XENOBIOCHEMIC SPECIFICITY OF THE DEOXYRIBONUCLEIC ACID INTERACTION WITH SOME CYTOSTATIC CHEMOTHERAPEUTICS

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Abstract:*The mostly recommended methods in oncotherapy are the surgical intervention, chemotherapy, radiotherapy, immunotherapy or a combination between them. The chemotherapy consists in the use of various drugs among which the most important are : the alkylating agents, antimetabolites, steroid hormones, antibiotics, phyto alkaloids, metal based drugs.*

In this review there are discussed the molecular mechanisms of the interaction of an alkylating cytostatic, i.e. cyclophosphamide (2 bis(-chloroethyl) amino-1-oxa-3-aza-2-phosphocyclohexan-2-oxide) – Cp and of a metal based cytostatic, i.e. cis-platinumum (cis-dichlorodiammineplatinum) - cDDP with deoxyribonucleic acid (DNA). Cyclophosphamide and cis-platinum present similar mechanism of interaction with DNA. Interacting with DNA these cytostatics give rise to mono- and bidentate adducts. The topics are of interest for comparative medicine (veterinary and human medicine).

A particular importance arising from the appearance of the DNA-chemotherapeutic adducts is that these compounds can be detected analytically and can provide information on the consequences of the biochemical injury. Detection of DNA adducts is useful not only for the diagnosis of neoplastic diseases, but also for biomonitoring the evolution during chemotherapy.

Key words *: deoxyribonucleic acid, xenobiotics, cytostatic chemotherapeutics*

INTRODUCTION

In cancer treatment the mostly recommended solutions are the surgical intervention, chemotherapy, radiotherapy, immunotherapy or a combination between them (Ghilezan, 1992; Price and Sikora, 2002; Manolescu, 2003). Chemotherapy consists in the use of chemical substances among which the most important are : the alkylating agents, antimetabolites, steroid hormones, antibiotics, phyto alkaloids, metal based drugs (Price and Sikora, 2002). The approached thematic in this review is of interest for comparative medicine. More exactly, there are presented data regarding the cytostatic drugs cyclophophosphamide (Cp) and cis-platinum (cDDP) which can be considered as chemical xenobiotics of pharmaceutical interest, from the point of view of comparative oncology.

Cyclophosphamide due to its chloroethyl group may become a bifunctional alkylating agent (Lindemann and Harbers, 1980; Gârban, 1986 a; Johnson et al., 2012).

Binding either to the phosphodiesteric groups of the DNA strands or to the guanine nucleobase of DNA a monoalkyl- or dialkyl guanine derivative is resulting which will generate adducts of type DNA-Cp.

Cis-platinum having in its structure a divalent platinum atom may also realize two bifunctional bonds (Gârban, 1986 b ; Lippert, 1999). The binding occurs particularly to the guanine (G) nucleobases of DNA and a lesser extent to the nucleobases adenine (A), thymine (T) and cytosine (C).

Generally it is considered that chemical xenobiotics are represented by organic/inorganic substances which belongs to various classes of compounds. In the acceptation of Biochemistry/Xenobiochemistry the chemical xenobiotics could be of food interest (e.g. additives, contaminants), of pharmaceutical interest (chemotherapeutics obtained by synthesis or by extraction) and of toxicological interest (biocides). The subject of this paper circumscribes two xenobiotics of pharmacological interest often used both in veterinary and human medicine for treatment (Martin, 1989; Gârban et al., 1990; Manolescu, 1997; Summers, 2014).

1. Chemical structure of cyclophosphamide and *cis*-platinum

One of the most frequently used antitumor compound is cyclophosphamide (fig.1-a, fig.1-b), an alkylating agent. Its pharmacologically active group is 2-bis (β-chloroethyl)-amino-2-oxo-1,3,2-ozazaphosphorinan often called nitrogen mustard (Lindemann and Harbers, 1980; Gârban et al., 1986 a).

Cis-platinum (fig.1-c) is a synthetic anticancer agent that belongs to the group of the metal-based alkylating agents (Haiduc and Silvestru, 1989; Reedijk, 2008).

