Na^+/K^+-ATPase inhibition by cisplatin and consequences for cisplatin nephrotoxicity

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Aims. Cisplatin is a widely used chemotherapeutic. However, it is associated with numerous adverse effects. The aim of our study was examination of cisplatin interaction with Na^+/K^+-ATPase (NKA, the sodium pump). This enzyme is of crucial importance for all animal cells and particularly for the kidney, which is frequently damaged during chemotherapy.
Methods. The entire NKA was isolated from porcine kidney. Its large cytoplasmic segment connecting transmembrane helices 4 and 5 (C45), was heterologously expressed in *E.coli* (wild-type or C367S mutant). The ATPase activity was evaluated according to the inorganic phosphate production and the interaction of isolated C45 with cisplatin was studied using chronopotentiometry and mass spectrometry.
Results. Our experiments revealed that cisplatin can inhibit NKA. The finding that other platinum-based drugs with a low nephrotoxicity, carboplatin and oxaliplatin, did not inhibit NKA, suggested that NKA/cisplatin interaction is an important factor in cisplatin adverse effects. The inhibitory effect of cisplatin could be prevented by preincubation of the enzyme with reduced glutathione or DTT. Using chronopotentiometry and mass spectrometry, we found that cisplatin is bound to C45. However, our mutagenesis experiment did not confirm that the suggested Cys367 could be the binding site for cisplatin.
Conclusion. Unintended interactions of drugs present serious limitations to treatment success. Although a large number of membrane pumps have been identified as potential targets of cisplatin, vis-a-vis nephrotoxicity, NKA inhibition seems to be of crucial importance. Experiments with isolated large cytoplasmic segment C45 revealed that it is the main target of cisplatin on NKA and that the reaction with cysteine residues plays an important role in cisplatin/NKA interactions. However, further experiments must be performed to identify the interacting amino acid residues more precisely.

Key words: cisplatin, Na^+/K^+-ATPase, nephrotoxicity, adverse effects, glutathione

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INTRODUCTION

Cisplatin (cis-diamminedichloroplatinum, CDDP) is one of the most potent chemotherapy agents used in human and veterinary medicine. It was approved for the treatment of both ovarian and testicular cancer in 1978 and it is also administered for many other types of tumors, e.g. sarcomas, small cell lung cancer and lymphomas[1].

Cisplatin utilizes copper- or organic cation transporters to overcome cellular membranes[2,3]. In the cell, cisplatin is activated by an aquation reaction involving the exchange of the two chloride leaving groups with water or hydroxyl ligands. Intracellular fluid has approximately 13-times lower concentration of chloride than extracellular fluid, and it is under these conditions that the aquation reaction effectively proceeds[4]. After this activation, cisplatin can form bifunctional adducts (the two sites are on the same DNA strand) with preference for the N^7-positions of adenine and guanine (>90%), the cross-links (the binding sites are located on different DNA strands) are rather rare (<2%) (ref.[5]). Formation of these adducts and cross-links inhibits the DNA replication, thus, interfering with the cell division by mitosis. The damaged DNA elicits the DNA repair mechanisms, which in turn activate apoptosis when repair proves impossible.

Unfortunately, administration of cisplatin is associated with numerous inevitable side effects. Of these, the most clinically significant and common disadvantage is its nephrotoxicity. In addition, severe nausea, vomiting, myelosuppression, ototoxicity and neurotoxicity were reported. Earlier studies estimated that ~30% of human patients receiving an initial dose of 50-100 $mg.m^{-2}$ of cisplatin develop acute renal failure and most patients who develop some degree of renal dysfunction never fully recover[6]. In current clinical practice, the nephrotoxicity is prevented by hydration management. However, the nephrotoxity remains the dose-limiting factor[7]. It has been reported that the other two platinum-based chemotherapeutics used in the clinical praxis, oxaliplatin and carboplatin, exhibit lower nephrotoxicity than cisplatin. However, they are effec-

tive against a different spectrum of tumors and cisplatin remains as the most effective chemotherapeutic against several types of tumors (e.g. testicular cancer) (ref.[8]).

The molecular mechanism responsible for the nephrotoxicity is unknown at present but several theories have been postulated[5]. The kidneys play a major role in the homeostasis of body fluid compartments. Despite large qualitative and quantitative changes in the dietary intake of solutes and water, this organ is able to maintain the composition of the body fluids within a very narrow range. An ultrafiltrate is generated during the passage of blood into the glomeruli. The reabsorption process taking place along kidney tubules results in the daily generation of 1 to 2 L of final urine containing 1% to 5% of the filtered Na^+ load. This work is accomplished by successive renal tubule segments which exhibit specific functional properties and, hormonal control[9].

