Influence of *cis*-Platinum Administration at Different Doses on Zinc, Cooper and Iron Concentration in Hapatic Tissue

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Interactions between metal ions and biological systems have many implications. Based on these facts, several platinum compounds have been obtained and evaluated as chemotherapeutical agents. It is generally believed that the anticancerous effects of cis-platinum are based on interactions with DNA. Our study is trying to reveal the interrelations between concentration of some metallic ions from hepatic tissue and different doses of cis-platinum (cDDP). The cytotoxic activity of cis-platinum can be correlate with the levels of platinum that bounds to DNA. For the experimental part, we used adults animals from Wistar rats strain divided in four experimental groups and one control group. Wistar rats from the experimental groups were injected intraperitoneally (i.p.) with cis-platinum (the drug called Sin-Platinum) in study days 3 and 8. The animals from control group were injected with saline solution (placebo), also in study days 3 and 8. The results showed that the concentration of metal ions in liver is modified proportionally with administrated doses.

Keywords: cis-platinum, metallic ions, adducts metals-DNA

In antitumoral chemotherapy a various number of drugs are used. Among them, *cis* - dichlorodiammineplatinum (abreviated *cis*-platinum, *cis*-DDP or cDDP) has been widely used in chemotherapy [1-3].

From the chemical point of view *cis*-platinum is a heavy metal complex containing a central platinum atom surrounded by two ammonia molecules and chloride atoms arranged in the *cis* – position. *Cis*-platinum, as a compound, was the first time described by Peyrone in 1845, and the separation of its *cis* and *trans* isomers was elucidated in 1895 by Alfred Werner [4-6]. In 1960s, Rosenberg and his team research discovered that electrolysis products from a platinum electrode inhibited mitosis in Escherichia coli bacteria.It is generally accepted that the major target in the interaction of *cis* – platinum with biological system id represented by deoxyribonucleic acid (abbreviated DNA) which is the carrier of genetic information.

Experimental part

For the experimental part, we used tumor free adults Wistar rats, supplied by the Animal Breeding Facility of the University of Agricultural Sciences and Veterinary Medicine of Timisoara. The animals were maintained on pathogen-free conditions, at 22–25°C room temperature, at 55–65% relative air humidity, and fed on normal rhythm and standard breeding food and water. All animals manipulation were performed according to the ethical principles for animal care.

The antineoplastic drug called *cis*-platinum used for thid study was the commercially available Sin-Platin (Sindan, Romania).

The animals were randomly divided in five groups: one control (C) and four experimental groups (E_1, E_2, E_3 and E_4) and injected intraperitoneally (i.p.) with *cis*-platinum in doses of 2.0 mg/kg body weight (b.w.) – in case of E_1 group, 4.0 mg/kg b.w. – in case of E_2 group, 6.0 mg/kg b.w. – in case of E_3 group and 8.0 mg /kg b.w. – in case of E_4 group. Each group contained 10 animals (males and females) with an average body weight (b.w.) of 200 ± 20 g. The animals from (C) control group, were injected i.p. with physiological saline solution. Animals doses have been received the doses on days 3th and 8th of the experiment. On day 13th of the experiment the animals were killed.

All the measurements of the concentration of Zn, Cu and Fe in liver tissue samples were performed with a Varian Spectra A 110 spectrophotometer and was determined by flame atomic absorption spectrophotometry (AAS). The liver samples have been previously ashed at the 450 °C and then extracted with a nitric acid solution pro analysis (Merck).

Statistical analysis

Results are expressed as means ± SD and to calculate the statistical significance the *t* test was used as appropriate and *p* values of less than 0.05 and 0.01 were considered as significant. Means of treated groups were compared with those of control groups by using MS Excell.

Results and discussions

Cis-platinum synthesis starts from the tetrachloro-platinate compound $PtCl_4^-$ (fig. 1). In case of the first NH_3 ligand, the addition is made to any of the four equivalent position, but the second NH_3 ligand could be added *cis* or *trans* to the amine.

Platinum can have different possible oxidation states, but especially two dominant oxidation states, +2 and +4. In case of platinum complexes in which platinum was in the +4 oxidation state the complexes are octahedral, and when platinum is in the +2 oxidation state, it forms square planar complexes [7,8,9]. The platinum complexes, having platinum with either the +2 or +4 oxidation state, are shown in figure 2.

