# INFLUENCE OF ANTINEOPLASTIC DRUG CISPLATIN AND PLATINUM NANOPARTICLES ON CHICKEN EMBRYO LIVER AND KIDNEY AMINO ACIDS

MILOSAVLJEVIC Vedran[1,2], CERNEI Natalia[1,2], BYTESNIKOVA Zuzana[1,2], KOPEL Pavel[1,2]

[1]*Department of Chemistry and Biochemistry, Mendel University in Brno, Czech Republic, EU*

[2]*Central European Institute of Technology, Brno University of Technology, Brno, Czech Republic, EU*

**Abstract**

Cisplatin (cis-diamminedichloroplatinum II) is cytostatic currently used in the treatment of a various type of solid tumors, including cancers of the ovary, testis, bladder, head and neck, lung, cervix and endometrium. The health effects of platinum are strongly dependent upon the kind of bonds that are shaped and the exposure level and immunity of the person that is exposed. An *in vivo* interaction study was performed as a comparison of amino acid profiles in chicken embryo tissue before and after application of cisplatin and two platinum nanoparticles covered with polyvinylpyrrolidone. We found that cisplatin directly influences kidney amino acid representation even at low concentrations. In addition, we performed an interaction study that resulted in the determination of breaking points for each of analyzed amino acids. Proline, leucine, valine, serine, glutamine and isoleucine were determined as the most sensitive amino acids. Additionally, we compared amino acid profiles of liver and kidney before and after exposure to cisplatin and platinum nanoparticles. The amount of amino acids after interaction with cisplatin and platinum nanoparticles was significantly reduced ($p < 0.05$). This fact points at an ability of cisplatin and Pt nanoparticles to induce changes in quantitative composition of amino acids in chicken tissues. Moreover, this confirms that the interactions between cisplatin, platinum nanoparticles and amino acids may act as another factor most likely responsible for adverse effects of cisplatin and platinum nanoparticles on chicken embryo tissues.

**Keywords:** Kidney, liver, toxicity, amino acids, ion-exchange liquid chromatography, platinum nanoparticles

## 1. INTRODUCTION

Nowadays, Cisplatin is one of the most widely used anticancer drug [1]. Related toxicities are dose-dependent; these may be more pronounced in the setting of a cisplatin overdose, resulting in significant morbidity and/or mortality [2]. Cisplatin is used to treat different kinds of malignancies, including ovarian, cervical, head and neck, esophageal and non-small cell lung cancer [1]. The incidence of cisplatin overdoses is unknown; however, early-phase clinical trials utilizing high-dose cisplatin and case reports in the overdose setting have characterized clinical features associated with cisplatin overdoses, highlighting some therapeutic strategies for consideration [3]. The major toxicities of cisplatin overdose include nausea and vomiting, renal insufficiency, electrolyte abnormalities, myelosuppression, ototoxicity, peripheral neuropathy, hepatotoxicity and retinopathy. Diarrhea, pancreatitis, seizures and respiratory failure have also been reported [4].

DNA repair is an important mechanism for resistance to platinum-based therapy and possibly the development of neurotoxicity [5]. Cisplatin, mitomycin C, and ecteinascidin form a covalent linkage with DNA and stabilize the double helical structure [6]. If the cell is able to repair the DNA attacked by the platinum agent, then that agent will be unsuccessful in inducing apoptosis [7]. It is known that cisplatin is significantly taken up in human liver and those high doses of the drug produce hepatotoxicity [8, 9]. Little is known about the mechanism of cisplatin induced liver damage, although apoptotic lesions seem to characterize the damaged liver parenchyma. Due to negative side effects of cisplatin, it is necessary to develop a new potential platinum based drug with high efficiency and low side effects. On the other hand, application of platinum nanoparticles

(PtNPs) leads to increased selectivity for tumor tissue, while lowering the side effects [10]. Excellent properties of PtNPs are reflected in the possibility of surface modification with various bioactive groups [11]. It is reported that PtNPs possess capacity to pass the cell membrane without limitation [12], and also to induce DNA damage, increase the cellular glutathione and genotoxic stress [13].