While the first step of cleaning that occurs in the glomeruli can be considered a relatively simple filtration, the reuptake of water, sodium and other valuable solutes (e.g. glucose) is driven by a sophisticated machinery of specific membrane transporters. Apparently, the fine control of Na^+ concentration is crucial in this process. First, the gradient of Na^+ is used by a variety of secondary active transporters, e.g. the transporters of glucose, Ca^{2+} or H^+ and others. Second, the concentration of sodium ions is an important contribution to the osmotic potential, and the resulting osmotic gradient favors the water reabsorption[10]. In contrast to many transporters that utilize the Na^+ gradient and in this way reduce it, there is only one transporter that creates the gradient – the Na^+/K^+-ATPase (also called sodium pump, NKA). Hence, while failure of any other transporter will decrease reuptake efficiency only of some specific solute, failure of NKA will result in the failure of the whole machinery.

Successful crystallization of the NKA provided the structural basis for understanding of the enzyme function and its interaction with other molecules[11]. The catalytic α-subunit has ten transmembrane helices (M1-M10) and two longer cytoplasmic segments that are organized into three well-separated domains. The one, designated as A, is formed by the C23 (cytoplasmic segment between the M2 and M3), the other two, designated as N and P are formed by C45. The cation-binding sites are located within the transmembrane region; the cations are coordinated by the residues of M4, M5, M6 and M8.

It has been shown that the large cytoplasmic segment C45 of the α-subunit, containing both the nucleotide binding- and phosphorylation sites, can be overexpressed in *E.coli* and purified without the rest of the enzyme. It has also been demonstrated that this isolated C45 retains its functional properties, such as ATP- or TNP-ATP-binding, suggesting that the 3D-structure is preserved[12]. Recently, we reported that the C45 also has dynamic properties that are expected for this part of the molecule within the entire enzyme[13]. This artificial system became popular, because (i) it uncouples the enzyme/nucleotide interaction from the cation transport, which facilitates the data interpretation, and (ii) solubility of the isolated C45 greatly facilitates the experimental work.

In our experiments, we focused on the NKA interaction with platinum-based chemotherapeutics. Both the entire NKA isolated from porcine kidney as well as heterologously expressed isolated C45 were used. In the previous study[14], it was suggested that cisplatin modifies the peptide GSHMASLEAVETLGSTSTICSDK. As the cysteines are typically the most reactive amino acids toward cisplatin[15], we decided to test the role of the only cysteine on this peptide by a mutagenesis experiment.

MATERIAL AND METHODS

Reagents

Unless otherwise stated, all chemicals were from Sigma-Aldrich Chemie (Steinheim, Germany). Cisplatin, oxaliplatin and carboplatin were dissolved in ddH_2O to a concentration of 1 mM. The cisplatin was activated by a 2-fold molar excess of $AgNO_3$ (24-h incubation), which irreversibly removes the chloride ligands as an AgCl precipitate, yielding the reactive diamminodiaqua-form. MALDI matrices were from Bruker Daltonik (Bremen, Germany).

Na^+/K^+-ATPase

The Na^+/K^+-ATPase from porcine kidney was isolated by the modified method of Jorgensen[16], pipetted into small aliquots, and stored in 20 mM Tris buffer, pH 7.4, containing the non-ionic detergent $C_{12}E_8$ at -20 °C. The molar concentration of Na^+/K^+-ATPase was estimated from the total protein concentration, considering MW (α+) = 165 000 and a protein purity of more than 90%, as estimated by SDS-PAGE.

Isolated C45

The large cytoplasmic segment connecting the transmembrane helices 4 and 5 (C45 loop, residues Leu^{354}-Ile^{777} of the mouse brain sequence) with a $(His)_6$-tag at the N-terminus was expressed in *E. coli* Rosetta cells (Promega, USA) and purified using a Co^{2+}-based affinity resin (Clontech, USA) as described previously[13]. Immediately after elution, the protein was dialyzed into TBS buffer (20 mM Tris, 140 mM NaCl, pH 7.6) and stored at -20 °C. Protein concentration was determined using the Bradford assay[17] with BSA as a standard.

The C367S mutant of C45

The cDNA sequence containing the point mutation in codon TGC → AGC (amino acid replacement Cys367 → Ser) and restriction sites NheI and HindIII for cloning into the final vector was designed. The 1275 bp DNA was commercially synthesized and cloned using the restriction enzymes NheI and HindIII and T4 DNA ligase (NEB) into the pET28b plasmid. The construct was multiplied in DH5α *Escherichia coli* bacteria and isolated using the NucleoSpin Plasmid (Macherey-Nagel) kit. The sequence was verified by DNA-sequencing using the primers T7-term_Inv (TAGTTATTGCTCAGCGGTGG) and T7-prom (TAATACGACTCACTATAGGG).Heterologous

expression in *E. coli* and purification of isolated C45 was in detail described in our previous manuscript[13].

Na^+/K^+-ATPase activity assay

Measurements of ATPase activity were performed according to ref.[18] with some modifications. This method is based on colored reaction of inorganic phosphate with ammonium molybdate, which is monitored as absorbance change at 710 nm. Experiments were performed using a microplate reader SynergyMx (BioTek).