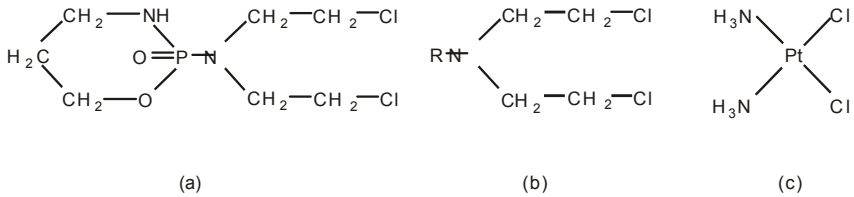

Fig.1. Chemical formula of cyclophosphamide and cis-platinum :
a) Cp - structural formula; (b) Cp- general formula; (c) cDDP - structural formula

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 (Lippert, 1999). Since the investigation of its cytostatic action by Rosenberg (1985), 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 (Reedijk, 2008).

In this context the molecular mechanisms of interaction for the alkylating agent cyclophosphamide and the metal-based drug cis-platinum (Pană et al., 1983; Johnson et al.,

2012; Galuzzi et al., 2014) will be exposed. Hereinafter will be discussed the chemical structure of the mentioned chemotherapeutics and details on their interaction with DNA.

A general mention is made with respect to that in case of Cp administration the cytotoxic effect target mainly the liver functions and in case of cDDP the renal functions.

A particular aspect in cytostatic chemotherapy is the fact that the DNA adducts make possible the biomonitoring of cases. In such circumstances there are discussed the investigations extended to biomarkers (Hemminki et al., 1998).

2. Mechanisms of action

Interaction of cyclophosphamide with DNA

The main target of Cp in biologic systems is represented by the nucleic acids, especially DNA. While the final alkylation targets of Cp are the guanine and cytosine bases of tumor cell DNA, it is activated in the liver to hydroxy-Cp, which enters tumor cells and converts to phosphoramide mustard (Gârban et al., 2000; Wolkenberg, 2001). The highly reactive metabolites of Cp interact with DNA, disturbing the structure – biologic activity relationship of the macromolecule, with dramatic effects on the cell life.

The mechanism of the interaction between Cp and DNA, which explains the antitumoral effects, is determined by the capacity of nitrogen mustards to bind to the N_7 atom of guanine, the most nucleophilic center, accessible from a steric point of view within the framework of the helical structure (Gârban, 2009). The interaction results in the formation of mono- or di-alkylated derivatives of the guanylic residue (the latter are typical for Cp). The attack of the alkylating agent Cp can lead to the appearance of both intrastrand and interstrand links. The interstrand links affect the guanines from different DNA strands, determining cross-linking which rapidly can block the replicative process of DNA. Whatever the linking manner, the final effect is the same: the modification of the chemical structure of the DNA macromolecules, followed by the disturbance of the biological activity – fig.2.

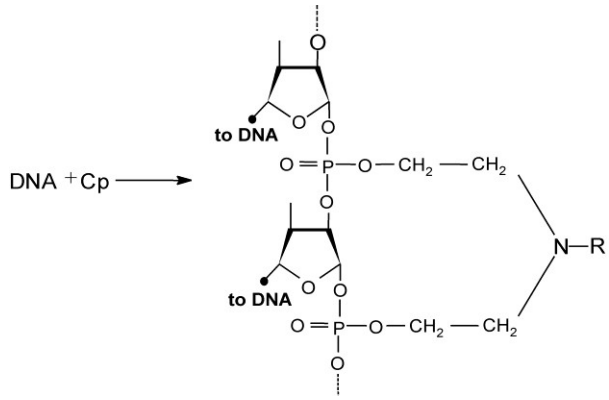

Fig 2. Binding mode of Cp to the phosphoric groups of DNA

The interaction of Cp with DNA, macromolecule which bears the genetic information, also implies the possibility that replication-transcription and translation processes could be affected (Johnson, 2012). As a result, the influence of protein synthesis is possible.

Cyclophosphamide is used in clinic to treat non Hodgkin lymphoma, Burkitt's lymphoma, chronic lymphocytic leukemia, various visceral solid tumors with rapid

proliferation tendency; Cp is often used in combination with surgical treatment, radiotherapy and other cytostatics. The indications of Cp in benign diseases include chronic evolutive arthritis, systemic lupus erythematosus, nephrotic syndrome, Waldenström disease, multiple sclerosis. Due to its immunoablation activity Cp is also used in preparative regimens for allogenic transplantation. Many studies have been made up to the present concerning the biologic effects of Cp, existing an extensive literature in the area.

