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ORIGINAL ARTICLE

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Ototoxicity, nephrotoxicity and pharmacokinetics of cisplatin and its monohydrated complex in the guinea pig

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Abstract *Purpose*: To evaluate and compare the ototoxicity and nephrotoxicity of cisplatin and *cis*-diammineaquachloroplatinum(II) ion (monohydrated complex of cisplatin, MHC, formed in vivo by hydrolysis of cisplatin) after their separate administration to guinea pigs. *Methods*: A dose of 4 mg/kg body weight of MHC was deemed suitable for the toxicity evaluation after dose titration. Electrophysiological hearing thresholds (auditory brainstem response, ABR), plasma creatinine and weight were measured in three groups of animals before and after receiving MHC 4 mg/kg (0.0141 mmol/kg), cisplatin 4.24 mg/kg (0.0141 mmol/kg, i.e. equimolar dose) or cisplatin 8 mg/kg (0.0267 mmol/kg) as an i.v. bolus injection. Cisplatin and MHC were analysed using liquid chromatography with post-column derivatization. *Results*: Administration of MHC 4 mg/kg caused a moderate ABR threshold shift, a significant increase in creatinine and a significant weight loss, changes similar to those seen after administration of cisplatin 8 mg/kg. Animals given cisplatin 4.24 mg/kg had a slight increase in creatinine, but had no ABR threshold shift and gained weight during the experiment. The pharmacokinetic parameters of cisplatin and MHC were estimated after administration of cisplatin 4.24 mg/kg and MHC 4 mg/kg. The area under the blood-ultrafiltrate concentration versus time curve (AUC) for cisplatin after administration of MHC 4 mg/kg was 23% (56 ± 5.0 μg·min·ml^{-1}) (means ± SD) of that after administration of cisplatin 4.24 mg/kg (240 ± 25 μg·min·ml^{-1}). The AUC for MHC after administration of cisplatin 4.24 mg/kg was 20% (30 ± 4.9 μg·min·ml^{-1}) of that after administration of MHC 4 mg/kg (149 ± 26 μg·min·ml^{-1}). *Conclusions*: MHC 4 mg/kg causes ototoxicity, nephrotoxicity and weight loss when administered to guinea pigs. The toxic effects were similar to those seen after administration of cisplatin 8 mg/kg and higher than those seen after administration of cisplatin 4.24 mg/kg.

Keywords Ototoxicity · Nephrotoxicity · Cisplatin · Monohydrated complex of cisplatin · Pharmacokinetics

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Introduction

Cisplatin [*cis*-diamminedichloroplatinum(II)] has been used in the treatment of neoplastic diseases for more than 30 years. It has been used against a variety of nonhematological malignancies, especially testicular and ovarian carcinoma [8]. Cisplatin is also used in concomitant chemoradiotherapy, for example in cervix cancer, oesophageal cancer and rectal cancer [9, 18, 34]. Even though newer platinum compounds have been developed, cisplatin can still be considered the most useful platinum-containing antineoplastic drug [29]. Prominent and potentially dose-limiting side effects of cisplatin are ototoxicity, nephrotoxicity and peripheral neuropathy [19, 26, 33]. Nausea is common but can be ameliorated by serotonin receptor antagonists [17]. Individual factors useful for predicting the risk of developing ototoxicity other than high dose and preexisting hearing loss have not been found (for review see reference 5).

The monohydrated complex (MHC), present in the blood of cisplatin-treated patients [3], is formed by hydrolytic biotransformation of cisplatin. Low chloride concentrations are believed to promote its formation in the intracellular environment where MHC is considered

to be the important cytotoxic agent mediating the reaction with DNA [21, 23]. It has been suggested that the hydrolysis products are more nephrotoxic than intact cisplatin [10] and that MHC may be responsible for all the major clinical toxicities of cisplatin [20] but the degree of involvement of MHC in ototoxicity has not previously been studied. A method for the purification of MHC in sufficient amounts to allow its administration to experimental animals has been developed [12], as well as methods for the accurate and selective analysis of cisplatin and MHC in biological fluids by liquid chromatography (LC) with post-column derivatization [1].