Nevertheless, the effect of applied PtNPs and their behavior inside the organs are still unclear. Amino acids play a crucial role in the function of liver, especially branched-chain amino acids (BCAA), which have the main role in prognosis of hepatocellular carcinoma (aHCC) [14]. However, the relevance of amino acid metabolism in the general population suffering from kidney and liver diseases still remains poorly elucidated [15]. The present study is a continuation of the earlier work, which was carried out to assess the cytotoxicity of PtNPs [16]. This study is focused on investigation of interactions between fundamental amino acids contained in liver and kidney of chicken embryo, before and after exposure to cisplatin by ion-exchange liquid chromatographic (IELC). However, the most important aims of this study were comparison of content and representation of amino acids in chicken embryo (liver and kidney), before and after treatment with synthesized platinum nanoparticles (PtNPs-10 and PtNPs-40), as potential replacement for toxic cytostatic cisplatin. Also the breaking points were determined, the critical amount of cisplatin and platinum nanoparticles sufficient for formation of mutual complexes for each amino acid.

## 2. METHODOLOGICAL BASES AND EXPERIMENTAL PART

### 2.1. Synthesis and characterization of platinum nanoparticles (PtNPs-10, PtNPs-40)

Platinum nanoparticles were produced using protocol published in our previous work [16]. Synthesis of platinum nanoparticles was conducted by mixing 0.07 g of $PtCl_4$ (Mr = 336.89) with addition of 33 µL of 37% HCl, followed by addition of 0.14 g of polyvinylpyrrolidone with different molecular weight PVP-10k and PVP-40k. Then 5 mL of $H_2[PtCl_6]$ was added and the mixture was stirred for 1 h. Finally, reduction was done by adding 50 mg of $NaBH_4$, and keeping the solution for 2 h under stirring. Prepared PtNPs were stored in dark at 4 °C. Resulting platinum nanoparticles were photographed using Tecnai F20 TEM (FEI, Eindhoven, Netherlands) at 120 kV. The average particle size and size distribution were determined by particles size analyzer (NANO-ZS, Malvern Instruments Ltd., Worcestershire, U.K.).

### 2.2. *In Vivo* distribution of cisplatin, PtNPs-10 AND PtNPs-40

The fertilized eggs of Lankenfeld roosters and ISA Brown hens (Integra, a.s., Czech Republic) were incubated in the incubator RCom 50 MAX (Korea) with temperature (37.5°C) and humidity control (55% rH). After ten days of the incubation the vitality of embryos was checked by digital egg monitoring system Avitronics (Vetronic services, England). The solution of PtNPs-10, PtNPs-40 and cisplatin (100 µL; 0, 3.5, 7.5 and 15 µg/mL) was applied through small hole in egg shell into the air cell on the chorioallantoic membrane using Chirana T. injection (maximal volume: 1 ml, size: 0.33 x 12 mm). The hole was covered with paraffin. After 10 days of the incubation the liver and kidney were analyzed.

Ethics Committee of Mendel University approved animal experiment according to resident governmental legislation. Reference number of accreditation is 57890l20l2-MZE-l7214. To minimize the distress, an opening into the air cell of the egg was made and the egg was placed into a chamber with inhaled anesthetics for 10 minutes.

### 2.3. Determination of content of amino acid in liver and kidney by IELC

The determination of AA content in liver and kidney before and after application of cisplatin, PtNPs-10 and PtNPs-40 was done by ion-exchange liquid chromatography (Model AAA-400, Ingos, Prague, Czech Republic) with post column derivatization by ninhydrin and an absorbance detector in the visible light range (VIS).