Biogenesis of DNA-Cp adducts (fig.3) occurs by the cyclophosphamide binding to DNA after releasing one Cl atom and generating a monodentate derivative (monoalkylated) and after releasing two Cl atoms giving rise to a bidentate derivative (dialkylated)

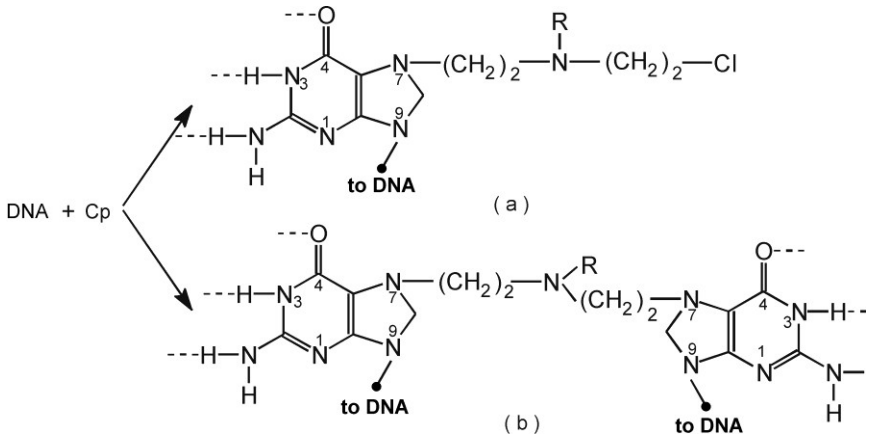

Fig. 3. Binding of Cp to the guanine nucleobase of DNA
a) mono(alkyl)guanine-derivate; b) di(alkyl)guanine-derivate

Interaction of cis-platinum with DNA

In the antitumoral chemotherapy different metallic compounds containing Pt, Pd, Ru etc. were studied. 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.

Cis-platinum is characterised by a planar geometry, has two inert ligands ($2NH_3$) and two labile ligands (2Cl) which are released during the hydrolysis (Rosenberg, 1985; Dasari and Tchounwou, 2014). Hydrolysis, developed in two steps, implies the mono- or diaquated platinum species formation (fig.4).

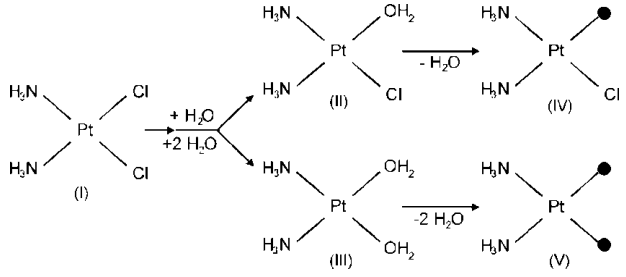

Fig.4. Formation of mono- and diaquated species of *cis*-platinum

Both species can form bindings with the nucleobases of DNA and, in some cases, with DNA (polyheteronucleotidic macromolecule) and protein (polyheteroaminoacid molecule) – symbolized by the amino acid chain (-aa-aa-). The bindings to the DNA nucleobases are made in the preferential order: guanine > adenine > cytosine, observing that thymine is not so often involved (Reedijk, 2008; Gârban et al., 2014; Manolescu et al., 2014). The binding may be homomacromolecular (DNA–cDDP) or heteromacromolecular (DNA–cDDP–Protein). As a result of the binding different derivatives with mono- or bidentate ligands are formed (fig.5)

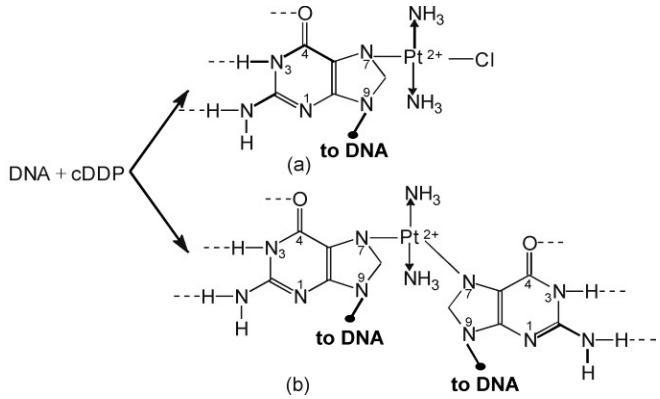

Fig. 5. Binding of cDDP to guanine nucleobase of DNA
a) fixing of monodentate ligand; b)) fixing of bidentate ligand