The objective of this study was to investigate the toxic properties of cisplatin and MHC in a dose-titration study and subsequently to evaluate the effect on auditory function, renal function and weight in animals receiving fixed doses of either cisplatin or MHC. The pharmacokinetics of cisplatin and MHC were also studied in two separate groups of animals after administration of cisplatin 4.24 mg/kg and MHC 4 mg/kg.

Materials and methods

Study design

The study consisted of three parts: dose titration, toxicity determination and pharmacokinetics.

Dose titration: Dose titration was performed to determine the approximate toxic potency of MHC (MW 283) as compared to cisplatin (MW 300) and to enable us to choose a dose level of MHC high enough to cause side effects in the guinea pig while maintaining a low mortality. Increasing doses of MHC (2, 2.5, 3, 3.5, 4, 5 and 6 mg/kg, $n=14$) were administered and the animals were monitored for weight loss and increases in plasma creatinine for 96 h after treatment. For comparison, the experiment was repeated with cisplatin (6, 10, 12 and 14 mg/kg, $n=9$). The lowest dose of cisplatin producing severe weight loss and an increase in creatinine appeared to be about twice that of MHC causing similar damage (8 mg/kg cisplatin vs 3.5 mg/kg MHC). Based on these results, a dose of MHC of 4 mg/kg was chosen since the incidence and severity of weight loss and the increase in creatinine were sufficiently high. The evaluation time used was 96 h for all toxicity parameters as the different toxicities had time to develop while mortality was comparatively low even for the higher doses of cisplatin. The time courses for development of auditory toxicity and mortality have previously been studied [25]. The 4.24 mg/kg dose of cisplatin was selected because it is equimolar and the 8 mg/kg cisplatin dose was selected because we had previous experience using this dose for the study of cisplatin ototoxicity [13, 14].

Toxicity: Side effects were determined in terms of auditory brainstem response (ABR) threshold shift, increase in plasma creatinine and weight change 96 h after injection of MHC 4 mg/kg (0.0141 mmol/kg) body weight ($n=10$), cisplatin 4.24 mg/kg (0.0141 mmol/kg, i.e. equimolar dose, $n=10$) or cisplatin 8 mg/kg (0.0267 mmol/kg, $n=10$).

Pharmacokinetics: The pharmacokinetics for cisplatin and MHC were compared in two separate groups of animals receiving either cisplatin 4.24 mg/kg ($n=5$) or MHC 4 mg/kg ($n=5$) as a bolus injection.

General procedure

A total of 63 pigmented guinea pigs of both sexes from a local breeder were used in the three parts of the study. They were housed four and four and provided with regular food and water ad libitum. The animals were anaesthetized with ketamine 65 mg/kg and xylazine 6.5 mg/kg administered intramuscularly. Additional doses of ketamine 25 mg/kg were administered when needed to maintain an adequate depth of anaesthesia during the experiment. MHC was prepared as described in the following section. Cisplatin (1 mg/ml; Platinol; Bristol Myers Squibb, New York, N.Y.) was diluted with normal saline to replicate the concentration of the MHC solution administered in each matched case to ensure that equal fluid volumes per weight were administered to all animals. During surgery the animals were kept on their back on a Harvard homeothermic pad (Harvard Apparatus, Boston, Mass.) maintaining a constant rectal temperature of 37°C. After the experiment, all animals were killed while anaesthetized. The project was approved by the local animal care and use committee, Stockholms Norra Djurförsöksetiska Nämnd (N138/99).

