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Review Article

BIOCHEMICAL AND MOLECULAR MECHANISMS OF ACTION OF CISPLATIN IN CANCER CELLS

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Abstract. *Cisplatin (cis-Diamminedichloroplatinum II) is one of the most important chemotherapeutic agents widely used in treatment of many types of solid cancer. Accumulating evidence suggests that the cytotoxic activity of cisplatin involves both nuclear and cytoplasm component, but its biochemical and molecular mechanisms of action are still unclear. Its mode of action is linked to the ability of cisplatin to interact with purine bases on the DNA, causing DNA damage, interfering with DNA repair mechanisms and inducing apoptotic cell death in cancer cells. The major limitations in the clinical application of cisplatin are the numerous side effects and the development of cisplatin resistance by tumors. Mechanisms that can explain cisplatin resistance include the reduction in drug accumulation inside the cell, higher concentration of glutathione and metallothioneins, faster repair of cisplatin adducts and modulation of apoptotic cell death in various cells. In this article we review the pathways that cisplatin can activate in cancer cell, the mechanisms of resistance and clinical toxicities. A deep knowledge of mechanisms of action may lead to design of more efficient platinum-based antitumor drugs and provide new therapeutic strategies in cancer treatment.*

Key words: *cisplatin, DNA damage, cancer cells, drug resistance, platinum-based drugs.*

Introduction

Cancer presents the second most common cause of death in Serbia, right after cerebrovascular disease. According to National cancer database cancer mortality rate is higher among men than women (181 per 100,000 men and 113.6 per 100,000 women) [1]. Lung cancer, colorectal cancer, and stomach cancer were among ten leading causes of death in men, whereas breast cancer, colorectal cancer, lung cancer, stomach cancer, and cervical cancer were among twelve leading causes of death in women [2]. Multidisciplinary approach to treatment of human malignancies includes surgery, chemotherapy or radiation therapy depending on the stage when cancer is diagnosed. Clinically useful chemotherapeutic drugs inhibit the processes essential for cancer cell growth and/or proliferation, such as blocking production of DNA, mRNA or proteins, directly damaging DNA or inhibiting components required for DNA replication or chromosome separation [3].

Cisplatin or *cis*-Diamminedichloroplatinum(II) is an effective chemotherapeutic agent that is used in nearly 50% of all cancer patients [4]. This complex was first synthesized in 1845 by Peyrone, but its antitumor activity was discovered by accident, thanks to the research of Rosenberg, the physics teacher at the University of Michigan in the late 1960s. The Food and Drug Administration approved the clinical use of this drug for treatment of genitourinary tumors in 1978, and since then it has been one of the most widely used drugs in cancer treatment [5]. It has been an important part of chemotherapeutic regimes for treatment of broad range of malignancies. Cisplatin success in treatment of testicular cancer is remarkable; its cure rate is more than 90 percent when it is used in combination with other chemotherapeutics [6]. It has been used in fight against ovarian, head and neck, bladder, cervical, esophageal, as well as small lung cancer. However, many patients eventually relapse and become refractory to the drug. Drug resistance is the major complication in cancer chemotherapy and accounts for the failure of chemotherapy to cure majority of patients. The development of platinum analogs that display similar effectiveness as cisplatin, but have better toxicity profile and lack cross-resistance is the major task in research centers worldwide.

Chemical Structure of Cisplatin

Cisplatin is a white or yellow crystalline powder, slightly soluble in water and soluble in dimethylpirimidine and N, N-dimethylformide. It is a neutral inorganic molecule with molecular weight of 301,1 g/mol, density of 3,74 g/cm^3 and melting point at 270°C, composed of platinum ion

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bound to two ammine groups and two chloride ions that are arranged in a square (Fig. 1). In metal complexes Pt can exist in either 2^+ or 4^+ oxidation state. The ammine groups represent carrier ligands, while chloride ions are leaving groups. In cisplatin the chlorides are next to each other. The presence of leaving groups is essential for biological activity of cisplatin [7]. Inside the cell, cisplatin loses two chloride ions and they are replaced by loosely bound water molecules, allowing the platinum to attack the DNA molecule in nucleus.