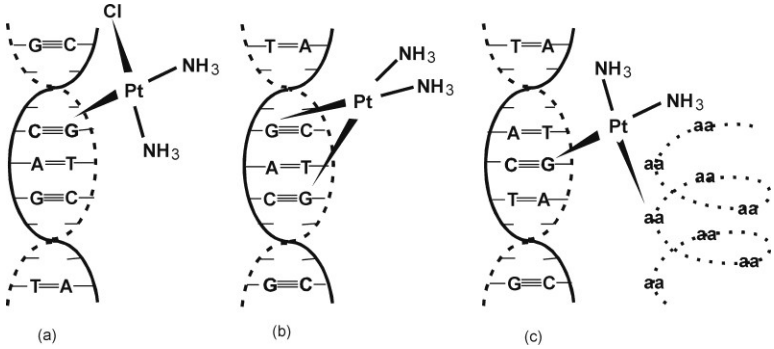

Fig.6. Binding of cDDP to DNA or to DNA and proteins:
a) monofunctional binding to a nucleobase; b) binding to different nucleobases;
c) heteromacromolecular binding between DNA strand and a polypeptide

Cis-platinum binding to the DNA can be made to the intrastrand nucleobases resulting adducts of type DNA-cDDP (i.e. homomacromolecular) or to nucleobases and protein generating adducts of type DNA-cDDP-Protein (i.e. heteromacromolecular) – see fig.6.

Generally, the usage of cis-platinum in the cytostatic chemotherapy is based on its interaction with DNA when various adducts of type DNA-cDDP result (Gârban, 2004). Adducts are formed by different bindings: (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) hetero-macromolecular cross-link DNA-cDDP-Protein.

Thus, the resulted DNA-cDDP adducts limit the division of cells and especially of the cancer cells. This means that cis-platinum, as a frequently used chemotherapeutic drug, affects mostly the cancer cells which a higher growth rates. In the organism the presence of the adducts DNA-cDDP can be detected analytically and can provide information about the efficiency of the anti-cancer action of the applied chemotherapeutic.

Discussing aspects related to the interactions of the DNA with cyclophosphamide (biogenesis of DNA-Cp adduct) and with cis-platinum (biogenesis of DNA-cDDP adduct) as well as their use in chemotherapy it is necessary to mention similarities and distinctions (Gârban et al., 1990; Gârban et al., 2009).

Similarities consist in the formation of adduct type complexes which reduce the multiplication of cells (having a more rapid rate in case of neoplasic cells) by disturbing their DNA biosynthesis . By this way the metastatic processes are limited, too.

Distinctions consist in their therapeutic efficiency in various types of neoplasis. In this regard it is known that cyclophosphamide is used with good results in the treatment of lymphomas, leukemia, breast cancer, malignant pheochromocytoma, Ewing's sarcoma a.o. Cis-platinum is more efficient in the therapy of testicular cancer, ovarian cancer, cervical cancer, sarcomas etc.

CONCLUDING REMARKS

Assessment of the adducts formation activity of Cp and cDDP – chemical xenobiotics of pharmacological interest - led to the knowledge of the specific molecular mechanisms and allowed to understand their cytostatic/cytotoxic effects.

In this context the structural characteristics, molecular speciation of Cp and cDDP as well as their interaction with the DNA macromolecule have been defined. The interaction is followed by the biogenesis of DNA-Cp, DNA-cDDP and DNA-cDDP-Protein adducts types. In the oncotherapy of humans and animals it is important to know also the neoplastic disease forms with better response to the use of these cytostatics

Presently, a domain of excellence in the cytostatic chemotherapy consists in the detection of DNA adducts useful in the biomonitoring of cases. But, in order to personalize the chemotherapy it must be taken into consideration the specificity of the resulting dyshomeostasis in metabolomics, too.

***Note**: The present paper was elaborated within the activity of the «Romanian Society of Comparative Oncology » Bucharest (Manolescu) and of the Working Group for Xenobiochemistry Timişoara (Gârban). Studies in the same domain were extended (after February 14, 2015) in the framework of the collaboration with the Faculty of Veterinary Medicine Timişoara.*

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