## 3. RESULTS AND DISCUSSION

PtNPs synthesis was conducted using two polymers (PVP-10k and PVP-40k), with different chain size as capping agent for stabilization of particles and reducing of toxicity effects. The morphology, size and zeta potential of tested platinum nanoparticles were conducted by transmission electron microscopy (TEM) and dynamic light scattering (DLS). Synthetized PtNPs have spherical shape with single particle size around 10 nm in the case of PtNPs-10 and 13 nm for the PtNPs-40 as shown in **Figures 1A** and **1C**. The TEM images show that spherical particles are formed in the case of both PtNPs. Dynamic light scattering (DLS) was applied for characterization of size and potential of tested PtNPs (**Figures 1C** and **1D**). DLS measurement confirms results obtained from TEM, where average size of PtNPs-10 was found to be 10 ± 2 nm, while PtNPs have average size 13 ± 3 nm. We found that PtNPs-10 have zeta potential at -10.6 mV (**Figure 1C**), while PtNPs-40 have zeta potential at -3.5 mV (**Figure 1D**), clearly indicating lower colloidal stability and formations of aggregates.

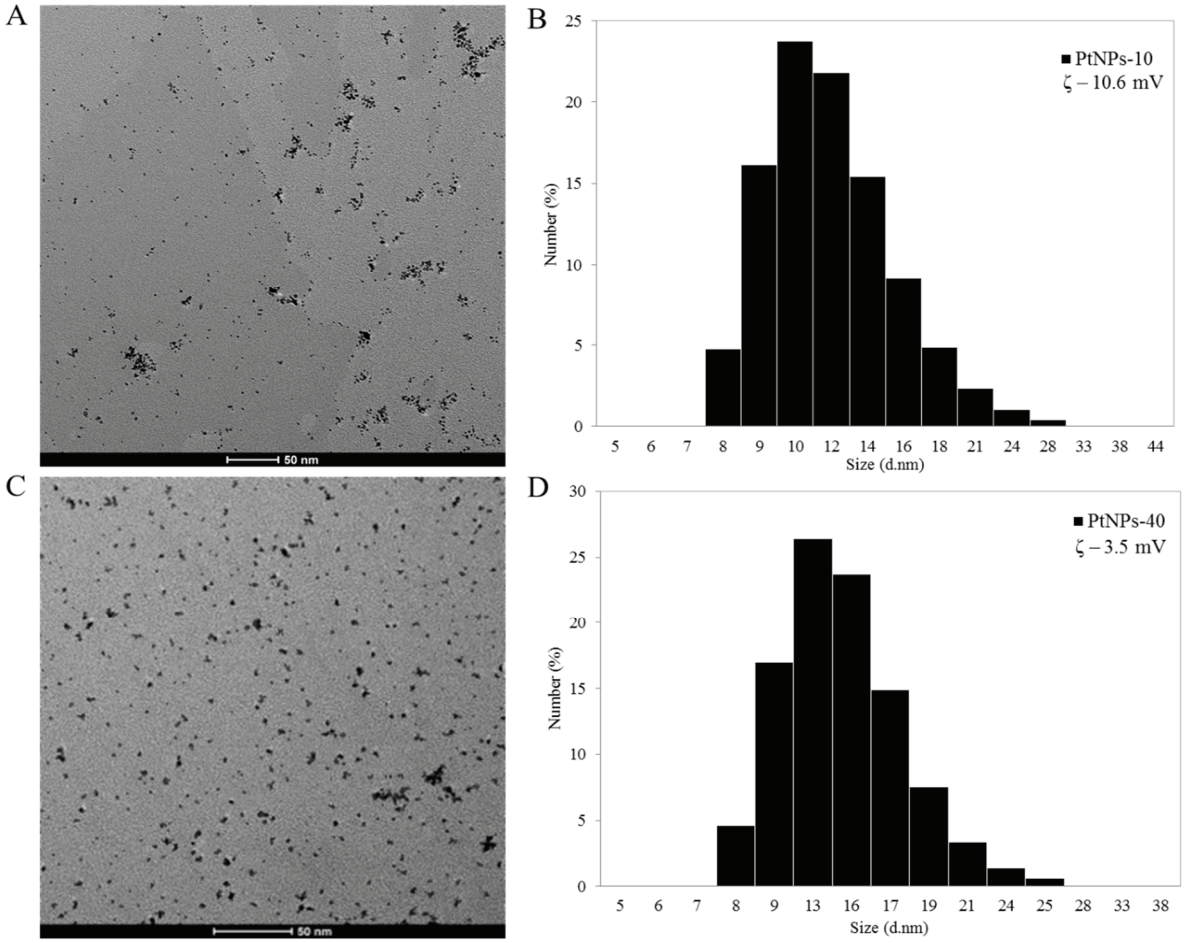


**Figure 1** The characterization of PtNPs; A) TEM images of PtNPs-10; B) Size distribution and zeta potential of PtNPs-10; C) TEM images of PtNPs-40; D) Size distribution and zeta potential of PtNPs-40

This study was focused on the investigation of cisplatin, PtNPs-10 and PtNPs-40 change effect to the amino acids profile