

2.3.1.1 Patients

In Paper V 15 patients with malignant melanoma and a reference group of 6 patients with cancer of the oesophagus are presented. The patients with malignant melanoma were given cisplatin 125 mg/m^2 intravenously as a one-hour infusion. Amifostine 910 mg/m^2 was administered as a 15-minute infusion starting 30 minutes before the cisplatin infusion. The reference group received cisplatin 100 mg/m^2 intravenously as a one-hour infusion.

2.3.1.2 Guinea pigs

The guinea pig has been used in hearing research for a long time. It is a docile animal, which is easily bred in captivity. The inner ear is accessible for both physiological and histological studies as most of the cochlea is protruding in the middle ear, not encapsulated in the temporal bone as it is in many other species. The behavioural hearing thresholds are similar to those of man (Prosen et al., 1978). Paper I-IV.

2.3.2 Methods

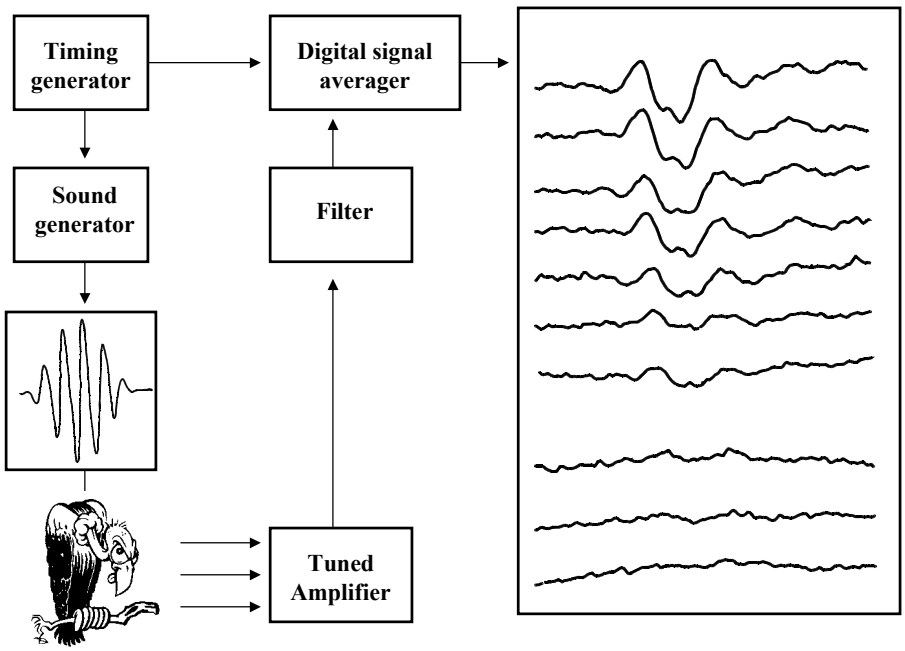
2.3.2.1 Drug administration

Cisplatin, MHC and D-methionine were administered under direct visual control through a catheter in the internal jugular vein to ensure a relevant, reliable and reproducible drug administration in the animal studies. In paper I a one-hour infusion was compared to a 15-second bolus injection. In Paper II-IV, 15-second bolus injections were used.

2.3.2.2 ABR

ABR (auditory brainstem response) was used to measure the electrophysiological hearing thresholds in the animals. It is a widely used method where the electric response from nerve cells in the auditory pathway is elicited by a brief auditory stimuli and recorded. A characteristic curve is obtained with peaks (classically numbered I-V) corresponding to the different relay stations in the auditory pathway from the cochlea to the brainstem. Thresholds are determined by varying the intensity of the stimulus in 5 dB steps around the threshold while observing the resulting curves. These should be visually reproducible above threshold. By using short sine wave stimuli a certain frequency resolution can be obtained. ABR thresholds can be used to estimate hearing loss as they follow behavioural thresholds frequency and intensity wise, albeit electrophysiological responses are elicited at a stimulus intensity level some dB higher

than behavioural responses (Borg and Engstrom 1983). All ABR measurements were performed in a soundproof test box using TDT equipment operated by a personal computer. Stimuli were presented through a speculum inserted in the external auditory canal connected to a calibrated earphone (TDH 39 or a TDT high-frequency transducer) with a flexible tube. For comparisons shift in pre and post exposure ABR thresholds was calculated. Papers I-IV.



