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SHORT COMMUNICATION

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Distribution of cisplatin in perilymph and cerebrospinal fluid after intravenous administration in the guinea pig

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Abstract The concentration of free cisplatin was followed in plasma, scala tympani perilymph and cerebrospinal fluid (CSF) after an intravenous injection (12.5 mg/kg) in guinea pigs. Liquid chromatography with postcolumn derivatization was used for quantitative determination of the drug. The distribution of cisplatin to CSF was fast; at 10 min after drug administration the concentration was 7 μg/ml and the CSF:plasma ratio was 0.37. Cisplatin seems to distribute more slowly to the perilymphatic compartment. The highest concentration measured was 4 μg/ml at 20 min after the injection, and the perilymph:plasma ratio was 0.40 at that time. The concentration-time curves generated for cisplatin in perilymph and CSF were similar. No accumulation in the perilymphatic compartment or CSF was observed.

Key words Pharmacokinetics · Blood-CSF barrier
Blood-perilymph barrier

Introduction

Cisplatin is the first of a number of platinum-containing compounds used in the treatment of human malignancies [19]. Even though other platinum analogs have been developed in an effort to minimize the toxic side effects, cisplatin remains at more than 20 years after its introduction the first-line treatment of several metastatized solid tumors. Cisplatin is the most important antineoplastic drug in the treatment of disseminated testicular cancer and ovarian adenocarcinoma [15, 24]. In spite of successful modifications of drug administration to reduce the serious and dose-limiting nephrotoxic effect [9], cisplatin-induced hearing loss continues to be a clinical problem in some patients receiving high-dose treatment [12, 13]. The ototoxic side effect of cisplatin shows large interindividual variability, and whether this variability is due to differences in drug concentrations in inner-ear tissues or to physiological factors has not been established. To the best of our knowledge, no information is available concerning the distribution of cisplatin in the inner ear following intravenous administration of the drug. The distribution of cisplatin to cerebrospinal fluid (CSF) has previously been studied in patients with brain tumors [2, 5, 22] and in rhesus monkeys [7]. However, total platinum, i.e., free and protein-bound [2, 7, 22] or ultrafiltrable "free" platinum [5], has been determined using atomic absorption or X-ray-dispersive spectrometry and, thus, the chemical nature of the platinum species has not been established. The present study demonstrates for the first time the distribution of intact drug to scala tympani perilymph (PLT) and CSF after intravenous administration of cisplatin to guinea pigs.

Materials and methods

Animal groups

Pigmented guinea pigs with a weight range of 250–550 g (median, 400 g) were used. The animals were divided into three groups for determination of cisplatin concentration in plasma ($n = 6$), PLT ($n = 21$), and CSF ($n = 13$).

Sampling technique

Each animal was anesthetized with diazepam at 5 mg/kg, droperidol at 5 mg/kg, and pentobarbital at 20 mg/kg (injected intraperitoneally). All surgical procedures were done with the animal placed on a thermic isolating sheet and a heater attached above it. The external jugular veins were exposed with the animal placed on its back and a catheter was inserted in each vein, one being used for cisplatin injection and the other, for obtaining blood samples. Cisplatin (12.5 mg/kg) at a concentration of 0.5 mg/ml (Platinol, Bristol-Myers Squibb Company) was injected i.v. over 3 min. Blood was drawn at the end of

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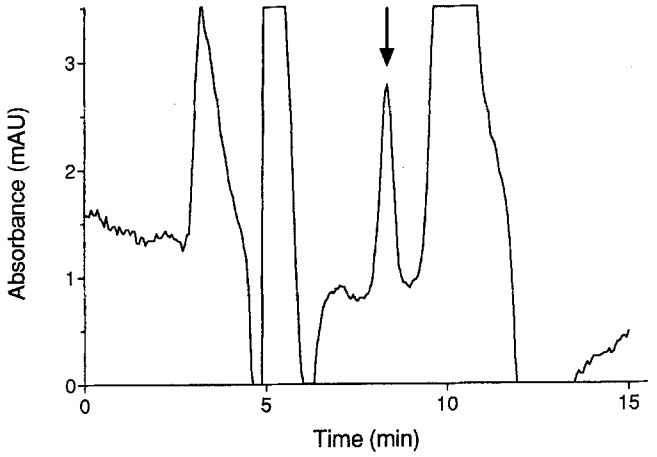