in liver and kidney. Amino acids show high affinity for platinum compounds, which makes them an important biomarker for study of the action and side effects of platinum based drugs [17]. Cisplatin also shows high affinity for cysteine, in order to increase the rate of platinum-DNA binding [18]. However, it is well known that cisplatin is highly toxic for liver and kidney [19]. Nevertheless, the effect of applied PtNPs and their behavior inside the organs are still unclear.

Due to that it was decided to treat the chicken embryo with alternative platinum drugs PtNPs-10 and PtNPs-40, in order to observe the interaction and binding of amino acids in liver and kidneys after treatment with platinum based drug. It was found that in the case of kidneys all amino acids increase their concentration with increasing concentration of cisplatin (**Figure 2 A**). Comparing control results with results obtained after application of PtNPs-10 and PtNPs-40 (**Figures 2B** and **2C**), it is clear that increasing rate of amino acids was found only in the case of highest applied concentration of 15 µg/mL. On the other hand, it is obvious that cisplatin treatment increases level of amino acids more than treatment with PtNPs. Fleck et al [20], reported that the increasing rate of amino acids was apparently mediated by nephrotoxicity via the metabolism of a glutathione-platinum conjugate to a cysteinyl-glycine-platinum conjugate, rather than by mobilization of amino acids from tissues.