ABR system with stimulus and ABR curves

2.3.2.3 Pure tone audiometry

Pure-tone air conduction audiometry was performed at the Department of Audiology at the Karolinska Hospital, Stockholm, and at the Academic Hospital, Uppsala, according to national standards. Test persons were seated in a sound proof testbox (Tegner AB) and pure tones were presented through TDH 39 earphones with MX 41 AR cushions, calibrated to ISO-389. Paper V.

2.3.2.4 Surface preparations

OHC damage is a prominent feature of cisplatin ototoxicity and can be quantified. Cochleae were perfusion fixed, and bone was dissected away. The cell cytoskeletons were stained with a fluorescent dye connected to the fungal poison phalloidin. The hair cells present were counted semi quantitatively and compared to a historical normative material. Papers II and IV.

2.3.2.5 Creatinine analysis

Increasing creatinine is an early and rather sensitive sign of renal damage. Guinea pig creatinine was determined with a spectrophotometric method. Papers I-III.

2.3.2.6 Cisplatin analysis

As cisplatin is rapidly distributed, eliminated and degraded a stringent sampling and sample treatment scheme has to be implemented. Blood was collected under direct vision from a sampling catheter in the internal jugular vein. All blood samples were immediately collected on ice, any deviations in sampling time noted, rapidly ultrafiltrated (4°C) and then stored at -70°C until analysis. Analysis was performed using separation on anionic (cisplatin) or cationic (MHC) columns with postcolumn derivatisation with DDTC and UV detection, as described in the present papers and by Andersson and Ehrsson (1994). Papers I-III and V. Platinum concentrations were measured by Inductively Coupled Plasma Mass Spectrometry (ICP-MS) a mass spectrometric method adapted for blood ultrafiltrate samples. Paper V.

2.3.2.7 MHC purification

MHC was purified by the method described by Ehrsson et al. (Ehrsson et al., 1995). Cisplatin was dissolved in distilled water and left overnight to attain hydrolysis equilibrium. MHC was subsequently separated by LC on a porous graphitic carbon column (Hypercarb®, Shandon, Astmor, UK), and detected by UV at 283 nm. The MHC fractions were collected on ice, shielded from light and then frozen at –80˚C until time of administration. Paper III.

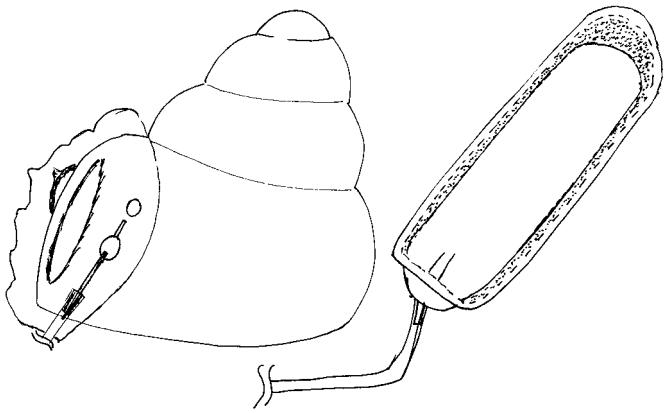
2.3.2.8 PET

The positron is the antimatter equivalent of an electron. It is formed as a result of certain forms of radioactive decay. Once formed it quickly reacts with a nearby electron emitting two gamma photons with equal energy travelling in the opposite directions. With a special detection device and signal analysis the radioactive decay can be resolved in both time and space. Comparing the observed radioactive decay within a subject with the measured activity of the administered substance, compensated for natural decay and metabolism of the administered substance, estimations of distribution and elimination of the radioactively marked substance can be made *in vivo*. Several isotopes are used for PET studies, for example ^{11}C and ^{18}F which are incorporated in suitable tracer compounds through one or more synthesis steps. In paper II [$^{11}CH_3$] D-methionine was synthesised from $^{11}CO_2$ and used to estimate D-methionine turnover and distribution. Paper II.