The reaction was started by the addition of 0.27 μM NKA into a solution with 130 mM NaCl, 20 mM KCl, 4 mM $MgCl_2$, 3 mM ATP and 30 mM imidazole, pH 7.2 (final volume: 50 μL). After 6 min of incubation, the reaction was stopped by the addition of 75 μL of a staining solution composed of 160 mM ascorbic acid, 3.7% (v/v) acetic acid, 3% (w/v) SDS and 0.5% ammonium molybdate. After 8 min, the staining was stopped by the addition of 125 μL of a solution composed of 0.9% (w/v) bismuth citrate, 0.9% (w/v) sodium citrate and 3.7% HCl; then OD_{710} was read. A solutions of 0-25 nM KH_2PO_4 were used as a standard.

The inhibitory effect of platinum-based drugs (10 μM) on the activity of 0.27 μM NKA was measured after 1-h incubation with these drugs at 37 °C. For cisplatin, the same experiment was performed also with an enzyme that had been incubated overnight with 0.5 mM dithiothreitol (DTT) or 10 mM reduced glutathion.

Ouabain serves as a specific inhibitor of the Na^+/K^+-ATPase, and in the presence of 10 mM ouabain, the ATPase activity of untreated NKA decreased to ~10%. This residual activity has been subtracted from the total estimated ATPase activity. Hence the data are presented as the ouabain-sensitive ATPase activity and each point is represented as the mean ± S.E.M. of five replicates.

Chronopotentiometric analysis of cisplatin interactions with C45 and its C367S mutant

The interactions of C45 or its mutant C367S (5 μM) with cisplatin were analyzed using an adsorptive transfer technique combined with constant-current chronopotentiometric stripping analysis (AdT CPSA) (ref.[19-21]). For this purpose, hanging mercury drop electrode (HMDE) was first dipped into a 10-μL aliquot of the studied sample. After an accumulation period (30 s), the electrode was washed by deionized water and placed in an electrochemical cell containing the supporting electrolyte (0.2 M phosphate buffer, pH 7.4). All CPS measurements were performed at room temperature using μAutolab III analyzer (EcoChemie, NL) in a three-electrode setup (Ag/AgCl/3M KCl electrode as a reference and carbon rod as an auxiliary electrode).

Mass spectrometry

Samples (~5 mg mL^{-1}) of unreacted C45 or its C367S mutant and their derivatives after overnight reaction with cisplatin (in a molar ratio of 1:20) were dissolved in 20 mM Tris-HCl, pH 7.4, containing 20 mM NaCl. The spectra were measured on a Microflex LRF20 MALDI-TOF mass spectrometer (Bruker Daltonik) using a combination of ferulic and sinapinic acids as a matrix (FA:SA, 5:15 mg mL^{-1} in acetonitrile/2.5% trifluoroacetic acid, 7:3, v/v). A standard dried droplet technique was used for sample preparation on an MSP AnchorChip™ 600/96 target. The instrument was calibrated externally using the Protein Standard II by Bruker Daltonik according to the manufacturer's instruction.

RESULTS

Na^+/K^+-ATPase activity

We tested the influence of platinum-based drugs on the activity of full-length Na^+/K^+-ATPase. Both carboplatin and oxaliplatin exhibited only a negligible suppression of Na^+/K^+-ATPase activity even at a 40-fold molar excess (Fig. 1), and thus they were not further examined. On the other hand, the cisplatin binding was able to completely suppress the Na^+/K^+-ATPase activity. The saturation was observed from a molar ratio of protein:cisplatin of 1:4 (Fig. 2). The presence of 10 mM reduced glutathione in the reaction buffer substantially protected NKA against the inhibitory effect of cisplatin (Fig. 1).

Electrochemical studies of cisplatin interaction with C45 loop

Cisplatin interactions with the C45 loop were studied using adsorptive transfer constant-current chronopotentiometric stripping analysis (AdT CPSA) at hanging mercury drop electrode (HMDE). In our experiments, C45 loop (5 μM) was incubated with increasing concentrations of cisplatin (1-50 μM). We monitored the peak S (around –0.7 V), which reflects the reduction of a Hg-S bond[22] that occurs between Cys residues in the protein and HMDE surface (Fig. 3A). In the experiment with BSA which was used as a positive control, we observed that peak S can diminish in the presence of cisplatin (Fig. 3B). The decrease is probably related to the cisplatin binding with Cys

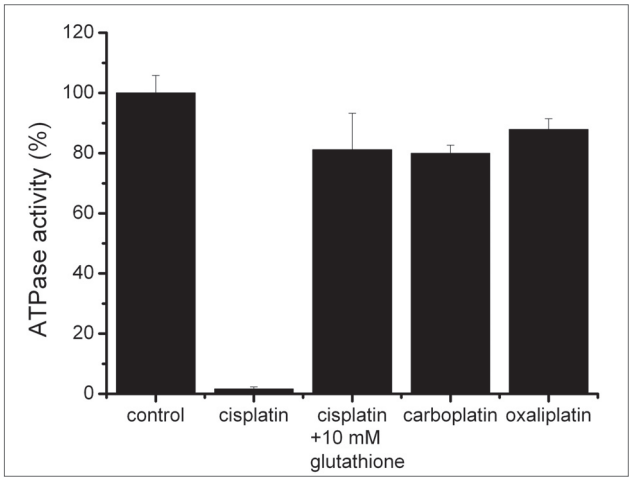

Fig. 1. Inhibition of Na^+/K^+-ATPase by platinum-based chemotherapeutics. The ATPase activity of 0.27 μM NKA was measured in the presence of 10 μM inhibitors and normalized to 100% for the untreated NKA. Preincubation of NKA with 10 mM glutathione prevented the inhibitory effect of cisplatin.