Because of the several side effects, over the years, many platinum complexes have been studied, some are shown in figure 3.

From all platinum compounds the most antitumoral agent has proved to be *cis*-platinum.

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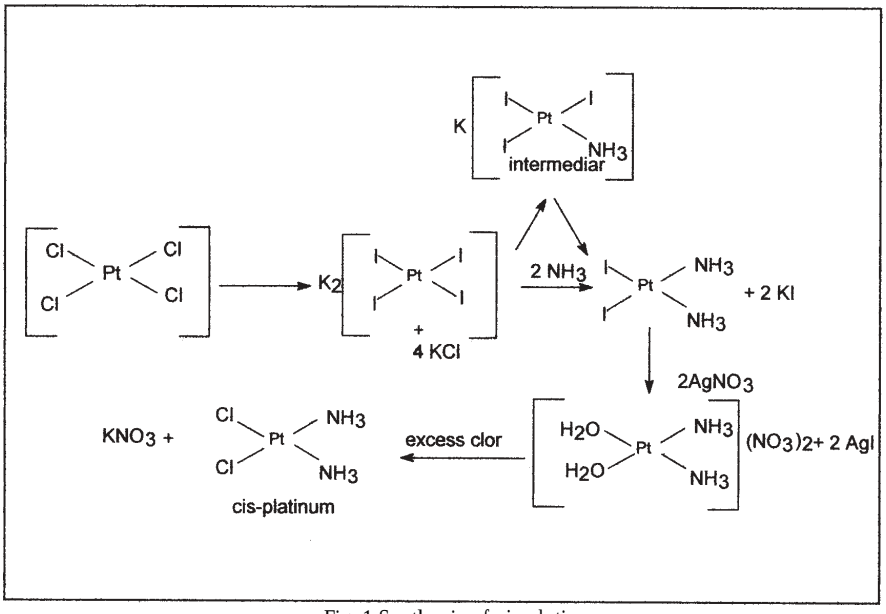

Fig. 1 Synthesis of *cis*-platinum

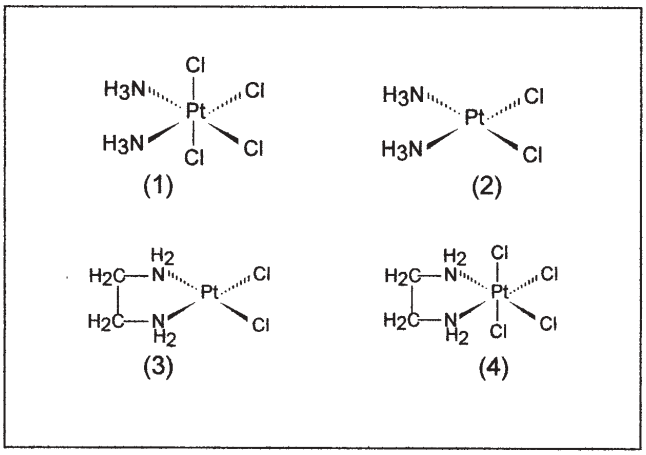

Fig. 2 Platinum compounds obtained by synthesis
(1) *cis*-$Pt^{IV}(NH_3)_2Cl_4$; (2) *cis*-$Pt^{II}(NH_3)_2Cl_2$;
(3) $Pt^{II}(NH_2CH_2CH_2NH_2)Cl_2$; °i (4) $Pt^{IV}(NH_2CH_2CH_2NH_2)Cl_4$