2.3.2.9 Continuous intracochlear administration

An implantable system designed to continuously deliver fluids directly to the cochlea at low rates has been developed and described (Brown et al., 1993; Prieskorn and Miller 2000). It has been used for infusions of a variety of substances such as nerve growth

factors (Ylikoski et al., 1998) and enzyme inhibitors (Ylikoski et al., 2002). Key features are an implantable osmotic pump, designed for exact and low flow rates, and a small diameter cannula with a seal consisting of a small silicon ball near the tip. Cannulas connected to pumps containing either TU or artificial perilymph (AP) were implanted in the left ears whereas the right ears were used as control ears. Access to the middle ear for implantation of the catheter in the inner ear was gained through a hole in the bulla. A small hole was drilled in the cochlea in which the catheter was seated. The catheter was subsequently secured in place and connected to the miniosmotic pump, which was seated in a subcutaneous pocket on the back. Care was taken when implanting the device, both in general regarding sterility and in particular when breaking the structural integrity of the cochlea to avoid damage to the sensory structures. Paper IV.



Direct intracochlear administration, schematic drawing. The cochleostomy and the catheter with its sealing silicone ball are seen near the round window niche. This is the ordinary view through the hole in the bulla during the operation.

2.4 RESULTS

2.4.1 Animal studies

2.4.1.1 Cisplatin and MHC pharmacokinetics

Bolus injection and infusion (Paper I)

Bolus injection of cisplatin 8 mg/kg caused a cisplatin peak concentration, which was considerably higher than after a one-hour infusion of the same dose. Cisplatin AUC after bolus injection was somewhat lower than after an infusion. For MHC the difference in AUC between bolus injection and infusion was not significant. The best-fit model for cisplatin elimination was a two-compartment model.

D-methionine treatment (Paper II)

Injection of D-methionine 300 mg/kg immediately before, 10 minutes before or one hour before injection of cisplatin 8 mg/kg caused a lowering of cisplatin AUC of about 30% which was not affected by the time between injection of protector and cisplatin administration. The lowering of MHC AUC was even more pronounced, about 60%. After a cisplatin dose increase by 20%, i.e. 9.6 mg/kg, there was no longer a significant difference in cisplatin AUC. However, MHC AUC was still lower in the group pre-treated with D-methionine. A two-compartment model best described elimination of cisplatin. Although not significant, there was tendency to a greater variability in cisplatin AUC among D-methionine treated animals.

MHC administration (Paper III)

Biotransformation of MHC to cisplatin and opposite *in vivo* is rapid. Biotransformation of MHC to cisplatin is favoured under normal physiological conditions. After administration of MHC 4 mg/kg the AUC of cisplatin was 23% of the AUC of cisplatin after administration of cisplatin 4.24 mg/kg. After administration of cisplatin 4.24 mg/kg the AUC of MHC was 20% of the AUC of MHC after administration of MHC 4 mg/kg. Cisplatin was found to be eliminated more slowly than MHC. About 30% of the administered dose of either cisplatin or MHC was eliminated through the urine. A two-compartment model best described elimination of the administered substance.

D-methionine kinetics (Paper II)

By using the decay of radioactivity in the heart region as a measure of D-methionine levels, D-methionine $T_{1/2}$ was found to be 58 minutes.

2.4.1.2 Ototoxicity

Bolus injection and infusion (Paper I)

There was no difference in ABR threshold shift between the animals receiving the bolus injection or those receiving the one-hour infusion of cisplatin 8 mg/kg. The

interindividual variability in ototoxic damage was significantly greater than variability in pharmacokinetic parameters after cisplatin bolus injection and infusion.

D-methionine treatment (Paper II)
After dose compensation, the difference in ABR threshold shift between dose compensated D-methionine treated animals and control animals was not significant. This result was substantiated by the cytocochleograms, which also showed no significant difference between dose compensated D-methionine treated animals and control animals. The small non-significant difference of means could, however, be found both in the cytocochleograms and in the ABRs, suggesting some remaining effect of D-methionine after dose compensation.

MHC administration (Paper III)
Administration of 4 mg/kg MHC caused a significant ABR threshold shift which was greater than that caused by the equimolar dose of 4.24 mg/kg cisplatin. It was similar to that caused by cisplatin 8 mg/kg, albeit smaller at the highest frequency.