Fig. 1 Chromatogram of a perilymph sample (3.5 µl) containing 3.0 µg cisplatin/ml

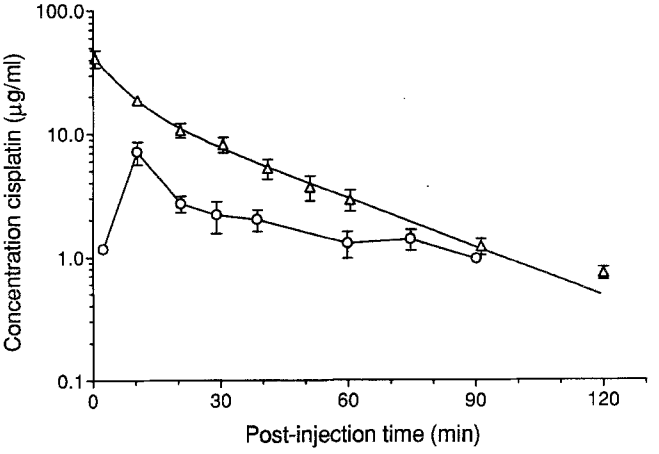

Fig. 2 Concentration of cisplatin detected in CSF (○) and plasma ultrafiltrate (Δ) in guinea pigs after an i.v. injection of 12.5 mg/kg. *Error bars* represent SEM

the injection, every 10 min for up to 60 min, and then at 90 and 120 min.

CSF pressure was reduced before perilymph aspiration by opening the dura mater. The bulla was exposed through a dorsolateral approach and the middle ear ossicles were kept intact. A hole was drilled in the otic capsule over the scala tympani in the basal turn. A 10-µl syringe filled with 2 µl normal saline was inserted into a holder attached to a micromanipulator and the tip of the syringe was lowered into the scala tympani. PLT (3–5 µl) was gently aspirated over 3–5 min. One sample of PLT was taken from each cochlea at up to 90 min after drug injection. CSF was collected from the subarachnoid space in the posterior cranial fossa. A wide hole was made in the occipital bone in the middle near the foramen magnum, exposing the dura mater. A sharp incision was made through the dura and arachnoidea and part of the cerebellum was retracted. Clear CSF (5–10 µl) was collected in a 10-µl syringe over 2–5 min. One to five consecutive CSF samples were collected within 90 min after drug injection, with a total volume of less than 40 µl being obtained from each animal.

Analytical procedure

The instrumentation and conditions for the liquid chromatographic (LC) system used for the quantitative determination of intact cisplatin has been described elsewhere [1]. Briefly, the column used was a strong anionic exchanger (Nucleosil SA 5 µm, 150 × 3.2 mm inside diameter) and a mobile phase composed of 0.125 *M* succinic acid adjusted to pH 5.2 with sodium hydroxide and methanol (2:3, v/v). The postcolumn reaction was performed in a packed-bed reactor using diethyldithiocarbamate as the derivatization reagent. The absorbance was monitored at 344 nm.

Blood samples (0.3–0.4 ml) were collected in heparinized tubes and were immediately centrifuged for 7 min at 500 *g* at room temperature. Each plasma sample was further ultracentrifuged at 4,000 *g* (4 °C) for 30 min using filters with a 10,000 Da cutoff (Filtron). The ultrafiltrates were immediately analyzed.

The PLT and CSF samples were stored at 8 °C until analysis was performed (< 3 h). Both PLT and CSF samples were directly injected into the LC system without any sample pretreatment. A chromatogram of a perilymph sample containing 3.0 µg cisplatin/ml is given in Fig. 1. The lower limit of detection was about 0.5 µg/ml.

Determination of in vitro degradation of cisplatin in CSF

CSF (50 µl) was pooled from two control animals and maintained at 37 °C in a water bath. Cisplatin was added to a final concentration of 150 µM. The degradation was followed with LC by injecting a 5-µl aliquot every 20 min for up to 3 h. The data were fitted to an equation expressing a first-order rate of degradation.