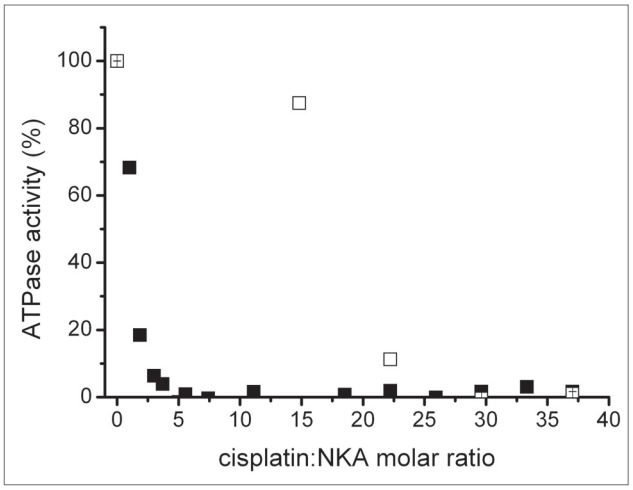

Fig. 2. NKA inhibition by cisplatin. The 0.27 μM NKA ouabain sensitive ATPase activity was inhibited by increasing concentrations of cisplatin (solid symbols). The data represent the mean of 5 independent measurements, the error bars are smaller than the symbols. The enzyme activity was not detectable for a cisplatin:NKA molar ratio of more than 4:1. On the other hand, the enzyme can be partially protected by a preincubation with 0.5 mM DTT (open symbols).

residues[23] localized on the surface of the protein, because upon cisplatin binding, the cysteine thiol group cannot form the reducible Hg-S bond.

For isolated C45, we also observed a significant peak S decrease with increasing cisplatin concentration. The complete disappearance of the peak S occurred at 10 μM cisplatin concentration, suggesting a cisplatin:C45 stoichiometry of 2:1 (Fig. 3).

MALDI-TOF experiments

Intact mass measurements of the C45 loop using MALDI-TOF mass spectrometry provided a molecular mass value of 48 419 Da (Fig. 4A), which is in agreement with the amino acid sequence. Upon the reaction with cisplatin, the molecular mass of the protein was shifted to a value of 50151 Da, indicating the presence of a modification (Fig. 4B). The observed mass difference of around 1730 Da suggests the formation of multiple adducts such as C45-[Pt], C45-[Pt(NH_3)], C45-[Pt(NH_3)$_2$], C45-[Pt(NH_3)$_2$(H_2O)] or C45-[Pt(NH_3)$_2$Cl] (ref.[24,25]).

Interaction of cisplatin with the C367S mutant

As the serine reactivity with cisplatin is rather low, the mutation C367S should eliminate one putative cisplatin binding site on C45. However, the peak S dependence on the cisplatin concentration was the same as for wild-type sequence C45 (Fig. 3B) and also the mass spectrometry of C367S mutant incubated with cisplatin provided a molecular mass value of 50168, which is in fact the same as that for the wild-type C45 (Fig. 4C) taking the experimental error into consideration. Hence, under given experimental conditions, the Cys367 is unlikely to be the cisplatin binding site.