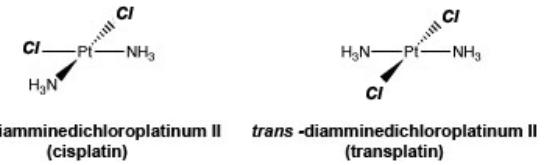

Fig. 1 Chemical structure of cisplatin and transplatin. Figure is modified from http://chemwiki.ucdavis.edu/Core/Inorganic_Chemistry/Coordination_Chemistry/Isomers/Geometric_Isomers%3A_cis-platin

Transplatin is an isomer that has both chloride ions opposite each other (Fig. 1); it causes different structural changes than cisplatin in cancer cells. Monoadducts, formed by transplatin, do not significantly change the structure and stability of DNA molecule [8].

Mechanisms of Action of Cisplatin

Cisplatin is administered to cancer patients intravenously as a sterile saline solution. In the circulation, chloride concentration is relatively high and cisplatin remains neutral and can be transported throughout the body. Once in the bloodstream, it binds strongly to plasma proteins, such as albumin and transferrin, leading to inactivation of large amount of the applied drug [9]. Passive diffusion across the plasma membrane has been proposed as process responsible for drug transport into the cell. In the last years, there is growing evidence that several proteins expressed on the cell membrane are involved in drug uptake. Copper transporter, that controls intracellular copper homeostasis, was shown to be involved in the uptake of cisplatin [10]. Many cellular components, such as cytoskeletal microfilaments, RNA and thiol-containing peptides and proteins, may react with cisplatin in the cytoplasm. Intracellular thiol-containing molecules such as glutathione and metallothionein, increase inactivation of the drug that results in cisplatin resistance.

Genomic DNA is the main cellular target for cisplatin, although only 1 percent of intracellular cisplatin is bound to nuclear DNA [11]. Cisplatin binds with DNA to form intrastrand crosslinks and adducts. DNA adducts formed by cisplatin inhibit DNA replication and/or transcription and activate several signal transduction pathways, culminating in the activation of apoptosis [12].

Cisplatin binds with DNA in two steps, first the bond with N7 guanine is formed, and then it binds with guanine or adenine in the same or opposite strand. The N7 atoms of guanine and adenine are the most accessible and cisplatin forms a broad spectrum of intra- and inter-strand crosslinks and all of them cause the distortion of the DNA. The great majority of DNA crosslinks are 1,2-d(GpG), and they represent 70 percent, while d(ApG) intrastrand adducts account for 20% of all lesions [13]. 1,2 intrastrand-crosslink is considered to be the most cytotoxic one, since inactive transplatin is not able to form this lesion. These lesions cause the bending and unwinding of the double helix and loss of function.

Several proteins can recognize the DNA bending induced by specific cisplatin adducts. High mobility group (HMG) proteins are non-histone chromosomal proteins involved in gene regulation and chromatin structure. Protein HMG1 binds with high selectivity to platinum adducts in DNA [14]. In this way, bounded proteins act as a shield and protect DNA from repair mechanisms. HMBG binding modulates signaling pathways in the cell by diminishing the efficiency of NER, and it has been connected to MMR, p53 activity and MARK pathway [15]. Recognition of 1,2-intrastrand adduct by these proteins may be the first step towards the initiation of apoptosis.

DNA lesions are recognized by damage recognition macromolecules, those can repair cisplatin DNA adducts. The most important families of DNA repair proteins are: 1) nucleotide excision repair (NER) proteins, 2) mismatch repair (MMR) proteins and 3) DNA-dependent protein-kinase (DNA-PK) proteins.