Local administration of Thiourea (Paper IV)
The intracochlear administration of TU did not offer a significant protection from cisplatin-induced ABR threshold shift. However, a highly significant protection of OHCs was evident in the cytocochleograms.

2.4.1.3 Nephrotoxicity

Bolus injection and infusion (Paper I)
A highly significant increase in s-creatinine was found but there was no difference between the bolus injection group and the infusion group in s-creatinine change. A correlation between s-creatinine increase and ototoxicity was found.

D-methionine treatment (Paper II)
A highly significant increase in p-creatinine was found both in dose compensated D-methionine treated animals and in the control group, but there was no significant difference between the two groups. No correlation between ototoxicity and nephrotoxicity was found.

MHC administration (Paper III)
Dose titration showed dose dependent increase in p-creatinine in both MHC treated animals and in cisplatin treated animals. A sharp increase in p-creatinine was noted in MHC treated animals receiving 3.5 mg/kg MHC and in animals given 8 mg/kg cisplatin, respectively. The p-creatinine increase was significant after cisplatin 4.24 mg/kg but it was significantly greater in both the MHC 4 mg/kg group and the cisplatin 8 mg/kg group. There was no significant difference between the latter two groups. No correlation between ototoxicity and nephrotoxicity was found.

2.4.1.4 Weight loss

Bolus injection and infusion (Paper I)
A highly significant weight loss was found in both groups. Weight loss was significantly greater in the bolus injection group.

D-methionine treatment (Paper II)
Weight loss was significant among the control animals but not among the dose adjusted D-methionine treated animals. Moreover, the control animals lost significantly more weight than the D-methionine treated animals. However, the interanimal variability was greater among D-methionine treated animals.

MHC administration (Paper III)
During dose titration weight loss appeared at a dose of 3.5 mg/kg of MHC and 8 mg/kg of cisplatin, respectively. There was a highly significant weight loss both among animals treated with MHC 4.0 mg/kg and animals treated with cisplatin 8 mg/kg but there was no significant difference between the groups. Animals treated with cisplatin 4.24 mg/kg (equimolar dose) showed a significant weight increase.

Local administration of Thiourea (Paper IV)
There was no difference between the TU treated animals and the control group in regard to weight change.

2.4.2 Clinical study

High dose cisplatin with amifostine: ototoxicity and pharmacokinetics (paper V)

Cisplatin 125 mg/m^2 and amifostine 910 mg/m^2 did not provide any objective tumour response in malignant melanoma patients. After the first course 80% of the patients had auditory symptoms, after the second course 92% suffered from such symptoms and all had audiometric changes of 15 dB or more at one or more frequencies. Ultimately, 3/15 patients had a hearing loss severe enough to motivate the use of a hearing aid after cisplatin treatment. Cisplatin pharmacokinetics during the first course were not predictive of hearing loss. Amifostine caused a lowering of dose normalised AUC for cisplatin. Pharmacokinetic data could not be fitted to a compartmental model. Using the unselective method for platinum analysis (ICP-MS) lead to a non-linear, time dependent overestimation of active drug.

2.5 DISCUSSION

2.5.1 General

Cisplatin ototoxicity is still considered a clinical problem after 30 years of use. To reduce the incidence of cisplatin-induced hearing loss efforts should be aimed at the development of methods for prediction of susceptible individuals and otoprotection. Pharmacokinetic measurements could possibly be used to predict the risk of ototoxicity. There is already an extensive body of experimental evidence suggesting that it is possible to prevent cisplatin-induced ototoxicity pharmacologically. From a clinical perspective, it is of the greatest importance that the preventive interventions do not interfere with the antitumour effects of the drug. Although the ultimate way to determine benefit of a treatment is a randomised controlled trial, valuable information for the planning and execution of such a trial can be extracted from animal experiments. The main aims of the present thesis were to study the relationship between cisplatin pharmacokinetics and ototoxicity and determine the effect of treatment with putative protectors on cisplatin pharmacokinetics to ascertain that cochlear protection is not simply caused by chemical elimination of the drug and lowered systemic exposure. To do this *in vivo* experiments are necessary. In earlier experimental ototoxic works a wide variety of administration modes have been used, making it difficult to compare the studies and to relate the findings to the clinical situation. To produce a reliable and reproducible mode of administration an intravenous 15-second bolus injection of cisplatin was compared to a one-hour infusion in paper I, the 15-second bolus injection was subsequently used in the experimental part of the present thesis, paper II-IV. This allowed a characterisation of the pharmacokinetics. Fairly young guinea pigs were chosen as they are more sensitive to cisplatin (unpublished data). A growing animal population can easily be size-matched and generally have a more uniform body composition than the fully grown. This makes general toxic effects easier to detect and, furthermore, decrease the influence of body composition on the pharmacokinetics. Using this animal model some important conclusions could be drawn that have a clinical applicability.