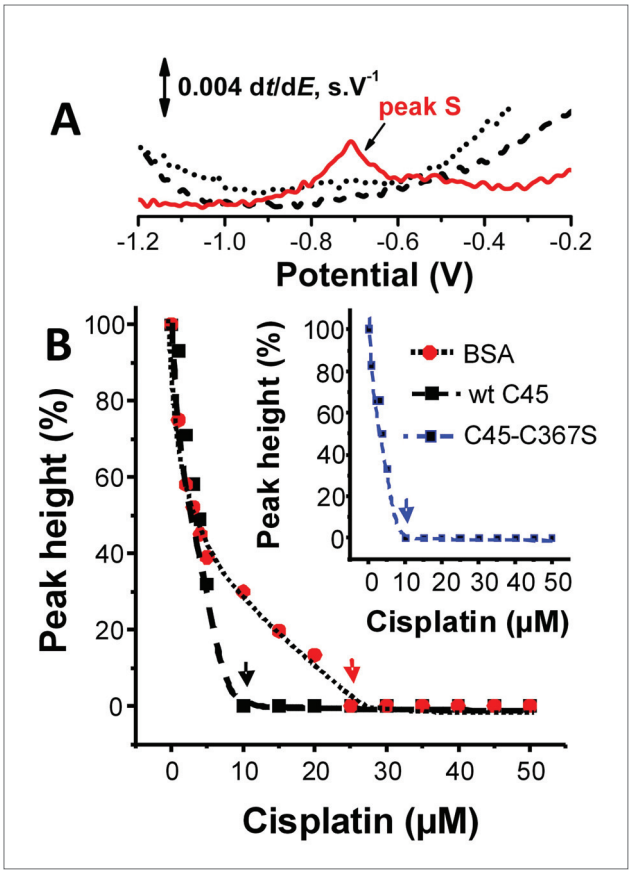

Fig. 3. (A) CPS records of 5 μM wt C45 before (–) and after (...) incubation with cisplatin; (---) supporting electrolyte. **(B)** Dependence of peak S height of wt C45 (■), mutant C45-C367S (in inset) and BSA (●) on the concentration of cisplatin. Arrows indicate stoichiometry for cisplatin:protein association via CPS peak S measurement. Concentration of cisplatin (for **A**) was 20 μM; incubation was performed at 37 °C for 20 min.

DISCUSSION

Na^+/K^+-ATPase is an enzyme of crucial importance for the metabolism of all animal cells. Consequently, any impairment of its function can inevitably influence a variety of cellular processes, and it seems to be also the case for cisplatin nephrotoxicity. Kidney metabolism is very rapid and it is finely tuned to maintain the body fluid homeostasis. Our finding that Na^+/K^+-ATPase activity was inhibited only by nephrotoxic cisplatin, while non-nephrotoxic carboplatin and oxaliplatin did not affect the enzyme, strongly supports our hypothesis that the molecular mechanism responsible for the well-known cisplatin nephrotoxicity may reside in cisplatin binding to Na^+/K^+-ATPase. This idea is not new, and our data obtained with the isolated enzyme are in a good agreement with previous study performed on rat kidney homogenate[26], which identified the Na^+/K^+-ATPase as cisplatin target.

The enzyme activity can be protected against cisplatin inhibition by preincubation with DTT or reduced glutathione (Fig. 1 and 2). However, the sulfur-containing species can interact also directly with cisplatin and other biomolecules within the cell, and hence their cellular effect may

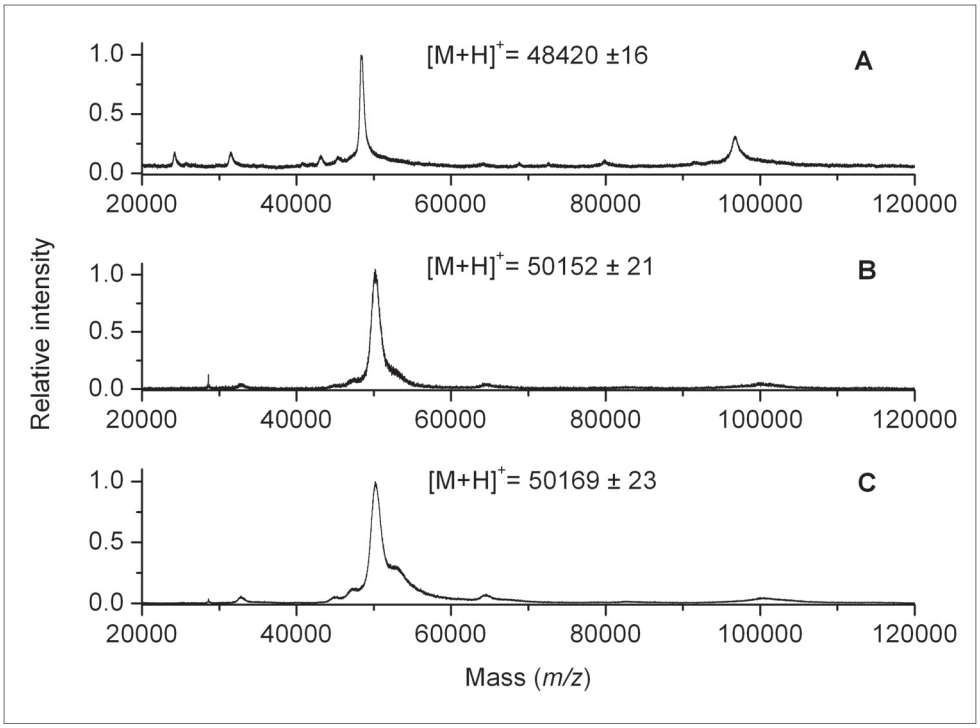

Fig. 4. Intact protein MALDI-TOF mass spectrometry of (A) unreacted C45 loop; (B) C45 loop after incubation with cisplatin; (C) C367S mutant after incubation with cisplatin.

be more complex. For example, it has been reported for reduced glutathione that on the one hand its interaction with cisplatin reduced the formation of cisplatin-DNA adducts[27], on the other hand, cisplatin-glutathione complex exhibited higher toxicity toward LCC-PK_1 cell line than cisplatin alone[28]. However, other sulphur-containing molecules, such as biotin or sulfathiazole, were shown to decrease nephrotoxicity of cisplatin, at the same time preserving its anti-tumor effect under *in vitro* conditions[29]. The prevention of nephrotoxicity by sulphur-containing species deserves attention and it could be a promising direction for further research.