Nucleotide excision repair (NER) system consists of at least 17 different proteins. This multiprotein complex recognizes intrastrand crosslinks and subsequently excises the DNA sequences of 27-29 base pairs oligonucleotides in length containing the damage [16]. The incision reaction on both sides of the lesion involves numerous protein factors such as XPA, RPA, XRC-HR23B, ERCC1-XPF and XPG. The enzyme DNA polymerase fills the remaining gap [17]. Over-expression of some genes involved in NER complex is associated with cisplatin resistance [18]. Mismatch repair (MMR) complex is ATP dependent multiprotein system that is crucial for normal *in vivo* response to DNA damaging drugs [19]. The MMR complex causes cell cycle arrest. The MMR proteins would try to insert the correct nucleotide on the non-damaged strand opposite to the intrastrand adduct between two adjacent guanines. When it does not succeed in the attempt to repair the damage, the apoptotic pathway is activated [20]. The Ku subunit of DNA-PK protein can also interact with cisplatin-DNA lesions, which leads to the activation of DNA-PK to phosphorilate itself or other transcription factors.

Oxidative stress is one of the most important mechanisms involved in cisplatin cytotoxicity (Fig. 2). Cisplatin causes oxidative stress by increasing the level of super oxide anions and hydroxyl radicals [21]. Under oxidative stress condition, excessive reactive oxygen species (ROS) can damage cellular proteins, lipids and DNA and may modulate survival signaling cascades.

Depending on the severity and duration of ROS exposure pro-survival or pro-apoptotic response pathways may be activated. Mitochondrial glutathione (GSH) is an essential molecule in the regulation of inner mitochondrial permeability. Cisplatin decreases intracellular concentration of GSH, leading in hydroxyl radical formation and oxidative stress, resulting in loss of mitochondrial protein sulfhydryl group, calcium uptake and reduction of mitochondrial membrane potential [22]. The molecular mechanisms that underlie the cytotoxic potential of cytoplasm cisplatin may involve the pro-apoptotic Bcl-2 family members Bak1, the voltage-dependent anion channel 1 (VDAC1) and the Bak1 homolog Bax [23]. It is well known that mitochondrial DNA (mtDNA) is more susceptible than nuclear DNA to damage from reactive oxygen species, due to either a limited capacity for DNA repair or the presence of nucleosome-free structure [24]. Cisplatin is a potent mtDNA-targeting agent. Cisplatin forms crosslinks with mtDNA that is more vulnerable than nuclear DNA. The mtDNA adduct levels are higher than the nuclear DNA adduct levels, due to significantly higher number of guanine stretch sequences (target sequences of cisplatin) in mtDNA than in nuclear DNA [24].

As previously noted cisplatin inter- and intra-strand DNA adducts can be recognized and safely removed by several repair systems that normally operate in the context of a temporary cell cycle arrest. There are two main checkpoints, G1/S and G2/M, in which cell cycle will be arrested to help the function of the repair machinery. The G1/S checkpoint allows DNA restoration before replication and G2/M facilitates the reparation of DNA damaged during S and G2 phases to prevent its segregation into daughter cells. Treatment with cisplatin usually induces G2 arrest through phosphorilation checkpoint kinases Chk1 and Chk2, activation of Cdc25C and its translocation to the cytoplasm which provoke cell arrest in G2 phase of cell cycle [25]. Meanwhile, when the damage is irreparable, the cell activates mechanisms that induce cancer cell death via apoptosis and prevent the passage of these cells into mitosis. Apoptosis, as a mode of programmed cell death, is energy-dependent process leading to membrane blabbing, phosphatidylserine externalization, cell shrinkage, chromatin condensation and activation of a family of cysteine proteases called caspases [26]. There are two major pathways of apoptotic cell death: the extrinsic pathway, activated by pro-apoptotic receptor signals at the cell surface, and the intrinsic pathway, activated by mitochondrial signals. In response to DNA damage, the Bcl2 family proteins regulate apoptosis through cytochrome c, apoptosis promoting activating factor 1 (Apaf-1) and caspases 9 and 3.