In the planning of the experiments of this thesis a few theoretical assumptions were made:

1) A useful chemoprotector protects only normal tissue.
2) For a certain mode of administration, both pharmacological effects and side effects are more related to the actual systemic exposure to cisplatin than to the dose administered.
3) Any attempt at chemo- and particularly otoprotection is of limited clinical interest if the antitumoural effect is lowered. The true goal of chemoprotection and particularly otoprotection is an increased TI. A lowered systemic exposure to

cisplatin would most likely lead to a lowered antitumour effect, as dose intensity is important to antitumour effects of cisplatin (Ozols 1989).

4) It is known that different tumour models show widely varying sensitivity to antineoplastic drugs. Therefore results regarding dose-response relationships from one tumour model cannot be generalised.
5) When studying pharmacokinetics as correlated to side effects, indirect measurements of biological effects of cisplatin on host cells, such as platination of DNA, cannot be assumed to be an equal or superior endpoint to physiological measurements of toxicity.

Several previous studies have shown a great interindividual variability for the ototoxic side effect of cisplatin. One general hypothesis is that this interindividual variability in sensitivity to cisplatin is caused by variability in pharmacokinetics, for example high peak concentrations. If so, tailoring of treatment schedules and careful monitoring of administration aimed at lowering the interindividual variability in pharmacokinetics could perhaps help to reduce the ototoxic side effect. However, within the limits of the present study, the observed differences in ototoxicity could be more ascribed to other factors than peak concentration differences, such as differences in AUC or the effects of protectors. While ototoxicity seemed more dependent on AUC than on peak concentration, weight loss was greater for the animals receiving a higher peak after injection of cisplatin which is in agreement with earlier clinical data (Salem et al., 1984). A pharmacokinetic explanation to this apparent discrepancy between ototoxic effect and weight loss might be the protected position of the inner ear, i.e. the blood-perilymph barrier, hampering diffusion and damping short peaks in the circulation (Laurell et al., 1995) and stopping the peaks from affecting the inner ear.

Cisplatin is a highly reactive drug and it is not surprising that ototoxicity is effected in several ways. Main targets for cisplatin are the OHCs and the stria vascularis. OHC damage is expected to be irreversible whereas experimental findings suggest that strial damage may be reversible (Klis et al., 2002) and also account for a significant part of ototoxicity (Klis et al., 2000). Cisplatin ototoxicity appears rather quickly after drug administration and is fairly stable after 3 days (Laurell and Bagger-Sjoback 1991). However, the recovery of strial function and cochlear electrophysiology takes a considerably longer time (Klis et al., 2002). The knowledge of the mechanisms of cisplatin ototoxicity on the sub-cellular level is at least as limited as it is for cells in other tissues. Why cisplatin is ototoxic whereas most other antineoplastic drugs are not is open for speculation. The specific action of cisplatin on the inner ear may be related to the small size of the molecule enabling it to cross the blood-labyrinth barrier or to the fact that cisplatin is not cell-cycle specific and thus can affect even the non-dividing cells of the inner ear such as the OHCs. Furthermore, mitochondria,

which are common in the OHCs and the metabolically active stria vascularis, are known to be important cellular targets for cisplatin.