Preparation of MHC

MHC was purified using the method described by Ehrsson et al. [12]. Briefly, cisplatin was dissolved in distilled water to a concentration of about 4 m*M*, and left overnight at room temperature to attain hydrolytic equilibrium. Prior to separation by LC on a porous graphitic carbon column (Hypercarb, 5 μm, 100×4.6 mm ID; Thermo Hypersil-Keystone, Astmoor, UK), 1% 1 *M* NaOH (v/v) was added to shift the equilibrium towards the less-reactive monohydroxo form of MHC [2, 15]. The mobile phase was 0.5 m*M* NaOH and detection was by UV at 283 nm. Fractions containing MHC were kept on ice. The concentration of MHC was approximately 500 μg/ml, as established using a 2 m*M* hydrolysis solution of cisplatin in distilled water containing 55% MHC (determined by atomic absorption spectroscopy) as a reference. The fractions were shielded from light and kept frozen at –80°C until the time of administration. Immediately before intravenous injection the stock solution was made isotonic with Na_2SO_4 and the pH was adjusted to 7.4 with HEPES buffer. Samples of the prepared MHC solution were immediately frozen after intravenous injection and analysed later by LC with post-column derivatization to verify the concentrations.

Toxicity evaluation

Standard procedure

Animals in the ototoxicity part of the study were anaesthetized and had a baseline ABR threshold determination 2 days prior to drug administration. Immediately before randomization and treatment, all animals in both the dose-titration and the toxicity study groups were stratified according to weight to ensure similar weight in all animals in the different groups. Anaesthesia was administered, a catheter was placed in the internal jugular vein and 1 ml of blood was obtained for creatinine determination. MHC or cisplatin was administered as a 15-s bolus injection. The catheter was removed from the vein and the wound was closed. The animals were allowed to regain consciousness before being put back in their cage. After 96 h the animals were weighed, the post-treatment ABR thresholds were determined where applicable and the second blood sample for creatinine determination obtained through an internal jugular vein catheter, and finally the animals were killed.

Ototoxicity

ABR is a commonly used method to estimate hearing that does not require the cooperation of the subject. An acoustic stimulus delivered to the ear causes an electric response in the auditory system, which can be recorded as a surface EEG (electroencephalogram). The EEG response is very weak but is amplified by digital averaging of repeated EEG samples, each obtained in a short time period after each acoustic stimuli. As the signal intensity approaches the threshold, successively weaker responses are obtained until the threshold is passed and the ABR waves disappear from the averaged EEG. By repeated measurements at different frequencies

the electrophysiological auditory threshold can be determined. It follows the behavioural auditory threshold fairly well in terms of both intensity and frequency [6]. The measurements were performed with a TDT standard modular system II (Tucker Davies Technologies, Gainesville, Fl.) operated via a PC using TDT BIO SIG ver. 2.0 software. Gross pathology of the ear canal and tympanic membrane was excluded by otoscopic examination. The stimuli were delivered to the right ear through a speculum from a TDT high-frequency transducer with the animal placed in a soundproof box. The auditory stimuli consisted of 2-ms full sine-wave tone bleeps at 2, 6, 12, 20 and 30 kHz, designed with a cosine gate, and delivered to the ear at a rate of 30/s. The electric signals were picked up by stainless steel electrodes placed subcutaneously in the right infra-auricular region and in the vertex with the reference electrode placed over the bridge of the nose. The EEG signal was amplified and sampled over a 10-ms period, digitally averaged over 2000 cycles, lowpass-filtered and presented on the computer screen for online comparison. The detection limit was considered as the lowest level where a visually reproducible response was evident on the resulting curves. The stimulus was varied in 5-dB SPL increments around the electrophysiological hearing threshold. All animals had a baseline ABR response within the normal range.

Nephrotoxicity

Renal damage was estimated in all animals by calculating the difference in plasma creatinine levels before the administration of cisplatin or MHC and immediately after weighing at 96 h and just before the animals were killed. Pre-exposure creatinine levels were thus measured 2 days after the baseline ABR determination in the ototoxicity part of the study, whereas the dose titration animals had no anaesthesia before the baseline creatinine measurement. Blood (1 ml) was withdrawn and substituted with an equal volume of normal saline. The samples were centrifuged, and the plasma transferred to Eppendorf tubes and frozen. Creatinine was analysed using the certified standard method used at the Karolinska Hospital laboratory, an enzymatic two-point reaction-rate method with photometric detection at 670 nm, using dry chemicals and a Vitros 950 instrument from Ortho Clinical Diagnostics (Rochester, N.Y.).

