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EFFECTS OF CIS-PLATINUM ON THE BIOCHEMICAL HOMEOSTASIS IN RATS NOTE I. INVESTIGATIONS ON THE HEPATIC DNA AND ON SERUM PROTEINS

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Abstract

Cytostatic chemotherapy induces changes in the homeostasis of the hepatic DNA and of serum proteins. Among the various drugs used in the chemotherapy the alkilating agents, antimetabolites, steroid hormones and antibiotics are better known. In the last decades there were discovered other new compounds such as platinum derivates which representia distinct class of compounds, having specific antitumoral action. Among platinum derivatives an essential effect has cis-platinum. Research on the cytostatic activity of cis-platinum implies the knowledge of the pharmacokinetic and biochemical effects. Experiments "in vivo" performed on Wistar strain rats pursued the action of the intraperitoneally injected cis-platinum on the hepatic deoxyribonucleic acid (DNA) biosynthesis and on serum proteins. The statistically processed analytical data revealed non-significant decreases of the hepatic DNA and of serum proteins concentrations. Electrophoretical fractions showed hypoalbuminemia and hyperglobulinemia. In case of globulin subfractions the decrease of α_1 - and α_2 - and increase of β - and γ - globulins was observed.

Key words: *cis-platinum effects - hepatic DNA and serum proteins in rats*

INTRODUCTION

In the acceptation of WHO, oncotherapy is aimed at the annihilation, elimination or neutralization of all cancer cells.

The main modalities of the antitumoral therapy - in actual use - are: surgical intervention; radiotherapy; chemotherapy and immunotherapy.

Presently, in the antitumoral chemotherapy there are used various types of drugs, such as: alkylating agents, e.g.: cyclophosphamide, chlorambucil, nitrosourea a.o.; antimetabolites, e.g. : 5-flurouracil, 6-mercaptopurine, methotrexate a.o.; steroid hormones**,** e.g.: estrogens and androgens; antibiotics, e.g. actinomycin D, bleomycin a.o.; alkaloids, e.g.: vincristine, vinblastine a.o. (Gârban et

al., 1997; Neidle and Waring, 2000; Manolescu, 2003).

The class of platinum coordination complexes was discovered during the investigations of the electric field effects on bacterial growth. Cis-platinum has the chemical denomination cis-diamminedichloroplatinum (abbreviated as cDDP) is the most important inorganic coordination compound (Rosenberg et al., 1969; Haiduc and Silvestru, 1989; Lippert, 1999; Gârban et al., 2014).

Secondary effects of cis-platinum include nausea, vomisment, nephrotoxicity, ototoxicity, neuropathia and mielosupression. In some cases appear arithmias, ischemic stroke, glucose intolerance and pancreatitis (Manolescu, 1997; Kelland and Farrell, 2000). Nephrotoxicity can be ameliorated by hydration, renal injury at the level of glomerules and tubules is cummulative, reason why serum creatinine is not longer a conclusive parameter for the glomelular filtration rate.

Nowadays a domain of major interest in comparative medicine and in biochemistry is represented by the study of metallomics related to xenobiotics of food interest, e.g. metals in foods and of pharmaceutic interest, e.g. metal based drugs like cis-platinum (Haiduc et al., 2008, Gârban, 2011).

MATERIALS AND METHODS

Experimental model. In vivo experiments were performed on laboratory animals - Wistar strain male rats, included in three groups : one control - C and two experimental (E_i) groups - noted E_1 and E_2. Each group comprised 10 animals with an average weight of 200±10 g. Animals of group C were injected intraperitoneally (i.p.) with physiological saline and those from groups E_i with cis-platinum in physiological saline, as follows : E_1 - a dose of 5 mg/kg b.w. and E_2 - a dose of 10 mg/kg b.w. After 72 hours the animals were anesthetized and killed. Blood samples were taken by the puncture of vena cava caudalis and a liver fragment for DNA analysis were excized.

Requirements for the protection of animals used in scientific or other experiments were respected according to Council Directive 86/609/EEC and National Governmental Ordinance No.37/30.01.2002.

Biochemical investigations. The hepatic DNA concentration was determined by the Ogur Rosen method modified by Spirin (1958) and adapted by us for UV spectroscopical methods (Gârban et al., 1986). From blood samples the total serum proteins by the biuret method and the electrophoretic protein fractions by paper electrophoresis (veronal-medinal buffer, bromo-phenol blue staining) were determined (Franke et al., 1977; Kaplan and Pesce, 2010).

Statistical evaluation. All the obtained experimental data were statistically processed, mean values (X) and standard deviations (SD) were calculated. One way ANOVA (Analysis of Variance) was also used.

RESULTS AND DISCUSSIONS

Investigations on the DNA interaction with cis-platinum revealed the possibility of complexes formation named "adducts". These adducts of DNA-cDDP type perturb the secondary structure of the macromolecule (Kelland and Farrell, 2000; Gârban, 2004).

In the present experiment we found the decrease of DNA concentration in the liver tissue of rats after the cis-platinum administration – see Table 1. The observed decrease was more obvious in group E_2 - animals to which a higher concentration of cis-platinum was administered.