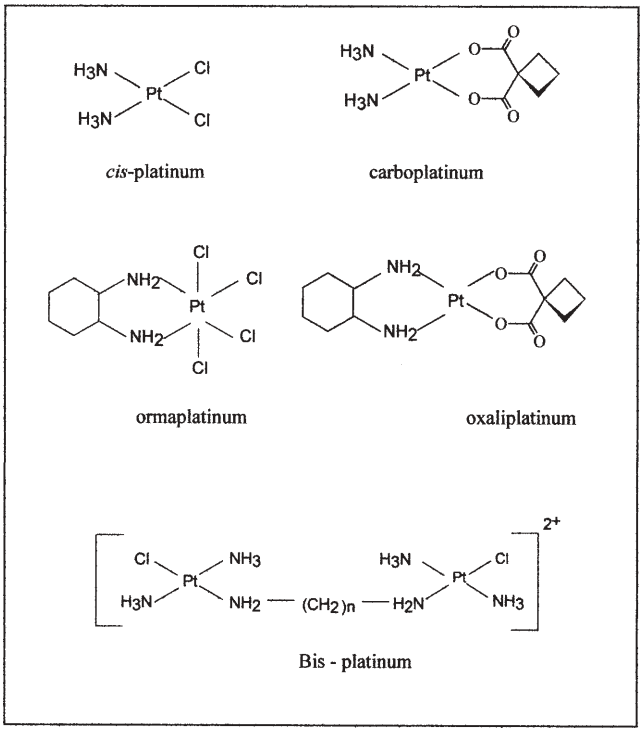

Fig.3 Chemical structure of platinum complexes

Because of the planar geometry, after the penetration into the blood, the two labile ligands 2 $(Cl)^-$ are released during the hydrolysis. The process of hydrolysis has two steps, and at the end of each step a mono-, respectively a diaquated species are resulting (fig. 4). The aquatated species of *cis*-platinum can form several types of covalent adducts with DNA.

The binding of *cis*-platinum to DNA can affect the structure of DNA and also the protein synthesis. The citotoxic activity of *cis*-platin correlates with the cuantum of platinum that is bound to DNA macromolecule [10].

Previous studies revealed that *cis*-platinum caused DNA interstrand cross-links and DNA – protein cross – links [11].

In double – stranded DNA the major site of platination (65%) is represented by the intrastrand cross-links between two adjacent bases deoxyguanosines (GG). Another 20% of DNA platination derives from intrastrand cross-links between adenosine and guanosine, and no adducts were realised between the nucleosides in opposite places. Other platinum derivates (9%) derives from a cross-link between

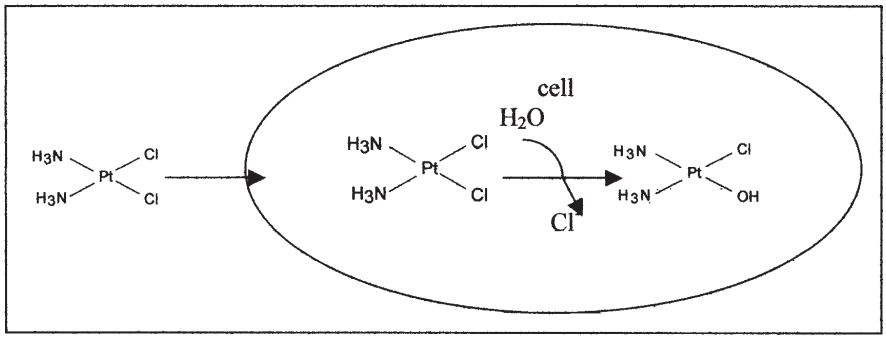

Fig. 4 Aquatation of *cis*-platinum after entering the cell

two deoxyguanosines separated by a third nucleoside [12, 13]. In all situations, platinum was bound to the N7 atom

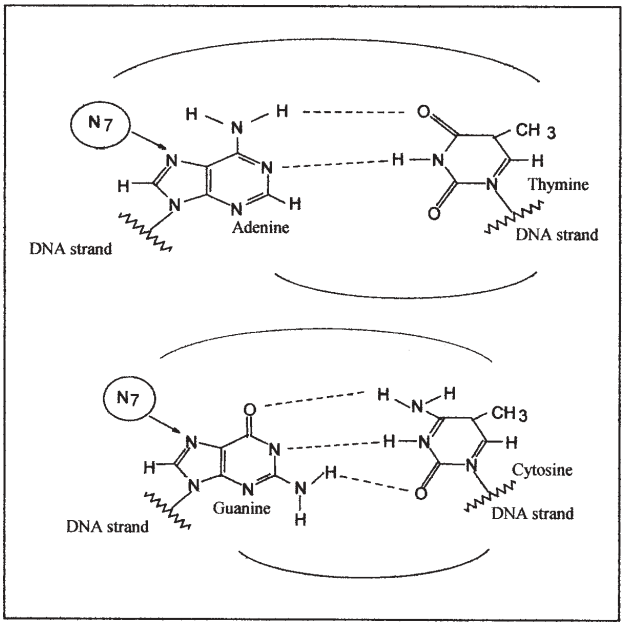

Fig. 5 Principal binding between DNA nucleobases

of purine bases (fig. 5).