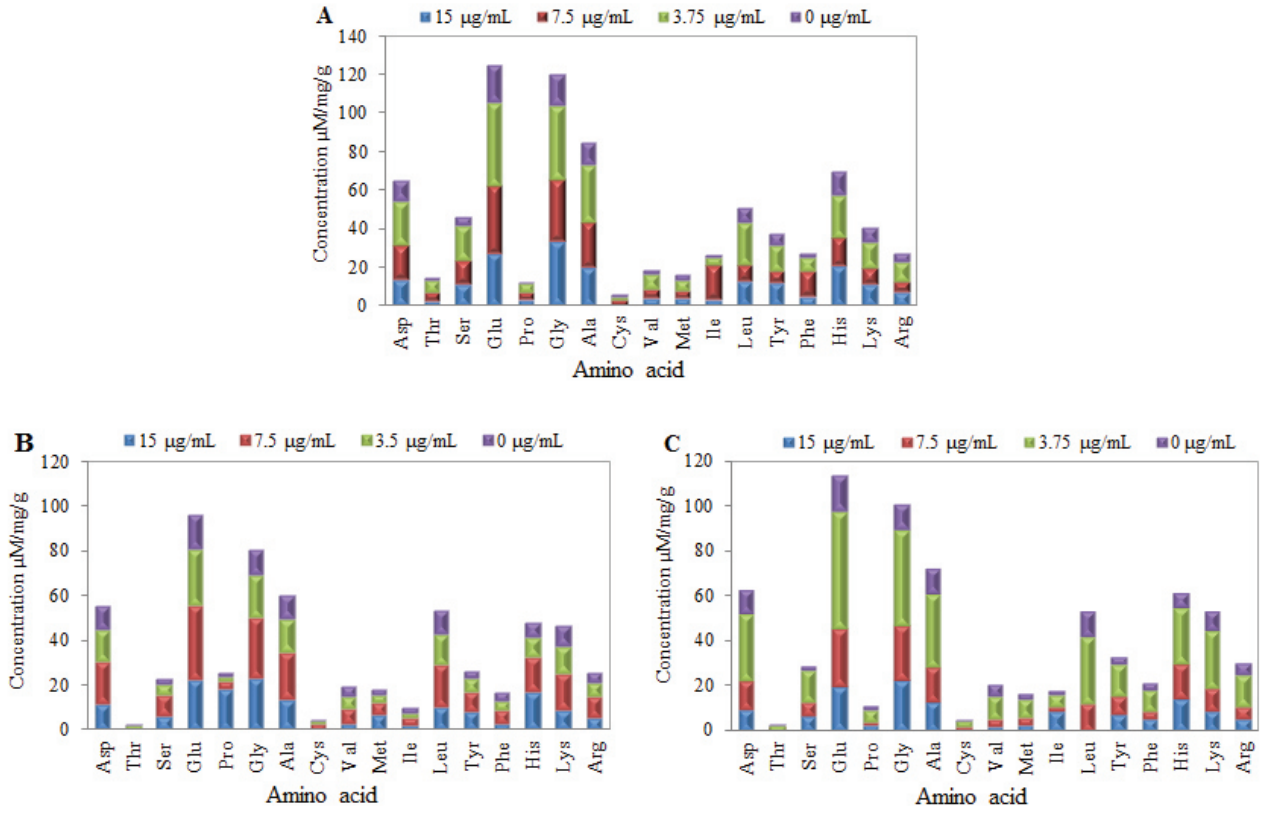


**Figure 2** Comparison of amino acids level in chicken kidney before and after application of cisplatin, PtNPs-10 and PtNPs-40 in different concentration of 0; 3.75; 7.5 and 15 µg/mL. (A) Chicken tissue kidneys before and after application of cisplatin; (B) chicken tissue kidneys before and after application of PtNPs-10; (C) chicken tissue kidneys before and after application of PtNPs-40

In the case of liver, results show that increasing rate of amino acids was found only in the case of highest applied concentration of 15 µg/mL. Significant increase of amino acids was found in the case of asparagine - 14.1 µg/mL, glutamic acid - 32.7, serin - 9.5 µg/mL, glycine - 36.4 µg/mL, alanine - 21.8 µg/mL, tyrosin - 13.2 µg/mL and histidine - 22 µg/mL after treatment with cisplatin comparing with control. (**Figure 3A**). After application of PtNPs-10 (**Figure 3B**), the significant increase was found in the case of glutamic acid, glycine and histidine. The similar results were also obtained in the case of PtNPs-40 (**Figure 3C**). Metal-catalyzed oxidation is the most common mechanism for proteins oxidation, where metal can bind protein metal binding site, leading to increase of hydroxyl radical after their interaction with hydrogen peroxide. In that case, amino acids can be modified directly via side chain reaction with ROS [21]. It was supposed that oxidation of protein

is the main reason for increasing level of amino acid in liver sample. However, a research on protein level is necessary to confirm our conclusion.