Cisplatin is a water-soluble compound that becomes activated after the penetration across the plasma membrane into the cell[4]. Hence, cisplatin interaction with the cytoplasmic part of the enzyme is presumed. Indeed, our experiments confirmed the direct interaction of cisplatin with the isolated C45 loop that constitutes the major part of Na^+/K^+-ATPase, which is exposed to the cytoplasm.

Mass spectrometric analysis of the C45 loop revealed a molecular mass increase after incubation with cisplatin of ~1730 Da, indicating that C45 could bind multiple molecules of cisplatin (Fig. 4). A comparison with the determined stoichiometry of the maximum Na^+/K^+-ATPase activity inhibition suggests that the C45 loop contains a sufficient number of cisplatin-binding sites and that it may serve as the main target for cisplatin binding to Na^+/K^+-ATPase.

The decrease of the electrochemical peak S reveals that cisplatin binds to cysteine residues (Fig. 3). This is in line with previous studies, where Cys residues were identified as the most reactive sites in the interaction of various proteins with cisplatin[30]. However, our mutagenesis experiment did not confirm that Cys367, which is found on the previously identified peptide GSHMASLEAVETLGSTSTICSDK (ref.[14]), could be the cisplatin binding site, and some other residues should be tested. It has been proposed that cisplatin can interact with several other amino acids[15,31]. Analysis of high resolution structures has revealed that particularly Met and His are very common binding partners[32].

The electrochemical experiment senses only the part of the C45 molecule, which is adsorbed onto the electrode surface. The rest of the molecule, which is not in a direct contact with the electrode, remains "invisible" in this type of experiment, and this is probably the reason why we observed only two cisplatin molecules per one C45 molecule binding stoichiometry in this type of experiment. Electrostatic surface potential maps obtained from a previously published molecular model of C45 loop revealed a large negatively charged cloud at the top of the N-domain[33]. Therefore, we presume that this part of the molecule is preferentially attracted to the positively charged HMDE surface during the AdT procedure which precedes Hg-S bond reduction (for (electro)chemical reactions of Cys with Hg-electrodes see[34]). Among others, it also contains a segment with Cys459, Cys463 and Cys464 (human α1 sequence numbering), all of which are accessible from the solvent (Fig. 5). The physiological significance of this enzyme segment is an issue of recent discussions. A recent work proposed that it could be the site of NKA redox regulation[35]. Moreover, it has been demonstrated that the N-domain interacts with Src kinase[36], although the precise interaction site has not been

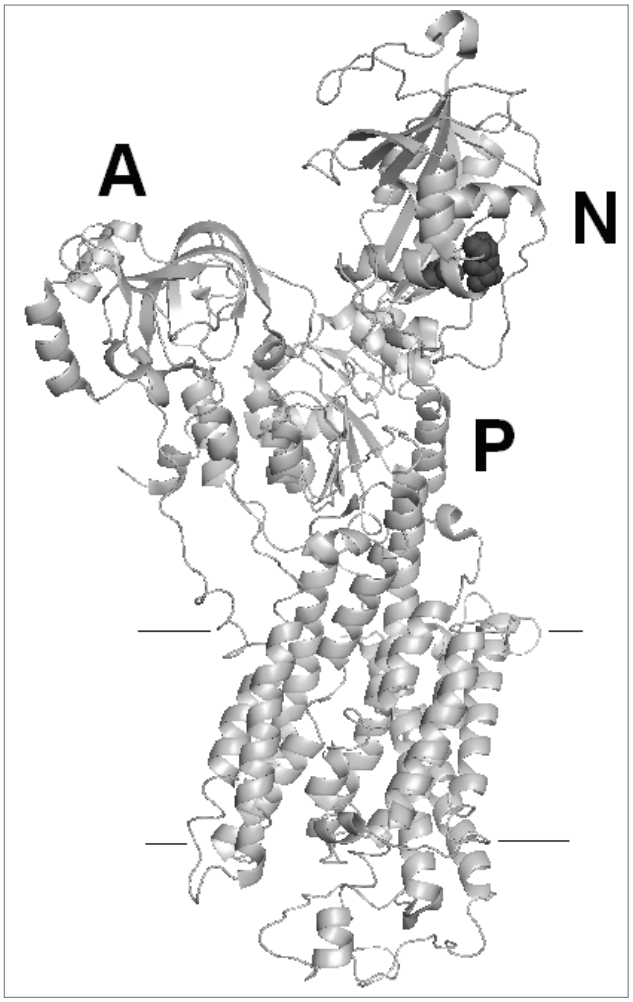

Fig. 5. High-resolution crystal structure of NKA (PDB entry 3b8e) (ref.[11]). The horizontal lines approximately delimit the plasma membrane, the cytoplasmic domains are denoted as A, N and P, the C45 is formed by domains N and P. The residues Cys459, Cys463 and Cys464 (human sequence numbering) on the N-domain are displayed as black spheres.

identified so far. Hence, a cisplatin binding could impair this protein-protein interaction, which would result in the modulation of the Src-mediated signal transduction pathway[37].