These results revealed that the 1,2 – intrastrand cross-links are the major adducts formed by *cis*-platinum. Also, the high selectivity N_7 atom of guanine in the major groove of double stranded DNA is common to *cis*-platinum and its analogues [14].

Our experiments performed on laboratory rats revealed that the distribution of cisplatinum in the organism lead to the c-DDP – DNA adducts formation [15]. As a consequence of interaction with proteins, some modifications regarding some metal concentration can be mentioned. Metal elements are implicated in biochemical homeostasis, which is very important for the normal development of chemical and biochemical processes from organism [16, 17].

In our study the concentrations of some trace elements were determined in liver after cis-platinum administration. The obtained data are presented in table 1.

Zinc plays a role in stabilizing the biomembrane structure, and polynucleotides conformation. The modifications of these metals concentrations are related to the lesions produced by cis-platinum on DNA macromolecule. Also it is a cofactor for many enzymes. Zinc values are higher in experimental groups compared

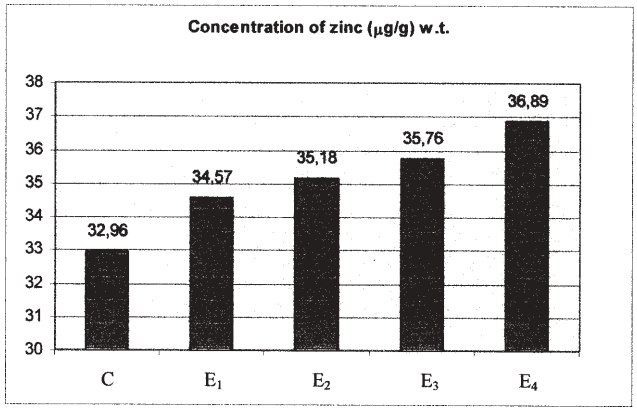

Fig. 6 Evolution, compared to control, of zinc concentration in liver of Wistar rats (C) control group, E_1 –2.0 mg/kg body weight (b.w.), E_2 - 4.0 mg/kg b.w. E_3 - 6.0 mg/kg b.w. ; E_4 - 8.0 mg /kg b.w

to control groups, and in case of experimental group (E_4) these difference are significant ($p < 0.01$), (fig. 6).

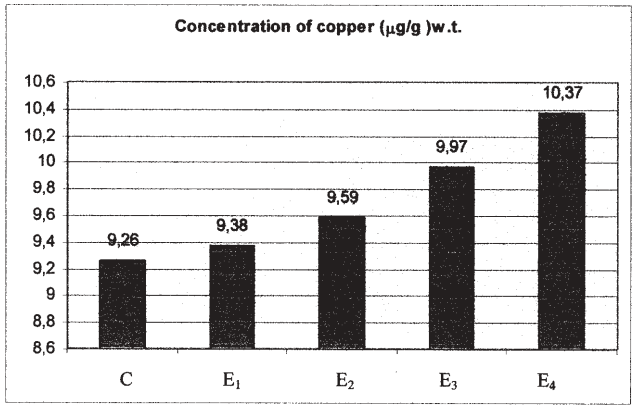

Fig. 7 Evolution, compared to control, of copper concentration in liver of Wistar rats (C) control group, E_1 –2.0 mg/kg body weight (b.w.), E_2 - 4.0 mg/kg b.w. E_3 - 6.0 mg/kg b.w. ; E_4 - 8.0 mg /kg b.w.

Zinc is also implicated in many biochemical processes, such as protein synthesis, and has an essential role in nucleic acid metabolism.

Copper is an essential cofactor for several enzymes (e.g. cytochrome oxidase, lysyl oxidase). The increased copper and zinc levels in liver of experimental groups of animals may show the increased capability of the enzymes against oxidative stress (fig.7) [18].