Thiol-containing compounds have been studied as potential chemoprotectors in cisplatin treatment for at least 15 years using various routes of administration. Among them D- and L-methionine have attracted a fair amount of interest, especially as they have shown promising otoprotective effects. It has even been proposed that their usefulness should be evaluated in clinical trials. It has been suggested that the protective mechanism is mediated via the antioxidant systems of the cochlea. However, theoretically thiol-containing compounds may interact chemically with cisplatin and therefore a study was designed to examine whether this occurred *in vivo* with D-methionine. If a chemical interaction occurs and causes a lowered systemic exposure to cisplatin, administration of a chemoprotector would likely not increase the therapeutic index. The risk of chemical interaction would increase if the two substances were mixed before administration but could also occur in the systemic circulation if both cisplatin and D-methionine were present in sufficient amounts. On the other hand, pre-administration of D-methionine a long time before cisplatin would be theoretically attractive if the mechanism of action is support of the antioxidant systems. A PET study showed a rather slow elimination of D-methionine as compared to what has previously been reported for L-methionine (Carter et al., 1999). This could be explained by the fact that D-methionine is not a part of normal amino acid metabolism. On the basis of this finding three time points for pre-administration were chosen, 0, 10 and 60 minutes before cisplatin administration. D-methionine was found to affect the pharmacokinetics of cisplatin but the degree of effect was not correlated to the time interval between the administration of D-methionine and cisplatin. This is interesting in view of the results from Campbell et al. who showed that administration of 150 mg/kg was as efficient as administration of 300 mg/kg D-methionine for otoprotection (Campbell et al., 1996). The longest time interval between administration of D-methionine and cisplatin in our study was 60 minutes, which theoretically would correspond to a halving of the D-methionine dose. Apparently, at these concentrations, the molar excess of D-methionine was still such that the concentration is not the critical rate-limiting factor for cisplatin elimination from the circulation. The next step in this study was to compensate the cisplatin dose so that the systemic exposure to cisplatin was equal among D-methionine treated animals and saline treated controls. However, even though the systemic exposure to cisplatin was similar in the two groups the MHC exposure was not fully compensated. When cisplatin dose compensation had been achieved a toxicity study was initiated. In the dose compensated D-methionine treated animals ABR threshold shift and OHC damage was slightly but not significantly lower than in the saline treated control group. It is tempting to ascribe this to the lower MHC exposure in the dose compensated D-methionine treated animals. The dose compensated D-methionine treated animals had a tendency to a higher creatinine increase. Moreover, in the D-

methionine treated group interindividual variability in creatinine levels was higher than in the saline control group, altogether indicating some interaction on the renal level. A higher creatinine increase among D-methionine treated animals is in agreement with what has previously been reported after L-methionine administration (Alden and Repta 1984). It can be emphasized that according to the present results, thiol-containing compounds should not be given to patients with the aim of decreasing cisplatin ototoxicity, unless pharmacokinetic studies are initiated to verify similar systemic exposure.

MHC formed intracellularly is believed to be the chemical species ultimately causing the cytotoxicity of cisplatin (Hausheer et al., 1998). When systemically administered MHC is believed to be a major cause of nephrotoxicity (Jones et al., 1991) but its importance for the ototoxic effect has not been studied previously. Different amounts of MHC may be formed both in the solution of reconstitution and in the circulation as a result of chemical manipulations. Thus, knowledge of the relative toxic effects of cisplatin and MHC is important when trying to relate the pharmacokinetics of different treatment schedules to the therapeutic effects and side effects. Earlier studies of MHC toxicity have used uncharacterised hydrolysis solutions of cisplatin instead of the purified substance. As a method for the purification of MHC in sufficient amounts for toxicity studies *in vivo* had been developed a study of the toxic effects of MHC, in particular on the auditory end organ, was performed. After administration of MHC, ABR threshold shifts were found to be caused by MHC at a considerably lower dose level than for cisplatin. Although the difference reached significance at only one frequency, the ototoxicity from MHC was somewhat smaller than that caused by slightly less than the double molar dose of cisplatin. There was no difference in weight loss and renal toxicity between the MHC group and the group receiving the double dose cisplatin. This slight imbalance between ototoxicity and weight loss/creatinine increase could indicate a different mechanism of ototoxicity from MHC as compared to cisplatin. One could for example speculate that MHC would cause more strial than OHC damage, which could be expected to recover with time (Klis et al., 2000). The difference could for example be due to a higher reactivity of MHC with strial components for example cell surface proteins or different diffusion capabilities. Another explanation could be that the increasing response part of the dose-response curve was passed for nephrotoxicity and weight loss but not for ototoxicity at this dose level of MHC. This remains to be explored as well as the relative importance of MHC and cisplatin levels during cisplatin treatment.
One pharmacokinetic observation was that the AUC for the MHC was approximately the same in paper I-III regardless of the mode of cisplatin administration and cisplatin dose. One possible explanation is that the rate of elimination of MHC in these experiments, rather than the rate of its formation, limits the systemic availability. The more rapid rate of elimination after administration of the purified compound, evidenced as a shorter elimination $T_{½}$, may partly be explained by an increased renal