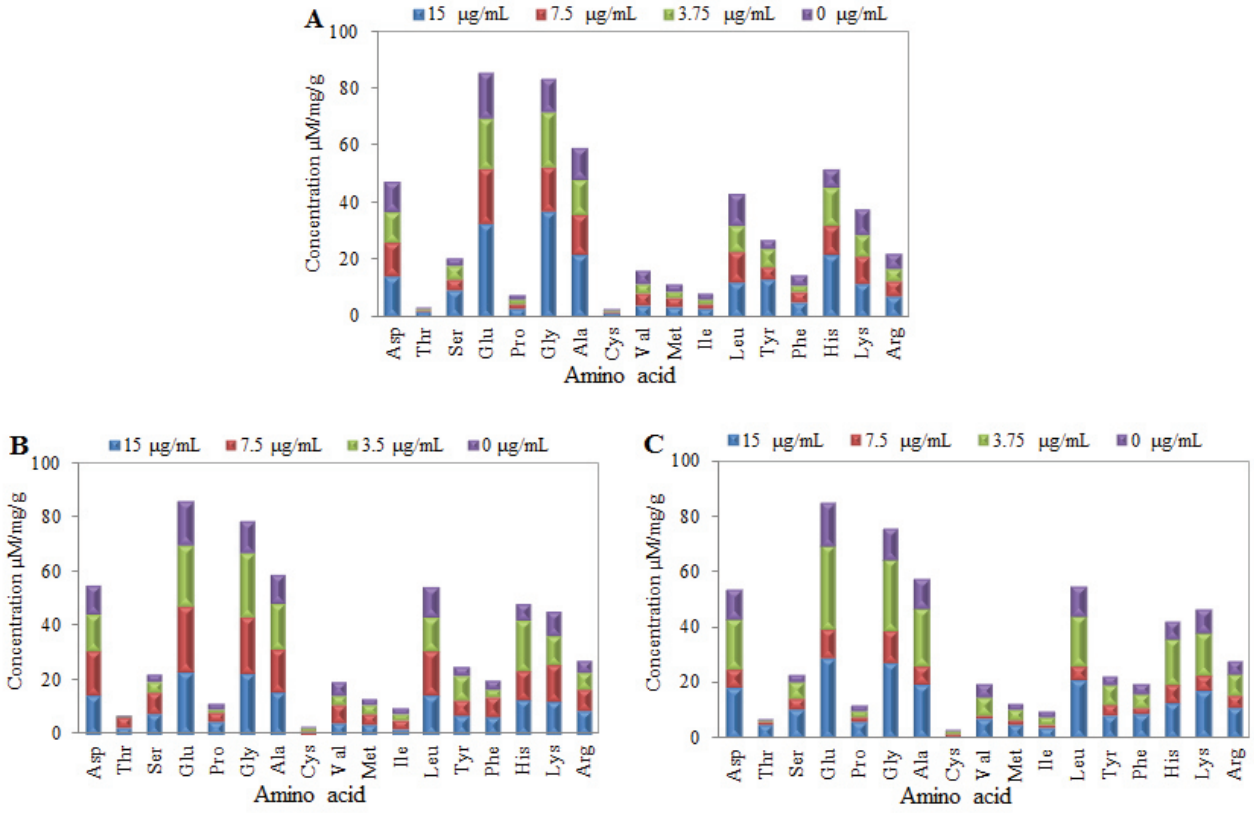


**Figure 3** Comparison of amino acids level in chicken liver before and after application of cisplatin, PtNPs-10 and PtNPs-40 in different concentration of 0; 3.75; 7.5 and 15 µg/mL. (A) Chicken tissue liver before and after application of cisplatin; (B) chicken tissue liver before and after application of PtNPs-10; (C) chicken tissue liver before and after application of PtNPs-40.

## 4. CONCLUSION

In this study, it was attempted to determine that cisplatin, PtNPs-10 and PtNPs-40 induce formation of complexes with amino acids in liver and kidney of chicken embryos. Possible formations of complexes may play an important role in the adverse effects of cisplatin and platinum nanoparticles. We also carried out comparison of the quantitative representation of amino acids in chicken tissues, such as liver and kidney before and after application of cisplatin and nanoparticles. It is important to reveal if cisplatin and platinum nanoparticles induced damage leads to alterations of development of neurotoxicity and if there is any chance to recognize the progressive and reversible damage. For these purposes we have plans to carry out next *in vivo* experiments in the future, in order to further explore this phenomenon.

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