Iron is a trace element important for physiological and enzymatic processes, implicated in the structure of hemoglobin, also in the oxygen transport [19]. In case of

Table 1
CONCENTRATION OF Zn, Cu AND Fe IN LIVER

Specification	n	Zn (µg/g) w.t. $\overline{X} \pm DS$	Cu (µg/g)w.t. $\overline{X} \pm DS$	Fe (µg/g)w.t. $\overline{X} \pm DS$
Group C	10	32.93 ± 5.48	9.26 ± 1.52	195.05 ± 27.50
Group E_1	10	34.57± 5.76	9.38 ± 1.64	189.87 ± 26.53
$\Delta\overline{X}$		+1.64	+0.12	-5.18
Group E_2	10	35.18 ± 5.93	9.59 ± 1.90	184.79 ± 24.31
$\Delta\overline{X}$		+2.25	+0.33	-10.26
Group E_3	10	35.76 ± 6.21	9,97 ± 2,16	178.33 ± 24.76**
$\Delta\overline{X}$		+2.83	+0.71	-16.72
Group E_4	10	36.89 ± 6.75*	10.37 ± 2.30*	171.71 ± 23.90*
$\Delta\overline{X}$		+3.96	+1.11	-23.34

n – number of animal for each goup *p< 0.01 ** p<0.05

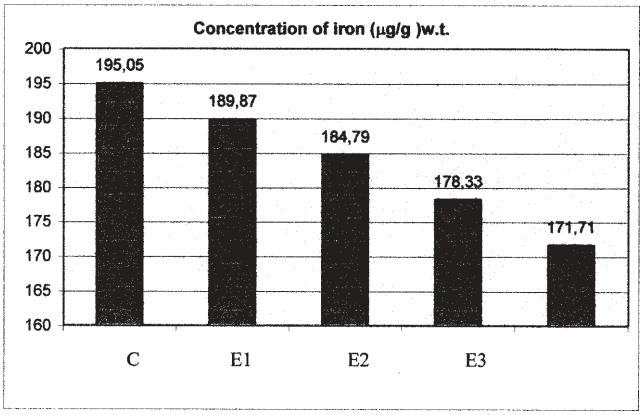

Fig. 8 Evolution, compared to control, of iron concentration in liver of Wistar rats (C) control group, E_1 –2.0 mg/kg body weight (b.w.), E_2 - 4.0 mg/kg b.w. E_3 - 6.0 mg/kg b.w. ; E_4 - 8.0 mg /kg b.w

iron, we can observe that his concentration in experimental groups is lower than in control group, and between experimental group (E_3) and control group, these differences are significant (fig.8).

Iron is a participant as a prostetic group for several enzymes in a number of biochemical pathways, in oxidative phosphorilation of xenobiotics. Iron is a cofactor for peroxisomal enzyme catalase. The enzyme contains heme group responsible for catalytic activity. Decreasing Fe content after *cis*-platinum administration have a negative influence on catalase activity with decreasing of H_2O_2 elimination. The decreased Fe levels in liver after *cis*-platinum administration may suggest the decreased activity of catalase. So, we can suggest that increasing H_2O_2 production by decreasing catalase activity can reduce the oxidative stress.

Conclusions

Concentration of metal elements from liver tissue are very important for defining the influence of chemotherapy on normal health status. Administration of *cis*-platinum in Wistar rats is characterized by increased concentration of zinc and copper and decreased concentration of iron in liver directly proportional with administrated doses.

Our results revealed that zinc concentration in samples of hepatic tissue are increasing proportionally with administrated doses. The zinc concentration is higher with 3.96 mg/dL in case of experimental group E_4 in comparison with control group, meaning an increasing of 12.02%. Also, copper concentration is higher in experimental group E_4 with 1.11 mg/dL, meaning an increasing of 11.98% in comparison with control group.

Iron concentration is lower in experimental groups in comparison with control groups. The decreasing is significant in experimental groups E_3 and E_4. In case of E_3 iron concentration is lower with 16.72 mg/dL, meaning 8.57%, and in case of E_4 with 23.34%, meaning 11.96%.

Our results revealed hepatic damages caused by *cis*-platinum administration. Metal elements zinc, copper and iron are implicated in biochemical homeostasis, which is very important for the normal development of several processes from organisms, such as: acido-basic balance, osmotic and colloid-osmotic equilibrium.

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