It is known that p53 protein plays a central role in chemotherapy-induced apoptosis. A primary mechanism by which p53 induces apoptosis is through transcriptional activation and repression of target genes whose promoters contain p53-binding sites. These genes may activate apoptotic process via multiple pathways (Fig. 2) [27]. The protein p53 is "guardian of the genome" because it activates a host of other genes (p21/waf1, mdm2, GADD45 and others) that lead to cell cycle arrest and activation of DNA repair [28]. On the other hand, p53 regulates cisplatin-induced apoptosis by several mechanisms like: activation of pro-apoptotic genes including PUMA [29], caspases [30], PIDD [31], MAPK protein family [32], as well as interaction with Bcl2 family proteins in mitochondria and/or cytosol

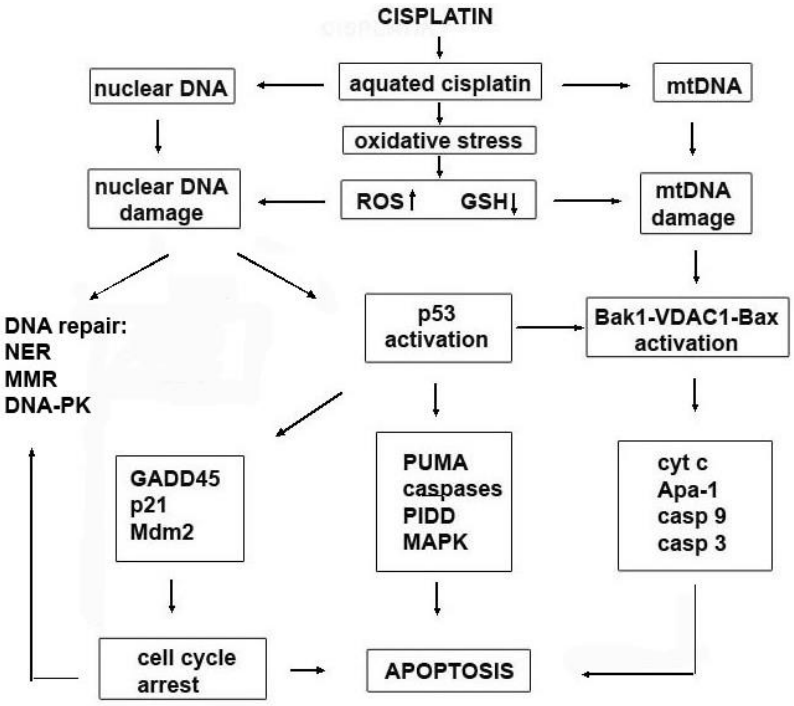

Fig. 2 Molecular mechanisms of cisplatin in cancer treatments

[33]. The p53-negative cells also respond to cisplatin-induced DNA damage that suggests the existence of alternate pathways upon the stress.

Side Effects of Cisplatin

Chemotherapy is associated with increased toxicity, especially in older patients. The efficiency of cisplatin administration is often limited by its side effects. Various studies confirmed that cisplatin induces the formation of ROS, responsible for the numerous side effects like nephrotoxicity, ototoxicity, hepatotoxicity, cardiotoxicity and neurotoxicity.

The kidney is the main route for excretion of cisplatin and it accumulates it to a greater degree than other organs, which is the reason for the cisplatin-induced nephrotoxicity. Tubular cell injury occurs in one third of cisplatin treated patients and manifests as an increase in serum urea and creatinine concentration and imbalanced electrolytes [34]. Proximal tubulocytes are the main point of cisplatin action. The concentration of the drug in these cells is five times higher than its serum concentration [35]. Pathological changes are most prominent in S3 segment and they are caused by multiple mechanisms such as oxidative stress, apoptosis, inflammation and fibrogenesis. Nephrotoxicity is cisplatin-dose-dependent [36]. Adequate hydration can decrease the reactive monohydrated cisplatin form and it is renoprotective.

Cisplatin is the most ototoxic drug known. Between 10 and 90 percent of treated patients develop some degree of hearing loss. These changes are irreversible and pediatric population is very vulnerable [37]. The destruction affects auditory sensory cells in the organ of Corti and both hearing and vestibular functions can be affected [38]. Ototoxicity is irreversible and it is associated with hipoalbuminemia, application of other medicaments, genetic factors, renal failure, and patient's age [39]. Otoprotective therapy should be administrated. Intratympanic application of the drug is the most effective, without compromising antitumor effect.

