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Dedicated to Professor Ionel Haiduc
on the occasion of his 70 th anniversary

INTERACTION OF DEOXYRIBONUCLEIC ACID WITH *CIS*-PLATINUM : STRUCTURE-ACTIVITY RELATIONSHIP

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The discovery of the cytostatic effects of *cis*-platinum led to more thorougoing studies concerning the chemical structure-biological activity relationship. Results of our studies presented in this paper reveal predilectly the mechanisms of interaction of *cis*-platinum (cDDP) with deoxyribonucleic acid (DNA) and their effects in vivo and in vitro. Investigations were performed in vitro by means of circular dichroism (CD) with respect to the interaction of cDDP with DNA and in vivo by experiments on laboratory animals concerning the effects of cDDP on hepatic DNA biosynthesis and on serumproteins.
In vitro investigations, based on studies by CD, followed the evolution of cDDP / DNA systems at the wavelenght λ =220-320 nm. The evaluation of dichroic spectra evidenced modifications in B-DNA (native form) with tendency to A-DNA and C-DNA forms. The modifications revealed a tendency to generate DNA-cDDP adducts.
In vivo experiments, performed on Wistar strain rats showed, after the i.p. injection of cDDP (in physiological saline) in doses: 5, 10 amd 15 mg / body weight, the depression of hepatic DNA synthesis (with -0.04 ; -0.09 and -0.15 as compared with the values obtained in the case of control groups). A reverse relationship was observed between the administered dose of cDDP and the quantum of hepatic DNA. Serumproteins revealed a decrease in experimental groups reverse proportional with the administered cDDP doses.

INTRODUCTION

Metal ions interact with biological systems and may have some biomedical implications. This ascertainment was made by investigating the role of some metallic compounds, *e.g.*: biometals (Mg, Zn, Cu, Co a.o.) in nutrition; heavy metals such as Hg, Cd, Pb in pathogenesis; Pt – especially cDDP in chemotherapy[1].

Chemotherapeutical aspects of various metallic compounds make the subject of pharmacology and pharmacotoxicology. In the antitumoral chemotherapy different metallic compounds containing Pt, Pd, Ru etc. are of interest[2-4]. Among them *cis*–dichlorodiammineplatinum (abbreviated *cis*-platinum, *cis*-DDP or cDDP) is mainly used, as a component of various drugs, such as: Cysplatyl, Neoplatin, Platidiam, Platinex a.o.

From the chemical point of view *cis*-platinum is an inorganic coordination compound. It was synthetised by Peyrone in 1845 and the separation of its *cis* and *trans* isomers was made by Werner in 1889. Since the discovery of its cytostatic action by Rosenberg et al. [4], various pharmacological studies were performed. It was concluded that the receptor substratum in the interaction of *cis*-platinum with biological systems is represented by nucleic acids and particularly by the DNA macromolecule which conveys the genetic information.

MATERIALS AND METHODS

Investigations "in vitro" with circular dichroism (CD) were made on DNA (Loba Chemie – Wien) and in cDDP/DNA system (dissolved in 0.14 M

NaCl) at the molar ratio 7.5 and 15.0. In order to record the circular dichroic spectra Jouan – Roussel dichrograph with a wavelength (λ) range of 220-320 nm was used. The CD spectra modifications may be expressed in: Δε – dichroic absorbtion, measured in M^{-1} cm $^{-1}$; [ψ] – specific ellipticity, measured in degree · ml · dm^{-1}; [θ] – molar ellipticity, measured in degree · cm^2 · dM^{-1} depending on wavelenghth as an expression of structural transitions.

Experiments "in vivo" were performed on Wistar strain rats (body weight 180 ± 20 g) included in four groups: one control (C) and three experimentals E_i (noted E_1, E_2, E_3). Animals from group C were injected intraperitoneally (i.p.) with physiological saline and those from groups E with cDDP: E_1 with a dose of 5 mg/kg and E_2 with a dose of 10 mg/kg body weight and E_3 with a dose of 15 mg/kg bwt. The experimental design is presented in Table 1.