Pharmacokinetics

Blood and urine sampling

Animals in the pharmacokinetic study were anaesthetized and had both internal jugular veins exposed and a catheter inserted on each side. Approximately 5 IU sodium heparin in 0.1 ml normal saline was administered via each catheter. One of the catheters was used for cisplatin or MHC administration and the other to obtain blood for analysis and to replace lost fluid. Blood samples of 0.35 ml were drawn at 1, 3, 6, 10, 20, 40, 60 and 90 min after drug injection. After each sampling the animal was given an equal volume of normal saline to rinse the catheter and replace fluid loss. The samples were collected in prechilled Microtainer tubes (Becton Dickinson, Franklin Lakes, N.J.) with lyophilized lithium heparin and kept on ice. Within 20 min the blood samples were ultrafiltered centripetally using a 10,000 Da cut-off filter (Centrisart, Sartorius, Göttingen, Germany) for 20 min at 4000 *g* and 4°C and the ultrafiltrates were frozen at –80°C until final analysis within 1 month. All animals had the bladder catheterized and emptied immediately before administration of drug. Urine from the indwelling catheter was then continuously collected on ice during the experiment. All urine samples were frozen at –80°C after volume measurement.

Analysis of cisplatin and MHC

Cisplatin and MHC were analysed in blood ultrafiltrates and urine using LC with post-column derivatization as described by Andersson et al. [1]. Briefly, cisplatin was separated by LC on a strong anionic exchange column (Nucleosil SB 5 μm, 200×4.6 mm ID) using 0.055 *M* succinic acid adjusted to pH 5.0 with sodium hydroxide and methanol (2:3 v/v) as mobile phase. Post-column derivatization in a packed bed reactor with *N*,*N*-diethyldithiocarbamate yielded a complex which could be quantitatively detected by UV absorption at 344 nm. MHC was separated on a strong cationic exchange column (Nucleosil SA 5 μm 200×3.2 mm ID) using 0.055 *M* succinic acid with added $NaClO_4$ to achieve a final concentration of 0.05 *M* (adjusted to pH 5.0 with sodium hydroxide) and methanol (2:3 v/v) as mobile phase, with detection as described for cisplatin. The precision of the analytical method is 11.5% (CV) for MHC at 20 ng/ml and 8% (CV) for cisplatin at 9 ng/ml [1]. Standard curves for the concentration determination were prepared for both cisplatin (0.1–50 μg/ml) and MHC (0.1–25 μg/ml) before each run. Known amounts of each drug were added to blood from untreated guinea pigs and the standard samples were handled in the same way as the samples collected from treated animals. All samples analysed had concentrations within the range of the standard curves.

Pharmacokinetic analysis

AUC (area under the blood-ultrafiltrate concentration-time curve) from zero to infinity and the selected pharmacokinetic parameters for cisplatin after cisplatin administration and MHC after MHC administration were estimated by pharmacokinetic modelling using WINNONLIN 1.5 SCI (Scientific Consulting, Cary, N.C.) software. The AUCs for MHC after cisplatin administration and for cisplatin after MHC administration were estimated according to the trapezoidal rule between 0 and 90 min. Elimination rates were calculated after curve fitting to a monoexponential or a biexponential model using JANA software [11]. Residual areas were calculated using the individually estimated elimination rate constants (k_{el}) and the measured concentration at 90 min. C_{max} and T_{max} for the biotransformation products were taken from the actual measurements.

Statistics

Calculations were made using nonparametric tests. $P < 0.05$ was considered significant. The Kruskal-Wallis test with Dunn's post test was used for comparison of the increases in creatinine, weight changes and pharmacokinetic data between groups. ABR data were compared using the Mann Whitney *U*-test.