CONCLUSION

Unintended interactions of drugs present serious limitations to the cure. Although a large number of membrane pumps have been identified as potential targets of cisplatin[1,38,39], from the point of view of nephrotoxicity, NKA inhibition seems to be of crucial importance. Experiments with isolated large cytoplasmic segment C45 revealed that it is the main target of cisplatin on NKA and that the reaction with cysteines plays an important role in cisplatin/NKA interactions. However, further experiments must be performed to identify the interacting amino acid residues more precisely.

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AUTHORSHIP CONTRIBUTIONS

MK: Study design and manuscript preparation; JG, MH: Isolation of Na^+/K^+-ATPase, C45 and its C367S mutant, participation on Na^+/K^+-ATPase activity measurement; MZ, JV: Electrochemical experiments; MS: Mass spectrometry experiments.

CONFLICT OF INTEREST STATEMENT

None declared.

REFERENCES

1. Miller RP, Tadagavadi RK, Ramesh G, Reeves WB. Mechanisms of cisplatin nephrotoxicity. Toxins 2010;2:2490-18.
2. Safaei R. Role of copper transporters in the uptake and efflux of platinum containing drugs. Cancer Letters 2006;234:34-9.
3. Yonezawa A, Inui K. Organic cation transporter OCT/SLC22A and H(+)/organic cation antiporter MATE/SLC47A are key molecules for nephrotoxicity of platinum agents. Biochemical Pharmacology 2011;81:563-8.
4. Rosenberg B. Anti-cancer activity of cis-dichloroammineplatinum(II) and some relevant chemistry. Cancer Treatment Reports 1979;63:1433-8.
5. Barabas K, Milner R, Lurie D, Adin C. Cisplatin: a review of toxicities and therapeutic applications. Veterinary and Comparative Oncology 2008;6:1-18.
6. Ries F, Klastersky J. Nephrotoxicity induced by cancer-chemotherapy with special emphasis on cisplatin toxicity. American Journal of Kidney Diseases 1986;8:368-79.
7. Tsang RY, Al-Fayea T, Au HJ. Cisplatin Overdose Toxicities and Management. Drug Saf 2009;32:1109-22.
8. McWhinney SR, Goldberg RM, McLeod HL. Platinum neurotoxicity pharmacogenetics. Mol Cancer Ther 2009;8:10-6.
9. Vinciguerra M, Mordasini D, Vandewalle A, Feraille E. Hormonal and nonhormonal mechanisms of regulation of the Na,K-pump in collecting duct principal cells. Seminars in Nephrology 2005;25:312-21.
10. Jorgensen PL, Hakansson KO, Karlish SJD. Structure and mechanism of Na,K-ATPase: Functional sites and their interactions. Annu Rev Physiol 2003;65:817-49.
11. Morth JP, Pedersen BP, Toustrup-Jensen MS, Sorensen TLM, Petersen J, Andersen JP, Vilsen B, Nissen P. Crystal structure of the sodium-potassium pump. Nature 2007;450:1043-9.
12. Kubala M, Teisinger J, Ettrich R, Hofbauerova K, Kopecky V, Baumruk V, Krumscheid R, Plasek J, Schoner W, Amler E. Eight amino acids form the ATP recognition site of Na+/K+-ATPase. Biochemistry 2003;42:6446-52.
13. Grycova L, Sklenovsky P, Lansky Z, Janovska M, Otyepka M, Amler E, Teisinger J, Kubala M. ATP and magnesium drive conformational changes of the Na(+)/K(+)-ATPase cytoplasmic headpiece. Biochimica Et Biophysica Acta-Biomembranes 2009;1788:1081-91.
14. Huliciak M, Vacek J, Sebela M, Orolinova E, Znaleziona J, Havlikova M, Kubala M. Covalent binding of cisplatin impairs the function of Na+/K+-ATPase by binding to its cytoplasmic part. Biochemical Pharmacology 2012;83:1507-13.
15. Dabrowiak DC. Metals in medicine. Wiley 2009
16. Jorgensen PL. Purification of Na+/K+ATPase - enzyme sources, preparative problems, and preparation from mammalian kidney. Methods in Enzymology 1988;156:29-43.