Pharmacokinetic analysis

All results are given as mean values ± SEM. The plasma concentration-time data were fitted to a two-compartment model using the NONLIN program (PC NONLIN 4.0 SCI Software, Lexington, Ky., USA). The calculation was based on an average of 5–6 animals for the first 60 min and on 3 and 2 animals for the 90- and 120-min time points, respectively. The elimination half-life for cisplatin in CSF was calculated from the elimination constant obtained by nonlinear regression analysis of data between 20 and 90 min. The area under the concentration-time curves (AUC) for cisplatin in PLT and CSF were calculated at between 0 and 60 min by the trapezoidal rule.

Results

In the present study the distribution of intact cisplatin in PLT and CSF after intravenous (i.v.) administration of cisplatin to guinea pigs was determined. In Fig. 2 the concentration of cisplatin measured in CSF and plasma is shown. The distribution and elimination phases in plasma had a half-life of 4.7 ± 0.7 and 23 ± 2 min, respectively. The first sample of CSF was taken at 2 min after the injection (1.2 µg/ml). At 10 min the concentration was 7.1 µg/ml and the CSF:plasma ratio was 0.37. The elimination half-life in CSF was 49 ± 6 min in vivo and 94 ± 3 min in vitro.

A comparison between the concentrations of cisplatin detected in CSF and perilymph is given in Fig. 3. The concentration measured in PLT at 20 min was determined to be 4.2 µg/ml, and at this time the PLT:plasma ratio was 0.40. It was not possible to determine the rate of elimination for cisplatin in PLT due to insufficient sensitivity of the analytical procedure. The concentrations of cisplatin in samples taken at 90 min in PLT were below the detection limit. No significant difference was found between the

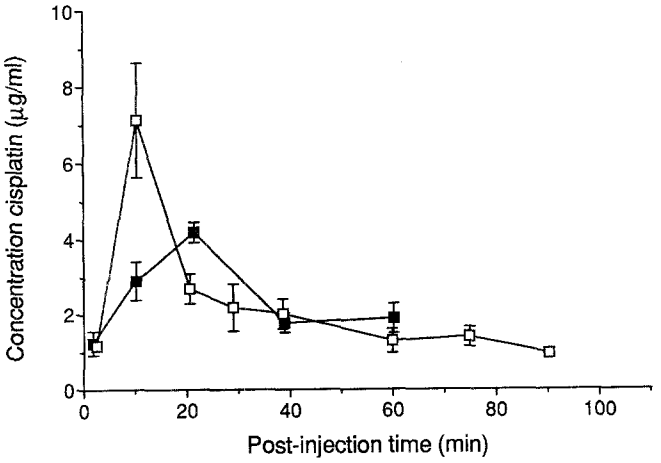

Fig. 3 Concentration of cisplatin detected in perilymph (■) and CSF (□) in guinea pigs given a dose of 12.5 mg/kg. One sample of perilymph per cochlea was taken (n = 3–8; *symbols without error bars* represent an average of two determinations). *Error bars* represent SEM

concentrations measured in PLT versus CSF at 10 min (Mann-Whitney test; $P = 0.143$, $n = 3$ and 5, respectively), but a significant difference was found at 20 min ($P = 0.004$, $n = 5$ and 6, respectively). The AUC values obtained for cisplatin in PLT and CSF were similar (148 ± 9 and 160 ± 17 µg ml^{-1} min, respectively).

Discussion

In the majority of pharmacokinetic studies thus far performed with cisplatin, total platinum or total ultrafiltrable platinum has been measured, not the intact drug. This is not satisfactory since cisplatin has a number of transformation products [14], which are vastly different in cytotoxic activity. It is generally accepted [3, 11] that cisplatin is first hydrolyzed before it reacts with its target DNA. In our previous work a method for the determination of *cis*-diamminaquachloroplatinum(II) ion (monoaqua) was developed [1], but it was not studied in the present investigation since the expected amount of monoaqua in perilymph is far below the detection limit of the method.