Results

Dose titration

Increasing doses of cisplatin and MHC caused successively larger increases in plasma creatinine and weight losses (Table 1). Cisplatin doses of 4.24 or 6 mg/kg caused only limited increases in creatinine and no weight loss whereas doses of 8 mg/kg and higher caused marked increases in creatinine and weight loss. A similar pattern was seen for MHC with 3.5 mg/kg being the dose at which increase in creatinine and weight loss appeared in the experiment. In the MHC group, one blood sample was lost from an animal treated with MHC 3.5 mg/kg. One animal in the MHC group treated with 6 mg/kg died immediately before a blood sample could be obtained on day 4. One of three animals receiving cisplatin 12 mg/kg and both animals receiving

Table 1 Weight change and increase in plasma creatinine level (means ± SD). Pretreatment weight was 224.9 ± 7.1 g and creatinine was 25 ± 2.1 μmol/l

	Dose (mg/kg)	Weight change (g)	Posttreatment creatinine (μmol/l)
Cisplatin	4.24	23.0 ± 8.8 (n = 10)	41 ± 18 (+56%, n = 10)
	6	14 and 5 (n = 2)	38 and 49 (n = 2)
	8	–19.7 ± 12.3 (n = 10)	113 ± 51 (+349%, n = 10)
	10	–28 and –29 (n = 2)	201 and 267 (n = 2)
	12	–25 and –28 (n = 2)	240 and 250 (n = 2)
MHC	2	28 and 16 (n = 2)	26 and 27 (n = 2)
	2.5	8 and 9 (n = 2)	31 and 36 (n = 2)
	3	6 and 8 (n = 2)	30 and 31 (n = 2)
	3.5	–23 and –48 (n = 2)	125 (n = 1)
	4	–22.2 ± 13.0 (n = 12)	106 ± 52 (+324%, n = 12)
	5	–26 and –48 (n = 2)	78 and 177 (n = 2)
	6	–26 and –32 (n = 2)	257 (n = 1)

14 mg/kg had to be killed on day 3 due to deterioration in general condition.

Toxicity evaluation

Ototoxicity

There was a significant threshold shift at stimulus frequencies of 30, 20 and 12 kHz in the groups of animals receiving cisplatin 8 mg/kg and MHC 4 mg/kg (Fig. 1). There was no significant change in ABR threshold at any frequency in the group receiving cisplatin 4.24 mg/kg. In the cisplatin 8 mg/kg and MHC 4 mg/kg groups, the threshold shift was significantly greater than in the cisplatin 4.24 mg/kg group at 30, 20 and 12 kHz. Cisplatin 8 mg/kg caused a significantly greater threshold shift at 30 kHz than MHC 4 mg/kg.

Nephrotoxicity and weight change

Increases in plasma creatinine levels were significant in all three groups. Cisplatin 4.24 mg/kg caused a low but uniform increase whereas cisplatin 8 mg/kg and MHC 4 mg/kg caused a large increase in most of the treated animals. There was no difference in the increases in creatinine between groups receiving cisplatin 8 mg/kg or MHC 4 mg/kg but both these groups had significantly higher increases in creatinine than the cisplatin 4.24 mg/kg group (Table 1). There was a significant weight loss in animals receiving cisplatin 8 mg/kg and those receiving MHC 4 mg/kg, but there was no difference between the two groups. In contrast, the animals receiving cisplatin 4.24 mg/kg showed a significant weight increase (Table 1).

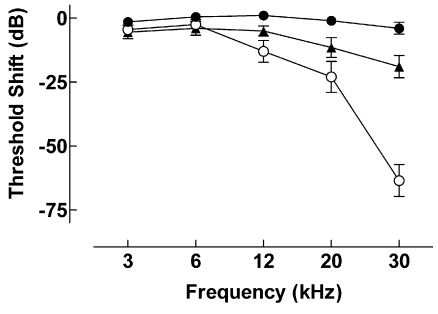

Fig. 1 ABR (auditory brainstem response) threshold shift 96 h after administration of cisplatin 4.24 mg/kg (*closed circles*, n = 10), 8 mg/kg (*open circles*, n = 10) and MHC 4 mg/kg (*closed triangles*, n = 10). Data are expressed as means ± SEM. Frequencies cited are centre frequencies of the presented auditory stimuli. The expected mean threshold shift for unexposed animals was 0 dB at all frequencies