Table 1

Experimental design

Groups	Administrated substance	Dose mg/kg b.w.	No. of animals	Way of admin.	Duration of experiment (hrs)
C	physiological saline	-	16	i.p.	48
E_1	cDDP in physiological saline	5.0	16	i.p.	48
E_2		10.0	16	i.p.	48
E_3		15.0	16	i.p.	48

After 48 hours the animals were killed. Blood samples were taken by the puncture of vena cava caudalis and a liver fragment for DNA analysis was excised. The hepatic DNA concentration was determined by the Ogur–Rosen method modified by Spirin [6] and adapted for UV spectrophotometry by Gârban et al.[7]. Serumproteins and electrophoretic fractions were also determined. From blood samples were determined the serumproteins (biuret method) and electrophoretic fractions (albumins and globulins).

RESULTS AND DISCUSSION

The circular dichroism spectra, determined by the unequal absorbtion of the circulary polarised components of the linearly polarised radiation, reveal the Cotton effect. Studies showed that this effect, in the case of DNA, is determined by the optically active deoxyribose, by purine and pyrimidine bases whose chromophores became optically active due to their binding to the deoxyribose and to the double helical structure of the macromolecule [8, 9].

The spectrum presents a geometrical minimum (negative Cotton effect) and a geometrical maximum (positive Cotton effect). Thus, the dichroic curve shows: an initial trough where $\Delta\varepsilon < 0$, at smaller λ. At the vanishing point of the specific ellipticity $\Delta\varepsilon = 0$, the curve changes its sense and according to Crabbé [10] it relates approximately to the wavelength for the absorbtion in ultraviolet.

It was established that the characteristic values of these parameters result from physico-chemical determinations and quantum mechanic calculations [11, 12]. Literature data refer to the A, B, C and D conformational types, among which B – DNA represents the classical Watson – Crick model. By means of CD only the A, B, C types were detected.

Correlations between the circular dichroism spectrum and the conformation of DNA macromolecule gave the first explanation for the conformational transitions to A and C types of DNA [14].

The CD spectra obtained in M^{2+}/DNA systems, compared with CD spectrum of DNA revealed modifications [14, 15]. Thus, in the case of B – DNA the CD spectra show the appearance of well defined positive and negative Cotton effects. Structural transitions to A – DNA type are characterised by the "smoothing" of the dichroic spectral curve in the domain of negative Cotton effect, and to C – DNA type by the "smoothing" of the curve in the domain of positive Cotton effect.

Investigations "in vitro" showed that DNA macromolecule in the presence of *cis* – DDP, *i.e.* the *cis* – DDP/DNA system, presents dichroic modifications. In fig. 1. one can see the normal CD spectrum of DNA and the spectra of the *cis* – DDP/DNA system at various molar ratios (r=7.5; r=15.0). The depression of the Cotton effect in the positive band was observed when the ratio increased and its augmentation when the ratio decreased. From our experimental data one concludes that in the system cDDP/DNA the different molar ratio determine the destabilisation of the B – DNA type. The conformational modifications of DNA may determine also functional ones. These aspects related to the problem of the chemical structure – biological activity relationship

acquire a particular importance in the case of *cis* – platinum which has oncostatic action.

The anticancer activity of cDDP is based on the binding types which occur during the interaction of *cis*platin with DNA. It was proven that *cis*platin may form bifunctional intra- and interhelical cross – links [16].

Intra– or interhelical bindings to DNA with adducts formation as well as the problem of binding to proteins was also mentioned in our previous studies [7].

From among all platinum compounds the most active antitumoral agent was proved to be *cis* – platinum – characterised by a planar geometry, having two inert ligands ($2NH_3$) and two labile ligands ($2Cl^-$) which are released during the hydrolysis. Hydrolysis, developed in two steps, implies the mono- or diaquated platinum species formation (fig. 2). Both species can form bindings with the bases of DNA and in some cases with DNA and protein. The bindings are made in the preferential order: guanine > adenine > cytosine, observing that thymine is not involved [1, 2, 9].

Investigations on the DNA interaction with *cis*-platinum revealed the possibility of adduct formation which perturbs the secondary structure of the macromolecule.