Intact cisplatin has previously been investigated in plasma and urine [4, 18] but not in CSF or perilymph. Gormley et al. [7] studied the total platinum in CSF and plasma in rhesus monkeys after a bolus injection and found the ratio between CSF and plasma at the time of peak concentration for CSF (20–40 min) to be as low as 0.02–0.03. The fact that only the unbound fraction of cisplatin reflects the active drug makes the results difficult to interpret. In the present study the ratio of cisplatin concentration between CSF and plasma was 0.37 at 10 min after the injection. This value is similar in magnitude to that found in a patient with neuroblastoma (0.25–0.44) receiving a 2-h infusion of cisplatin [5], whereby platinum in plasma ultrafiltrate was measured.

It is generally accepted that the blood-CSF barrier is formed mainly by the epithelium of the cells of the choroid plexus. Clear-cut evidence for the site of perilymph production is lacking, but it has been proposed that the perilymph composition is maintained only by a local mechanism in the cochlea without any contribution from the CSF [16]. No active transport mechanism has been proven for cisplatin [6]. It is not possible by the present methodological approach to establish whether a passive or active mechanism is responsible for the fast distribution and the rather high concentration of intact drug found in PLT and CSF. The ototoxic effect may be explained by altered transport properties in the cochlea at other sites, e.g., the stria vascularis. Additionally, the blood-CSF barrier is only one of several extracellular pathways via which blood-borne substances may enter the central nervous system.

Even though the calculated AUC values for CSF and PLT did not differ, the shape of the curves were different. This supports an earlier hypothesis of structural differences in barrier function between the blood-CSF and the blood-perilymph barriers [10]. The blood-CSF barrier is characterized by selective permeability properties [17]. Previous studies have reported that the penetration of non-electrolytes through pores in the blood-perilymph barrier may depend on the molecular size [21]. The elimination phase of cisplatin in PLT and CSF might reflect elimination from the extracellular fluid to the plasma compartment, chemical degradation, and distribution to deeper compartments, i.e., to the cochlear sensory structures and brain cortex, respectively. Since cisplatin is hydrophilic, its access to cerebral parenchyma is expected to be limited. The pathway for cisplatin to the sensory structures in the cochlea is not known but does not appear to be mediated through the endolymphatic compartment (Laurell et al., manuscript in preparation). The results of its in vitro degradation in CSF show that a significant proportion of cisplatin is most probably eliminated by chemical reaction. Although the protein content of CSF is considerably lower than that of plasma [20], there are other substances capable of reacting with cisplatin, e.g., sulfur-containing amino acids. There is no apparent accumulation of drug in PLT, since the concentration at 90 min was below the detection limit.

Studies on perilymph pharmacokinetics are difficult to perform. Several methodological factors may influence the results, e.g., the sampling technique and the analytical procedure used for determination of the drug concentration. Different methods have been proposed to avoid methodological errors that may influence the results, i.e., mixture of PLT with CSF, minimization of the volume of the sample [8], relief of CSF pressure before aspiration of PLT [20], and blockage of the cochlear aqueduct [20]. A drawback in this study was the volume of perilymph sample needed (3–5 µl) for the quantitative determination of intact cisplatin. The detection limit of the analysis technique (2 ng injected amount) made it impossible to use the small volume (200 nl) proposed by Hara et al. [8] for kinetic studies of cisplatin in perilymph. It cannot be excluded that the connection between PLT and CSF through the cochlear aqueduct partly accounts for the similarities in the AUC

values obtained for PLT and CSF, although under physiological conditions there is no fluid transport from CSF to PLT [16].

The perilymph concentration has previously been extensively studied for the ototoxic drug gentamicin [23]. It is noteworthy that the concentration ratio determined for gentamicin between perilymph and plasma seems to be in the same range (0.24) as that found in the present study for cisplatin. This may be due to certain similarities between the molecules with regard to molecular weight, polarity, and low-level reversible protein binding. Even though the drug concentration in the sensory epithelia does not seem to be related to the concentration in the perilymph [23], the initial distribution to the inner ear is expected to be mediated through the blood-perilymph barrier and the vessels in the stria vascularis. The initial step of cisplatin-induced cochlear toxicity, i.e., the passage to and clearance from the perilymph, should therefore be considered in attempts to reduce the ototoxicity. At a certain dose level it might be possible to find a method of administration that reduces the amount of drug that is eliminated from the perilymph to the sensory structures.

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