Pharmacokinetics

Cisplatin and MHC pharmacokinetics

The main pharmacokinetic data for cisplatin and MHC after administration of cisplatin 4.24 mg/kg and MHC 4 mg/kg are presented in Table 2. The relationships between the AUCs of cisplatin and MHC in the two groups are displayed in Figs. 2 and 3. The AUC of cisplatin in the MHC 4 mg/kg group was 23% of the AUC of cisplatin in the cisplatin 4.24 mg/kg group. The AUC of MHC in the cisplatin 4.24 mg/kg group was 20% of the AUC of MHC in the MHC 4 mg/kg group. For both administered compounds, cisplatin or MHC, the best fit of concentration data as judged by the F-ratio test [7] was obtained with a two-compartment model in all animals except one in the MHC group. Comparing the elimination rates of the administered compounds, cisplatin was found to be eliminated more slowly than MHC ($t_{1/2}$ 20.7 vs 13.0 min). The concentrations of the formed biotransformation products showed a slow increase and a slow elimination.

Renal clearance of cisplatin and MHC

A significant amount of both the administered parent compound and the formed secondary cisplatin or MHC was excreted in the urine. In total 30.4% of the dose administered was excreted in the urine as either cisplatin (26.4%) or MHC (4.0%) in animals receiving cisplatin 4.24 mg/kg. In animals receiving MHC 4 mg/kg, 33.4% of the administered dose was excreted as either MHC (25%) or cisplatin (8.4%). Renal clearance was calcu-

Table 2 Pharmacokinetic parameters of cisplatin and MHC after administration to guinea pigs. The AUC values presented are the values from 0 to infinity. Residual areas and $t_{1/2}\beta$ are presented as mean (range) and AUC and C_{max} as mean ± SD. The T_{max} values for the administered compound were assumed to be at the end of the 15-s injection period. For the biotransformation products the actual median sample time of C_{max} is cited as T_{max}

Parameter	Group	
	Cisplatin 4.24 mg/kg	MHC 4 mg/kg
Cisplatin AUC (μg·min·ml^{-1})	240 ± 25 (n = 5)	56 ± 5.0 (n = 5)
Residual area (%)	3.7 (2.7–4.3)	11.0 (5.2–16.9)
Cisplatin C_{max} (μg/ml)	15.2 ± 0.74	1.05 ± 0.06 (T_{max} 10 min)
MHC AUC (μg·min·ml^{-1})	30 ± 4.9 (n = 5)	149 ± 26 (n = 4[a])
Residual area	20.4 (6.2–26)	1.7 (0.3–3.1)
MHC C_{max} (μg/ml)	0.6 ± 0.7 (T_{max} 10 min)	12.5 ± 3.4
Cisplatin $t_{1/2}\beta$ (min)	20.7 (17.8–24.8, n = 5)	
MHC $t_{1/2}\beta$ (min)		13.0 (10.0–16.9, n = 4[a])

[a]The samples from one animal treated with MHC could not be evaluated for MHC due to interference in the chromatogram

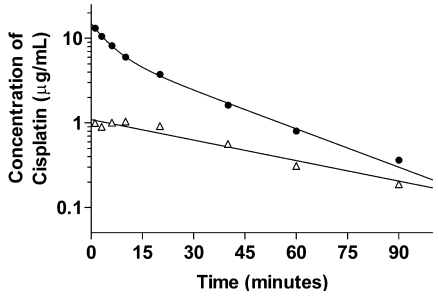

Fig. 2 Plasma ultrafiltrate elimination profile of cisplatin after administration of cisplatin 4.24 mg/kg (*closed circles*, n = 5) and MHC 4 mg/kg (*open triangles*, n = 5). The values shown are mean concentrations