Stereochemical pecularities of the binding to DNA imply the transition of macromolecule from B–DNA type (native state) to A–DNA and C–DNA. The conformational transitions facilitate the adducts formation. This interaction is preceded by the hydrolysis of cDDP: the two inert ligands ($2NH_3$) are not modified, while the labile ligands ($2Cl^-$) are released.

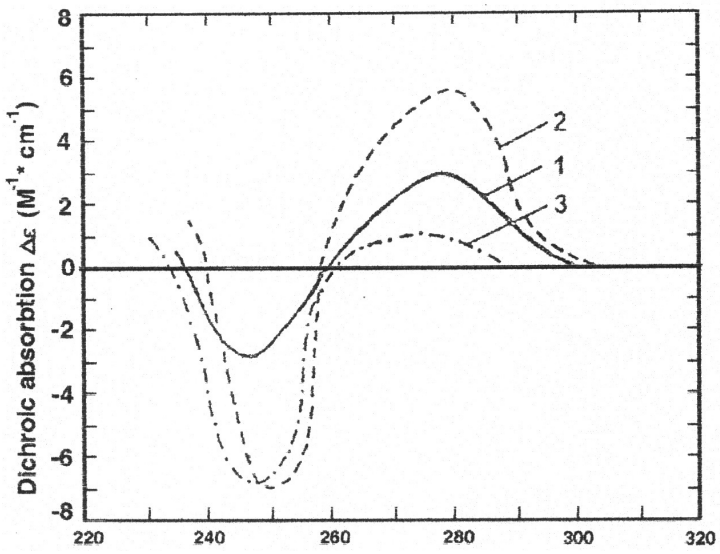

Fig. 1 – Circular dichroic spectra: for DNA (1); for DNA-cDDP Adducts at r = 7.5 (2) and DNA-cDDP r = 15.0 (3).

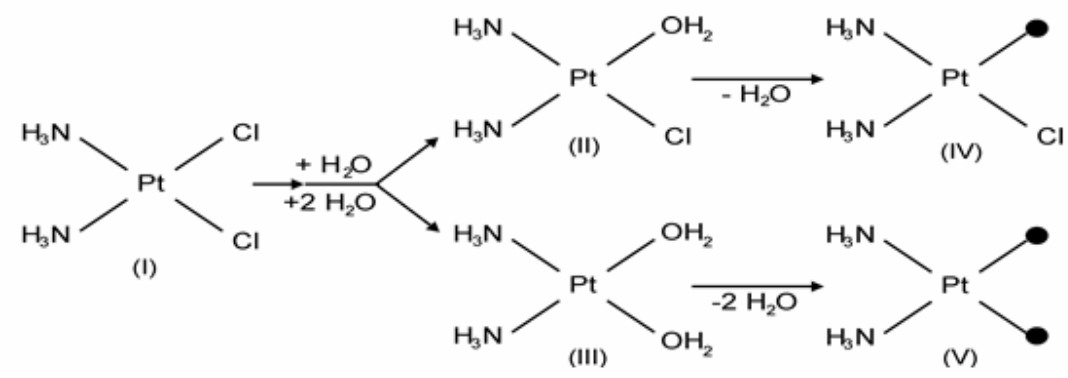

Fig. 2 – Formation of mono- and diaquated species of *cis*-platinum.

Quantitative evaluation of cDDP – DNA adducts were made in various mice tissues by an immunohistochemical method[17]. This method permitted the visualisation and quantitation of *cis*platin in its active position, i.e. bounded to nuclear DNA. With PAP technique (specific in cytometry), using an antiserum elicited against cDDP – DNA adducts, the various tissue sections were stained and than the image analysis were estimated.

Thus mono- and diaquated platinum compounds can bind to bases of DNA or to DNA and protein. The binding may be homomacromolecular (DNA–cDDP) or heteromacromolecular (DNA–cDDP–Protein). As a result of the binding different adducts are formed by intercatenar or intracatenar cross – links, by monofunctionally binding and by DNA–cDDP–Protein cross-link (fig. 3).