17. Bradford MM. Rapid and Sensitive Method for Quantitation of Microgram Quantities of Protein Utilizing Principle of Protein-Dye Binding. Analytical Biochemistry 1976;72:248-54.
18. Baginski E, Foa P, Zak B. Determination of phosphorus. Clinical Chemistry 1966;12:546-52.
19. Trnkova L, Kizek R, Vacek J. Catalytic signal of rabbit liver metallothionein on a mercury electrode: a combination of derivative chronopotentiometry with adsorptive transfer stripping. Bioelectrochemistry 2002;56:57-61.
20. Zatloukalova M, Orolinova E, Kubala M, Hrbac J, Vacek J. Electrochemical Determination of Transmembrane Protein Na+/K+-ATPase and Its Cytoplasmic Loop C45. Electroanalysis 2012;24:1758-65.
21. Vacek J, Zatloukalova M, Havlikova M, Ulrichova J, Kubala M. Changes in the intrinsic electrocatalytic nature of Na+/K+ ATPase reflect structural changes on ATP-binding: Electrochemical label-free approach. Electrochem Commun 2013;27:104-7.
22. Dorcak V, Palecek E. Electrochemical Determination of Thioredoxin Redox States. Anal Chem 2009;81:1543-8.
23. Odenheimer B, Wolf W. Reactions of cisplatin with sulfur-containing amino-acids and peptides I. Cysteine and glutathione. Inorg Chim Acta 1982;66:L41-L43.
24. Hartinger CG, Ang WH, Casini A, Messori L, Keppler BK, Dyson PJ. Mass spectrometric analysis of ubiquitin-platinum interactions of leading anticancer drugs: MALDI versus ESI. Journal of Analytical Atomic Spectrometry 2007;22:960-7.
25. Zhao T, King FL. Mass-spectrometric characterization of cisplatin binding sites on native and denatured ubiquitin. Journal of Biological Inorganic Chemistry 2011;16:633-9.
26. Daley-Yates PT, McBrien DCH. The inhibition of renal ATPase by cisplatin and some biotransformation products. Chemico-Biological Interactions 1982;40:325-34.
27. Gosland M, Lum B, Schimmelpfennig J, Baker J, Doukas M. Insights into mechanisms of cisplatin resistance and potential for its clinical reversal. Pharmacotherapy 1996;16:16-39.
28. Townsend DM, Deng M, Zhang L, Lapus MG, Hanigan MH. Metabolism of cisplatin to a nephrotoxin in proximal tubule cells. J Am Soc Nephrol 2003;14:1-10.
29. Jones MM, Basinger MA, Holscher MA. Control of the nephrotoxicity of cisplatin by clinically used sulfur-containing-compounds. Fundam Appl Toxicol 1992;18:181-8.
30. Boal AK, Rosenzweig AC. Crystal Structures of Cisplatin Bound to a Human Copper Chaperone. Journal of the American Chemical Society 2009;131:14196-203.
31. Hartinger CG, Tsybin YO, Fuchser J, Dyson PJ. Characterization of platinum anticancer drug protein-binding sites using a top-down mass spectrometric approach. Inorganic Chemistry 2008;47:17-9.
32. Bischin C, Lupan A, Taciuc V, Silaghi-Dumitrescu R. Interactions Between Proteins and Platinum-Containing Anti-Cancer Drugs. Mini-Review in Medicinal Chemistry 2011;11:214-24.
33. Kubala M, Grycova L, Lansky Z, Sklenovsky P, Janovska M, Otyepka M, Teisinger J. Changes in Electrostatic Surface Potential of Na(+)/K(+)-ATPase Cytoplasmic Headpiece Induced by Cytoplasmic Ligand(s) Binding. Biophysical Journal 2009;97:1756-64.
34. Heyrovsky M, Mader P, Vesela V, Fedurco M. The reactions of cystine at mercury-electrodes. Journal of Electroanalytical Chemistry 1994;369:53-70.
35. Petrushanko IY, Yakushev S, Mitkevich VA, Kamanina YV, Ziganshin RH, Meng XY, Anashkina AA, Makhro A, Lopina OD, Gassman M, Makarov AA, Bogdanova A. S-Glutathionylation of the Na,K-ATPase Catalytic alpha Subunit Is a Determinant of the Enzyme Redox Sensitivity. Journal of Biological Chemistry 2012;287:32195-205.
36. Tian J, Cai T, Yuan ZK, Wang HJ, Liu LJ, Haas M, Maksimova E, Huang XY, Xie ZJ. Binding of Src to Na+/K+-ATPase forms a functional signaling complex. Molecular Biology of the Cell 2006;17:317-26.
37. Li ZC, Cai T, Tian J, Xie JX, Zhao XC, Liu LJ, Shapiro JI, Xie ZJ. NaKtide, a Na/K-ATPase-derived Peptide Src Inhibitor, Antagonizes Ouabain-activated Signal Transduction in Cultured Cells. Journal of Biological Chemistry 2009;284:21066-76.
38. Bhatnagar V, Ramalah A. Characterization of Mg2+-ATPase activity in isolated B16 murine melanoma melanosomes. Mol Cell Biochem 1998;189:99-106.
39. Shiraishi Y, Nagai J, Murakami T, Takano M. Effect of cisplatin on H+ transport by H+-ATPase and Na+/H+ exchanger in rat renal brush-border membrane. Life Sci 2000;67:1047-58.