High dose of cisplatin may cause hepatotoxicity. Oxidative stress appears to play an important role in cisplatin-induced hepatotoxicity liver injury [40]. Cisplatin therapy has been associated with mild elevation of transaminases and bilirubin in circulation [41]. Recent studies show that administration of high doses of selenium and vitamin E has protective effect on liver injury [42]

Antineoplastic therapy with cisplatin induces lipid peroxidation of cardiac membranes leading to serum elevation of lactate dehydrogenase and creatine kinase. Arrhythmias and prolongation of QT-interval have been reported in vulnerable individuals [43].

Cisplatin is thought to act on the dorsal root ganglion to generate both transient and chronic neuropathies, which explain the primary sensory neuropathy commonly observed in patients treated with cisplatin [44]. Anti-oxidant compounds are being developed to prevent these toxic side effects.

Cisplatin administration results in side effects common to most cytotoxic agents such as nausea, vomiting, myellosuppression, gastrotoxicity and some reproductive toxic effects [45].

Development of Cisplatin-induced Resistance

Tumor cell resistance to chemotherapeutic drugs is a barrier to improving outcomes in these patients. Cisplatin resistance is a multifactorial phenomenon and may include changes in cellular uptake, decreased influx or increased efflux of drug, glutathione or metallothionein conjugation or drug detoxification. The increased DNA repair and inhibition of apoptosis is the significant mechanism of resistance. The resistance can be intrinsic, in which the drug is ineffective from the onset or acquired resistance, in which a drug is initially beneficial but becomes ineffective over time [46].

Reduced drug accumulation is predominantly caused by defect in the uptake of a drug. It has been further confirmed in human ovarian carcinoma cell line that cisplatin, at plasma concentration, rapidly downregulates protein expression of Ctr1 [47]. Two other copper transporters have also been implicated in resistance to cisplatin: ATP7A and ATP7B. These copper transporters are responsible for the export of copper from the cell. High levels of ATP7A and ATP7B expression lead to cisplatin resistance [48].

In the cytoplasm aquated cisplatin reacts with thiol containing compounds including glutathione and metallothioneins. Glutathione-S-transferase catalyses the reaction where cisplatin is conjugated with glutathione and therefore, cisplatin can not bind with DNA and other cellular targets. In some malignant tissues, there is a positive correlation between resistance to treatment and cellular level of glutathione as well as over expression of GST and other enzymes involved in glutathione metabolism [49, 50]. Metallothioneins, a family of low molecular weight thiol-rich proteins, can bind cisplatin in cytoplasm leading to drug inactivation in some tumor cell lines [51, 52, 53].

Alterations of the DNA repair pathways are important for mediating resistance. Studies of testicular and ovarian carcinoma cell lines showed a deficiency in NER mechanism in cells that were sensitive to platinum therapy [54, 55]. The NER is the main repair pathway that involves recognition of the damage and incision that requires various proteins including ERCC-XPF. The level of ERCC1 protein inversely correlates with the response to chemotherapy in gastrointestinal and non-small cell lung carcinoma [56, 57].

Resistance mechanisms, therefore, arise as a consequence of intracellular changes that either prevent cisplatin from interacting with DNA, interfere with DNA damage signals for activating the apoptotic machinery, or both. More than one mechanism is usually observed in resistant cells, and this contributes to the multifactorial nature of cisplatin resistance. To minimize cisplatin resistance, combinatorial therapies were developed and

have been proven to be more effective in defeating cancer. The main goal is to find compounds that are less toxic, have no cross-resistance and possibly are more efficient than cisplatin. Drug resistance is the single most common reason for discontinuation of the drug.

Development of New Platinum-based Antitumor Drugs

Different modifications of cisplatin have been investigated in order to obtain a drug that has better toxicity profile and wider therapeutic spectrum than cisplatin. In order to reduce toxic side effects and overcome cancer cell resistance, new platinum drugs have been developed. Although a large number of platinum compounds underwent *in vitro* testing, less than a thirty entered clinical trials [58].

Cisplatin, carboplatin and oxaliplatin (Fig. 3) are worldwide approved drugs that have a major role in human oncology.