clearance and partly by a higher rate of binding to endogenous nucleophilic sites. The hypothesis that elimination is the limiting step for systemic exposure is further supported by the fact that MHC levels were more affected than cisplatin levels by co-administration of the nucleophilic protector D-methionine and that dose compensation, which normalised cisplatin levels, did not fully normalise MHC levels. It was shown that renal elimination of cisplatin is important in paper III where about 30% of the administered dose was excreted in the urine. The observation that a bolus injection caused a lower cisplatin AUC than a one-hour infusion of the same dose and that injection of cisplatin 4.24 mg/kg caused a cisplatin AUC greater than half that caused by administration of 8 mg/kg could be explained by a saturable renal re-absorption in the presence of a high fraction of renal excretion, which is in accordance with an observation by Reece et al. (Reece et al., 1989).

As systemic protection was shown for the first time to cause systemic interaction between the protector and cisplatin, intracochlear administration of TU was tested. Although local administration of TU showed promising preservation of OHCs the ABR thresholds were still affected by cisplatin. Even though full otoprotection could not be achieved the observed preservation of OHCs is still of interest, especially in view of the results by Klis et al (Klis et al., 2002). They studied cisplatin ototoxicity and found a long-term recovery of CAP (compound action potential, an electrophysiological measurement of cochlear acoustic sensitivity) which was attributed to restoration of strial function but limited by OHC damage. By the intracochlear route of administration even larger molecules than TU can be delivered to the perilymphatic compartment. However, it introduces a considerable risk of traumatic damage to the cochlea and it can not yet be used in humans. Middle ear administration of gentamicin has been used clinically for the treatment of Meniere's disease (Harner et al., 2001). As TU can penetrate from the middle to the inner ear (Laurell et al., 2002) it is possible that middle ear administration could be used in the experimental situation, thereby facilitating the necessary long-term follow up of cisplatin effects on cochlear targets.

The concept of correlating biological markers of cisplatin effects in tissues to treatment outcome and use them for dose adjustment is not new. For example, platination of leukocyte DNA has been correlated with therapeutic effects (Schellens et al., 1996) and used to individually adjust cisplatin dose (Schellens et al., 2003). There is a morphological similarity between the stria vascularis and the proximal tubule of the kidney. Both tissues are ion-transporting epithelia and drugs which damage the kidney, such as aminoglycoside antibiotics and cisplatin, are also ototoxic. If a correlation between ototoxicity and nephrotoxicity could be found a measure of nephrotoxicity could perhaps be used as a biological marker to predict the ototoxic outcome. Therefore the correlation between ABR threshold change and creatinine increase was examined. Such a correlation was found only in paper I but

not in paper II and III. The lack of correlation between p-creatinine increase and ABR threshold shift in papers II and III is in agreement with data from Lautermann et al. (1995). However, this does not exclude a common mechanism of damage. First, the present studies are not completely comparable as D-methionine was co-administered as a protector in paper II. Second, the use ABR threshold shift as a measure of ototoxicity may confound the comparison, as ototoxicity likely represents the combined lesion of the OHCs and the stria vascularis. Renal damage would, from a morphological point of view, primarily be assumed to be correlated with strial damage.