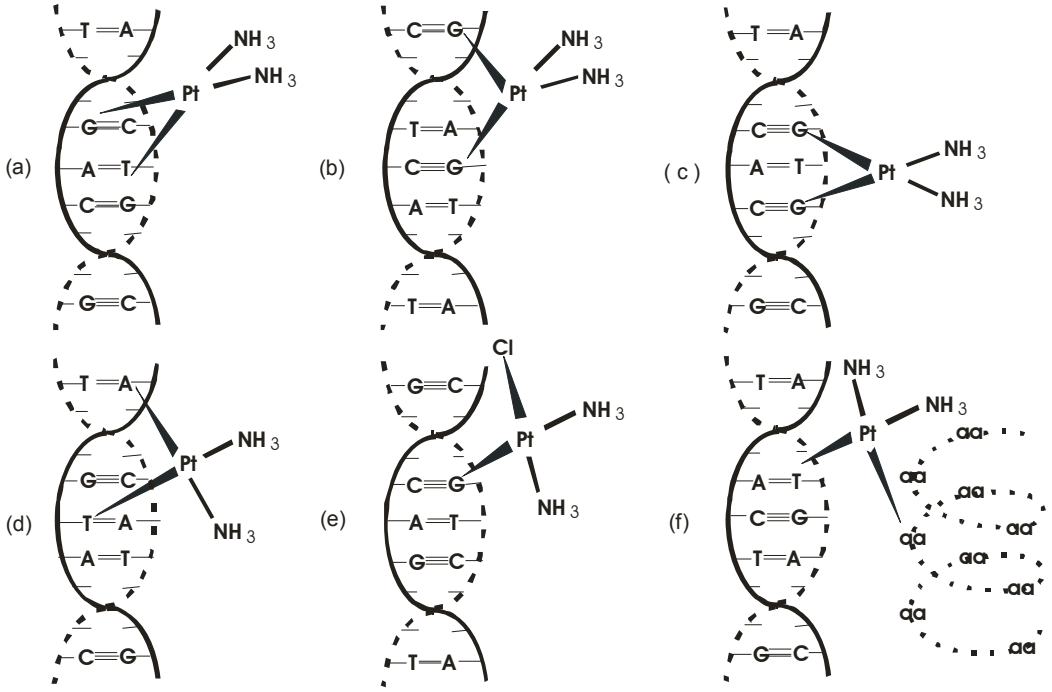

Fig. 3 – Adducts of DNA with cis-platinum:
(a) interstrand cross-link to different nucleobases; (c) interstrand cross-link to identic nucleobases; (b) intrastrand cross-link to identic nucleobases; (d) intrastrand cross-link to different nucleobases; (e) intrastrand cross-link to nucleobase-monoadduct; (f) heteromacromolecular cross-link DNA-cDDP-Protein

Cis-platinum interaction with DNA, studied on laboratory animals, revealed changes in the hepatic DNA concentrations.

The obtained data on DNA concentration are presented in Table 2. One can remark the depression of hepatic DNA concentration, in direct relation with the increasing dosis of the administered cDDP.

Beside the above mentioned data there are presented also the results obtained on serum proteins concentrations (see Table 3).

Table 2

Concentrations of hepatic DNA

Group	No. of animals	Hepatic DNA (μg/mg tissue) $\overline{X} \pm SD$	$\Delta\overline{X}$ $(X_C\text{-}X_E)$
C	16	3.03 ± 0.28	-
E_1	16	2.99 ± 0.31	- 0.04
E_2	16	2.94 ± 0.53	- 0.09
E_3	16	2.88 ± 0.42*	- 0.15