Table 1 . Hepatic DNA concentration after cis-platinum administration in Wistar rats

Groups	No. of animals	Admin.dose (mg/kg b.w.)	Duration (hours)	DNA (μg/mg tissue) X ± SD	ΔX $X_C - X_{E_i}$
C	10	-	72	2.74 + 0.19	-
E_1	10	5.0	72	2.65 + 0.20	- 0.09
E_2	10	10.0	72	2.61 + 0.17	- 0.13

The decrease of hepatic DNA concentration might be explained by the interaction of cis-platinum with the cellular DNA. Bindings occur to the nucleobases of DNA, i.e. guanine (G), cytosine (C), adenine (A) and thymine (T). As a consequence of this interaction structural modifications will occur in DNA which are followed by the disturbance of the replication process and consequently of other biological functions, too.

Aquation of cDDP in two steps leads to the formations of its pharmacologically active form which will bind to the nucleobases of DNA. These steps are given below :

$$cis\text{-}Pt(NH_3)_2Cl_2 \xrightarrow[-\,Cl^-]{+H_2O} \left[cis\text{-}Pt(NH_3)_2(OH)Cl^-\right]^+ \xrightarrow{-\,Cl^-}$$

$$\longrightarrow \left[cis\text{-}Pt(NH_3)_2(OH)_2\right]^{+2} \xrightarrow[-2H_2O]{+\,2H} \left[cis\text{-}Pt(NH_3)\right]^{+2} \longrightarrow$$

$$\longrightarrow cis\text{-}Pt(NH_3)\left[nucleobase\right]_2$$

It is to mention that $[\ cis\text{-}Pt(NH_3)_2\ ]^{+2}$ binds to the N and O atoms of the nucleobases, preferentially to N_7(G), N_3(C), O_2(C), O_6(G), N_3(A), N_7(A), O_4(T) and O_2(T), i.e. in the decreasing order of the binding energy level. These bindings will destabilize the secondary structure of the DNA and thus the replication-transcription-translation processes, involved in the protein synthesis, will be disturbed, too. One can consider that effect of cis-platinum on the protein synthesis is due to the changes in the chemical structure-biological activity relationship at the level of DNA by resulting adducts (Gârban, 2011).

The results obtained in our experiment revealed homeostatic changes in serum proteins and electrophoretic fractions and are given in Table 2. One can observe that with the increasing dose of the administered cis-platinum the concentration of serum proteins decreased. It is to notice the existence of an inverse proportionality: the increase of the cis-platinum dose induces the decrease of serum proteins concentration.

Table 2. Concentration of serum proteins and electrophoretic fractions

Specification		C (n = 10) X ± SD	E_1 (n =10)		E_2 (n = 10)	
			X ± SD	ΔX	X ± SD	ΔX
Serum proteins (g%)		5.87± 0.23	5.39 ± 0.35	- 0.48	5.15 ± 0.42	- 0.72
Electrophoretic fractions (%)	Albumin	54.30± 1.70	53.60 ± 2.06	- 0.70	52.60 ± 1.71**	- 1.70
	Globulins - total	45.70± 1.70	46.40 ± 2.06	+ 0.70	47.40 ± 1.71**	+ 1.70
	α_1-globulins	12.90± 1.28	12.70 ± 1.63	- 0.20	12.70 ± 1.05	- 0.20
	α_2-globulins	10.70± 0.82	10.50 ± 0.84	- 0.20	10.80 ± 0.91	+ 0.10
	β-globulins	14.80± 1.54	15.10 ± 1.52	+ 0.30	15.20 ± 1.22*	+ 0.40
	γ-globulins	7.30± 1.15	8.10± 1.44*	+ 0.80	8.70 ± 1.05*	+ 1.40

Note: n - number of animals; * $P < 0.01$; ** $P < 0.05$

In case of electrophoretic fractions a decrease of albumin and increase of total globulins in the experimental groups (E_1 and E_2) as compared with the control group (C) was found. As to globulin subfractions α_1 - and α_2 - globulins decreased while β - and γ - globulins increased in E_1 group. In case of animals from E_2 group - experiment with a higher cis-platinum dose, the globulin subfractions showed a decrease of α_1 - globulin and increase of α_2 -, β- and γ - globulins. Regarding the concentration of serum proteins and of the albumin fraction Saleh et al. (2014) found also a decrease after the administration of cis-platinum in rats. The occurred biochemical homeostasis changes in hepatic DNA, serum proteins and electrophoretic fractions are the consequences of the cis-platinum interaction with DNA and tissue proteins, more exactly with certain target functional groups, e.g. amino, hydroxyl a.o. (Lippert, 1999; Keeland and Farrell, 2000). At molecular level these interactions concern with the pharmacon-receptor interaction and explain the mechanism of cytostatic action of cis-platinum.

CONCLUSIONS

The in vivo interaction of cis-platinum with DNA evidenced a statistically non-significant decrease of DNA concentration in case of both doses: 5 mg/kg b.w., 10 mg/kg b.w., respectively. These slight decreases could be due to the low administered dose of cis-platinum. The effect is explained by adducts formation which binds a part of DNA resulting cis-platinum-DNA type adducts.

In both experimental groups (E_1 and E_2) the concentration of serum proteins decreased. The electrophoretic fractions revealed decrease in case of albumin concentration and increase of globulins concentration. Globulin subfractions in case of E_1 group showed a decrease of α_1 - and α_2- globulins, and an increase of β- and γ-globulins. In case of group E_2 only α_1-globulins decreased while α_2- , β- and γ-globulins increased. Higher increases were found in case of γ-globulins (immunoglobulins) which confirm the effect of cis-platinum.

Note. This paper was elaborated within the activity of the *Comparative Medicine Forum – Romania*. The next note will approach the homeostasis of the non-protein nitrogen metabolites.

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