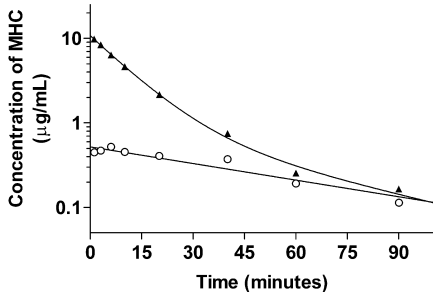

Fig. 3 Blood ultrafiltrate elimination profile of MHC after administration of cisplatin 4.24 mg/kg (*open circles*, n = 5) and MHC 4 mg/kg (*closed triangles*, n = 4). The values shown are mean concentrations

lated using the formula: clearance = (amount in urine$_{0-90\ min}$)/($AUC_{0-90\ min}$). The renal clearance of MHC after administration of MHC 4 mg/kg (1.66 ± 0.21 ml·min^{-1}, n = 4) was significantly higher than the renal clearance of cisplatin after administration of cisplatin 4.24 mg/kg (1.07 ± 0.19 ml·min^{-1}, n = 4).

Discussion

To the best of our knowledge this is the first study investigating the toxic properties of MHC. It is important to consider the relative toxicities of MHC and cisplatin for several reasons. Apart from being an important cisplatin biotransformation product in humans [3], much indirect evidence points to its involvement in the induction of side effects during cisplatin treatment. For example, Jones et al. found that a hydrolysis mixture of cisplatin in sterile deionized water is approximately three times as nephrotoxic as cisplatin on a molar basis [22]. Daley-Yates and McBrien reported that a hydrolysis product of cisplatin in plasma ultrafiltrate is nephrotoxic at a concentration at which cisplatin is not, but the exact composition of the product was not determined [10]. Using hydrolysis mixtures of cisplatin, Zheng et al. have shown greater cellular accumulation of platinum and greater cytotoxic and nephrotoxic activity as compared to cisplatin [36]. The interpretation of these results is confounded by the fact that hydrolysis mixtures not only contain varying amounts of MHC and cisplatin, but also dihydrated complexes and possibly platinum-containing dimers [4, 15]. Using purified MHC instead of a hydrolysis mixture, Yachnin et al. found greater cytotoxicity for purified MHC than for cisplatin in the small-cell lung cancer line U-1285 [35]. Litterst et al. studied the effect of different NaCl concentrations in the reconstitution fluid [28]. High NaCl concentrations, which decrease the amount of MHC formed, decrease toxicity. Hypertonic saline as a reconstitution fluid was introduced clinically by Ozols et al. [31] based on these data.

The toxic effects of cisplatin and MHC were compared in three groups of ten guinea pigs receiving MHC 4 mg/kg (0.0141 mmol/kg), cisplatin 4.24 mg/kg (0.0141 mmol/kg, i.e. equimolar dose) or cisplatin 8 mg/kg (0.0267 mmol/kg). Most probably the main part of the observed toxicity was produced by the administered compound, as opposed to its biotransformation product, in all three groups since the AUC of MHC in the MHC 4 mg/kg group was five times larger than in the cisplatin 4.24 mg/kg group and the AUC of cisplatin in the cisplatin 4.24 mg/kg group was 4.5 times larger than in the MHC 4 mg/kg group.

The most commonly used method to quantify cisplatin ototoxicity in animal models is to measure the effect

on electrophysiological hearing thresholds (ABR). Histological methods such as surface preparations with outer hair cell counts may also add information. In our opinion, measurement of ABR threshold shift, when applicable, offers the most relevant functional information regarding MHC and cisplatin ototoxicity. Both cisplatin 8 mg/kg and MHC 4 mg/kg caused a significant and similar ABR threshold shift. The shape of these two ABR curves was typical for cisplatin ototoxicity with the characteristic extension to the higher frequencies and was similar to the curve shapes observed after cisplatin administration in this animal model found both in the study presented here and previously [13]. Cisplatin 8 mg/kg produced a significantly more pronounced threshold shift at 30 kHz than MHC 4 mg/kg, indicating a greater ototoxic effect. Cisplatin 4.24 mg/kg on the other hand caused no significant threshold shift at any frequency. Consequently, MHC 4 mg/kg was more ototoxic than cisplatin 4.24 mg/kg. As for cisplatin 8 mg/kg, the interindividual variability in susceptibility to the ototoxic effect was also high among the animals in the MHC 4 mg/kg group, some being slightly affected and others severely affected. This is consistent with our previous observations on cisplatin [13].