The second generation platinum drug carboplatin was introduced into cancer therapy in 1989, for treatments of ovarian cancer. The replacement of the chloride groups of cisplatin by cyclobutanedicarboxylate ligand of carboplatin (Fig. 3) provides good aqueous solubility and greater stability and leads to diminishing side effects. Carboplatin can be applied in higher doses with possibly better effects. The downside is that carboplatin and cisplatin are cross resistant.

Newly acquired knowledge about mechanism of tumor resistance to platinum drugs enabled discovery of third generation drugs such as oxaliplatin that is effective in colon cancers, which were thought to be resistant to platinum compounds. Oxaliplatin has a different carrier ligand diaminocyclohexane (DACH) [59], that has less cross-resistance and a more favorable toxicity profile.

Satraplatin, lipophilic platinum (IV) complex is the first platinum compound active after oral administration and is currently in different phases of clinical research [60]. Platinum (IV) complexes are less reactive in ligand substitution reactions compared to their platinum (II) analogues, and they have reduced toxicity and a smaller fraction of the drug deactivated on its pathway to target cell [61]. The platinum (IV) complexes are in focus and they have been tested in various cancer cell lines [62, 63]. Newly synthesized platinum (IV) complexes are tested for cytotoxic activity against various cell lines and some of them showed similar activity as cisplatin towards human ovarian carcinoma, breast cancer and colon carcinoma cell lines [64, 65].

Picoplatin is platinum coordination complex which, during in vitro testing, showed activity against several cisplatin-resistant and oxaliplatin-resistant cell lines. Unfortunately, it failed to produce significant clinical results compared to standard therapy for lung cancer [66].

Multinuclear complexes are another class of platinum complexes that showed activity in both cisplatin resistant and cisplatin sensitive cell lines. They are di-, three-, or tetra-nuclear compounds, in which platinum centers are connected by rigid or flexible bridges [67, 68]. The DNA binding of these compounds is structurally different from binding of cisplatin and its analogues and they exhibited cytotoxicity in cancer cell models, and some of them entered clinical trails [69].

Platinum drugs resistance can also be circumvented by improved delivery of the drug to tumor tissue. This can be achieved by linking platinum-based drug to a water soluble, biocompatible co-polymer [70]. In some cases, such as an ovarian cancer, local application of a drug, through intraperitoneal injection might be adequate [71].

Conclusions

Cisplatin plays a major role in the treatment of a variety of malignances. Cisplatin and other platinum-based compounds are cytotoxic drugs which kill cancer cells by damaging nuclear and mitochondrial DNA, inhibiting DNA replication and mitosis and inducing apoptotic cell death. Cisplatin-induced damages are considered to be an important trigger of p53 activation that leads to cell apoptosis. On the other hand, cisplatin can also react with other cellular components such as membrane phospholipids and proteins, cytoskeletal microfilaments, thiol-containing biomolecules and cytoplasm proteins, resulting in cell death depending upon the mechanism of DNA damage. Unfortunately, the therapeutic effects of cisplatin are often limited due to cell resistance which develops through changes in drug transport, detoxification, DNA repair and apoptosis signaling pathways. Dose dependent toxicity and acquired and intrinsic resistance are still the major obstacles in platinum based therapy. Therefore, the comprehensive understanding of the mechanisms of action and tumor resistance might be useful in defining new strategies in the search for the new therapeutics with improved pharmacological properties.

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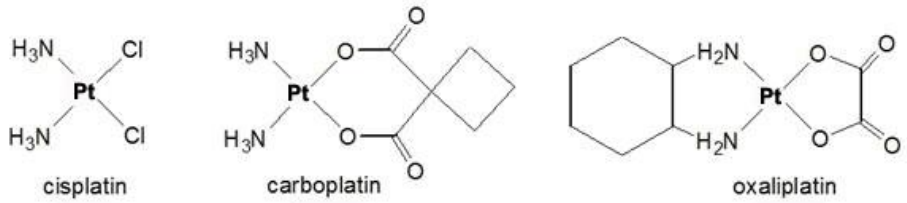

Fig. 3 Chemical structures of cisplatin, carboplatin and oxaliplatin. Figure is modified from http://www.dscf.units.it/ricerca_grp.php?name=inorgani1group&menu=research&file=ruthenio/ruthenio

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