* $0.95 < p < 0.99$

Table 3

Total serum proteins and electrophoretic fractions – analytical data

Specifications		Group C		Group E_1			Group E_2			Group E_3		
		n	$\overline{X} \pm SD$	n	$\overline{X} \pm SD$	$\Delta \overline{X}$	n	$\overline{X} \pm SD$	$\Delta \overline{X}$	n	$\overline{X} \pm SD$	$\Delta \overline{X}$
Serum proteins (g%)		16	5.77 ± 0.68	16	5.61 ± 0.73	– 0.16	16	5.54 ± 0.91	– 0.23	16	5.46 ± 0.84	– 0.31
Electrophoretic fractions (%)	Albumins	16	54.96 ± 3.47	16	54.12 ± 3.72	– 0.84	15	53.52 ± 4.19	– 1.44	16	52.99 ± 4.97	– 1.97
	Globulins-total	16	45.04 ± 3.47	16	45.88 ± 3.72	+ 0.84	15	46.48 ± 4.19	+ 1.44	16	47.01 ± 4.97	+ 1.97
	α_1-globulins	16	12.02 ± 0.76	16	11.93 ± 0.64	– 0.09	15	11.85 ± 0.85	– 0.17	16	11.64 ± 0.72	– 0.38
	α_2-globulins	16	11.07 ± 0.83	16	11.11 ± 0.71	+ 0.04	15	11.16 ± 0.80	+ 0.09	16	11.21 ± 0.67	+ 0.14
	β-globulins	16	13.97 ± 0.48	16	14.31 ± 0.70	+ 0.34	15	14.58 ± 0.73	+ 0.61	16	14.73 ± 0.79 *	+ 0.75
	γ - globulins	16	7.98 ± 0.88	16	8.53 ± 0.97	+ 0.55	15	8.89 ± 1.10	+ 0.91	16	9.43 ± 1.03 *	+ 1.45

* $0.95 < p < 0.99$; n – number of animals per each working group

These data reveal that total proteins concentration decreases with the increasing dose of the administered cDDP. Serum electrophoretic fractions show a decrease in albumin and an increase of globulins concentration. As to globulin subfractions the decrease of α_1- globulins and increase of α_2- β- and γ-globulins was observed. The increase of γ-globulins concentrations reveal a disturbance in immunitary processes.

Experiments performed on mice revealed that from pharmacokinetical point of view the distribution of cDDP in the organism lead to the cDDP-DNA adducts formation. If the absorbtion and distribution of the drug occurs rapidly (i.p. administration) the process of cDDP-DNA adducts formation will be slower. The maximal levels were achieved between 30 min and 4hrs after the drug administration and was followed by a steady state lasting for at lease 24 hrs. There is a tissue specificitiy of adducts biogenesis studied by immunohistochemical staining. When cDDP doses increased, a linear or almost linear increae of Pt concentrations and cDDP-DNA addcuts levels was observed in all samples types examined [17].

Modifications in the protein metabolism reveal renal dysfunction. It was established that the non-protein nitrogen metabolites: creatinine, uric acid and urea are modified, pleading for the nephrotoxicity of *cis*-platinum [18, 19].

From the point of view of pharmacodynamics it is necessary to mention that *cis*-platinum interacts also with some proteins and especially with glutathione (G-SH), forming the G-S - cDDP complex [20]. Hematological and neurological effects appear as consequence of interaction with proteins. It was observed that sodium selenite reduces the toxicity of *cis*-platinum without inhibiting the antitumoral activity. In conclusion the authors stress the protective role of selenium. More recent studies [20] showed that the target of *cis*-platinum are proteins p53 and p73 which act as transcription factors in cell cycle control, regulation and cell development and/or in apoptotic pathways.

CONCLUSIONS

Changes in the positive and negative Cotton effects as well as a hypsochromic shift of the spectrum were observed by the in vitro circular dichroic investigations of the cDDP – DNA (at ratios 7.5. and 15.0) interactions. These modifications certify disturbances in the secondary structure of the DNA macromolecule.

Variations of the dichroic absorption spectra, compared with B-DNA type (present in normal conditions) plead for a conformational transition to C-DNA type (at r = 15.0) and a slight tendency to develop into A-DNA type (at r = 7.5).

A dose-dependent depression in the hepatic DNA concentrations of rats was observed meaning that DNA biosynthesis was diminished by cDDP administration.

As to serumproteins concentrations a reverse proportional decrease with the administered cDDP doses was observed in the experimental groups.

Regarding electrophoretic fractions: hypoalbuminemia, respectively hyperglobulinemia were found in experimental groups. As to globulin subfractions: hypo-α_1-globulinemia and hyper-α_2-, hyper-β- and hyper-γ- globulinemia were observed.

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