The inner ear consists of a membranous labyrinth divided by tight barriers into three compartments or scalae. The middle scala is filled with a fluid with a unique composition resembling that of the intracellular fluid in its high potassium content [32]. It is possible that the ionic milieu affects the hydrolysis reactions between cisplatin and MHC. Cisplatin has multiple toxic effects on the inner ear both on the ion-transporting epithelia of the middle scala, the stria vascularis, and on the outer hair cells [24, 25, 30]. Cisplatin is known to penetrate to the perilymph of the inner ear from where it can reach the hair cells and exert its toxic action [27]. It is possible that the mechanism behind ototoxic injuries from MHC differ from that causing cisplatin ototoxicity, for example due to different transport properties across the inner ear barriers, but this remains to be explored.

Plasma creatinine level was used as an approximate measure of renal function. It is not specific and tends to underestimate clearance loss [16], and may be affected by the degree of hydration and catabolism in the animals. There was a significant increase in creatinine in all three groups. The animals receiving MHC 4 mg/kg and those receiving cisplatin 8 mg/kg were not different from each other, but differed from the animals receiving cisplatin 4.24 mg/kg. Judging from the increase in creatinine, MHC 4 mg/kg is thus more nephrotoxic than cisplatin 4.24 mg/kg. The growth of animals receiving cisplatin 8 mg/kg or MHC 4 mg/kg was inhibited and these animals lost weight. The weight loss is interpreted as a compound measure of catabolism and gastrointestinal toxicity. The animals in the group receiving cisplatin 4.24 mg/kg increased significantly in weight. This would be expected among untreated animals at this age and points to the limited toxic effect of this dose.

The toxic dose potency of MHC was greater than that of cisplatin for all studied parameters. One possible explanation could be that both substances cause toxic effects by the same type of reaction but at different cellular levels. MHC being more reactive and polar may cause more localized damage to cell surface proteins causing a rapid demise of the cell. Cisplatin, on the other hand, may cause more widespread damage at the cellular level that is easier to repair and/or sublethal and/or with a different time course.

Cisplatin and MHC were analysed by LC with post-column derivatization [1]. The elimination of respective parent drug was biphasic with a rapid distribution phase and a slower elimination phase. The blood ultrafiltrate concentration time curves for cisplatin formed after MHC treatment and for MHC formed after cisplatin treatment were rather flat. This could possibly have been due to a rapid initial elimination of both parent drug and formed biotransformation product cutting the peak. In humans the AUC of MHC is about 15% of the AUC of cisplatin after cisplatin administration [3]. In animals in the MHC 4 mg/kg group, the AUC of MHC was about 13% of the AUC of cisplatin in animals in the cisplatin 4.24 mg/kg group, agreeing well with the previous results from humans. The AUC of cisplatin in the MHC 4 mg/kg group was 37% of the AUC of MHC. These findings suggest that biotransformation of MHC to cisplatin is favoured under normal physiological conditions in vivo. We believe the more rapid elimination of MHC as compared to cisplatin is due to its higher chemical reactivity. The urine was continuously sampled on ice through an indwelling low-volume catheter over 90 min. Thus small changes in the amounts and proportions of biotransformation products in the urine during this period cannot be ruled out. There were also possibly individual factors in the composition of urine that may have affected degradation. The data must therefore be interpreted with care. Notwithstanding the technical limitations, renal excretion was found to be an important route of elimination by which approximately 30% of the administered dose was eliminated after both cisplatin and MHC administration. The higher renal clearance of MHC than of cisplatin might have been due to the more polar character of MHC. However, the fate of cisplatin and MHC in the kidney is complicated, and the process was not specifically studied.

In conclusion, MHC causes ototoxicity, nephrotoxicity and weight loss at dose levels at which cisplatin does not. Interventions such as administration of protective substances [14] or alterations in the vehicle salinity may affect MHC concentrations to a greater extent than cisplatin concentrations. Thus it is imperative to quantify both substances when performing pharmacokinetic/pharmacodynamic studies.

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