Weight loss was used in papers I-III as a measure of general toxicity. Weight loss could be interpreted as a compound measure of decreased appetite and increased catabolism. It is a sensitive measure of cisplatin toxicity and probably correlates well to the long-term mortality rate. The normal development for animals of this age is a weight increase during the study period. This was evident only among the animals treated with cisplatin 4.24 mg/kg in paper II, which increased in weight during the observation period. Considering the data from the dose titration study as well as an observation on the interindividual variability among the cisplatin treated animals it can be postulated that the retarded growth rate of the animals in papers I-III was due to toxic effects rather than to surgical trauma or post-anaesthetic effects.

2.5.2 Clinical study

In the clinical situation otoprotection can be more difficult to assess than in experimental studies. Amifostine is a clinically used radio- and chemoprotector (Capizzi and Oster 2000). Its main metabolite is WR-1065, which contains a reactive thiol-group. In view of the previous experimental findings the pharmacokinetics of cisplatin was evaluated in patients with malignant melanoma receiving high-dose treatment in conjunction with amifostine. The few earlier clinical studies are showing conflicting results regarding the otoprotective effects of amifostine. Paper V is the first clinical study where an attempt has been made to correlate cisplatin pharmacokinetics based on drug concentrations measured by a selective method to the ototoxic outcome on the individual level. It is also the first clinical study where the effect of a chemoprotector on cisplatin pharmacokinetics has been evaluated with a selective method. The ototoxic side effects were troublesome and truly dose limiting despite concomitant administration of amifostine. Hearing loss could, however, not be predicted on the basis of cisplatin pharmacokinetics during the first course. It is reasonable to speculate that the lack of otoprotection could be explained by the chemical properties of amifostine. Previous experimental findings suggest that amifostine and WR-1065 do not cross the blood brain barrier unless it has been permeabilised (Lamperti et al., 1988; Utley et al., 1984) explaining the lack of

protection on the central nervous system (Capizzi 1996). As a similar system exists in the inner ear, the blood-labyrinth barrier, amifostine might simply not be able to penetrate into the vulnerable structures in the deep compartments of the inner ear. Protection would thus have to be exerted on the systemic level. In fact, the dose normalised AUC was lower in the amifostine treated group as compared to the reference group indicating a systemic interaction promoting the elimination of cisplatin. For good reasons one can speculate that the nature of interaction was similar to what was found in the D-methionine treated animals in paper II.

2.5.3 Conclusion

The most important findings of this study with a clinical applicability are listed below:

The interindividual variability in pharmacokinetic parameters was small as compared to the interindividual variability in the ototoxic outcome. The peak concentration was of minor importance for ABR threshold shift as compared to other factors. A rapid injection and a slow infusion caused similar ototoxicity. However, a higher AUC and yet similar ototoxicity and nephrotoxicity together with lower general toxicity would theoretically favour the use of an infusion of cisplatin in the clinical situation.

D-methionine affected cisplatin pharmacokinetics *in vivo* and MHC pharmacokinetics to an even greater extent. The major part of the otoprotective effect of D-methionine can be explained by its effect on cisplatin pharmacokinetics, which considerably limits its usefulness in clinical practice.

MHC caused ototoxicity, nephrotoxicity and general toxicity at a dose where cisplatin did not and was approximately twice as toxic as cisplatin. MHC pharmacokinetics should be monitored during pharmacokinetic/pharmacodynamic studies for two reasons: under normal circumstances it contributes significantly to toxicity and its turnover is affected by chemoprotection to an even greater degree than the turnover of cisplatin.

Intracochlear administration of thiourea offered partial protection from cisplatin ototoxicity as OHCs were preserved while ABR thresholds were still depressed in the TU group.

High dose cisplatin caused an unacceptable incidence of ototoxicity in spite of amifostine treatment. Thus, amifostine does not provide any important reduction of cisplatin-induced hearing loss in high dose treatment. Amifostine affected cisplatin pharmacokinetics in patients and this was not detected by the use of an unselective analytical method (ICP-MS).

It is recommended that before any clinical trial is undertaken with systemic administration of a putative chemoprotector, pilot studies of cisplatin pharmacokinetics using a selective method should be performed to ascertain similar systemic exposure of cisplatin.

It is also recommended that the pharmacokinetics of current treatment schedules should be evaluated by a selective method to obtain baseline material for future comparative purposes. This information could also possibly be used to establish non-ototoxic levels of cisplatin exposure for a patient population.

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