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Mini-Review

Cytotoxicity and resistance mechanisms of cisplatin

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ABSTRACT

In recent years, cisplatin has found wide application in different types of cancer. Among the different platinum compounds, cisplatin was found to be able to cross the cell membrane due to its simple chemical structure and, form adducts with DNA, to cause cell death. The removal of these adducts is mediated mainly by the excision repair cross complementing group 1 (ERCC1) protein of the nucleotide excision repair (NER) system whose activity affects tumor response. However, the use of cisplatin may be burdened by an acquired resistance due to the combination of different mechanisms. The purpose of this manuscript is to discuss the mechanisms of cytotoxicity and resistance to cisplatin.

KEYWORDS: cisplatin, toxicity, resistance.

INTRODUCTION

The use of metal complexes in anticancer therapy began after the discovery of the antiproliferative properties of cis-diamminedichloroplatinum(II) (cisplatin) by Rosenberg in 1965 [1]. In his studies he tested the effect of an electric field generated by two platinum foil electrodes immersed in a culture medium on the growth of E. Coli bacteria. Rosenberg realized that cell division was inhibited by a platinum compound that might be produced in the culture medium as a result of electrolysis and the consequent reactions of the platinum ions with Cl^- and NH3 in the culture medium [2]. Later studies led to the identification of cisplatin [3]. The characteristics that have made cisplatin so important in antitumor therapy are its antiproliferative activity against a wide range of tumors and its remarkable effectiveness [4]. Cisplatin, after intravenous administration, penetrates the cells by passive diffusion, undergoes a hydrolysis reaction then forms adducts with DNA filaments causing the activation of the process of cellular apoptosis [5-7].

The clinical limits in the use of cisplatin in cancer therapy are the risk of developing side effects and drug resistance. Mild side effects are nausea, vomiting and asthenia. They occur in the hours immediately following administration and are commonly controlled by the administration of antiemetic drugs [8-10]. Among the most severe side effects, there may be a deficiency of kidney function. Since nephrotoxicity can be limited by pre- and post-treatment hydration, the most severe side effect of cisplatin treatment is ototoxicity and neurotoxicity [6, 11-13].

Symptoms of neurotoxicity include numbness, tingling, and motor difficulties of various kinds and severity, which may persist for up to 4 years from the end of treatment [14-17]. However, clinically, nephrotoxicity is usually the dose-limiting toxicity [18]. In order to reduce this toxicity, intravenous administration of cisplatin requires adequate hyperhydration to begin 6-12 hours before the dose of chemotherapy and continue over the following 12-24 hours [18-22].

The need for adequate hydration of the patient to reduce the risk of renal toxicity has led to the search for oral drugs contributing significantly to the improvement of the quality of life of the

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patients [23-26]. For this reason, several platinum complexes have been studied to be administered orally, such as satraplatin [27-29]. Unfortunately, none of the compounds studied were approved for clinical use, having demonstrated poor anticancer activity in phase II clinical trials [27].

Mechanism of action of cisplatin

Rosenberg and colleagues in 1965 during experiments conducted on *Escherichia coli* discovered the inhibitory effects of cis--diamminedichloroplatinum (cisplatin), a complex containing bivalent, inorganic and water-soluble platinum, on cell replication (Figure 1) [2]. Following these discoveries a number of basic and clinical studies that have led to the approval for the use of these compounds, initially for testicular cancer alone, then for many types of cancer, have been conducted. Over the past 30 years, cisplatin has been used in the treatment of bladder, ovarian and lung cancer and is currently considered the most widely used cancer drug in North America and Europe.

However, due to the toxic effects of cisplatin, such as vomiting and kidney/audiological damages, similar drugs that contain platinum, such as carboplatin and oxaliplatin, have been investigated. Data from previous studies have demonstrated that when these compounds are utilized, there is a reduction of undesiderable side effects [30-32].

However, carboplatin and oxaliplatin, synthesised for potential clinical use, were found to be actually less toxic than cisplatin but also had reduced antineoplastic efficacy. The cellular effects of platinum compounds have been extensively studied using cisplatin, carboplatin and oxaliplatin. Cisplatin was found to be able to cross the cell membrane due to its simple chemical structure despite there being numerous transmembrane transport systems for the extrusion of drugs from a cell. Recent studies, comparing cisplatin with transplatin, show that the effects of cisplatin on DNA lead to cell death thanks to the cis conformation of its reactive groups. At the physiological pH of 7.4 chlorine atoms can be displaced directly by reaction with nucleophilic substances, such as thiol groups; the substitution of chlorine is probably responsible for the formation of activated products of the drug, which then react with nucleic acids and proteins.

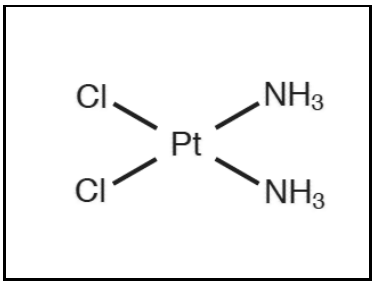

Figure 1. Structure of cisplatin.

Once inside the cell the cisplatin can follow three different paths: 1) immediate extrusion from the cell through one of the many specific transmembrane transport systems; 2) neutralization of proteins with sulfide groups such as glutathione; 3) reaction with intracellular molecules such as RNA and DNA, both cellular and mitochondrial. Platinum complexes can interact with DNA by forming intra- and inter-stranded crosslinks (covalent Platinum–DNA bonds).

Several studies have confirmed that N7-d(Gpg) intrafilament adducts and N7-d(Apg) are probably responsible for cisplatin-induced cell death (Figure 2). The adducts N7-d(Gpg) and N7-d(Apg) represent more than 80% of the actual damage on DNA strand following exposure to cisplatin and are associated with a strong folding of the double helix of the DNA. The nitrogen in position 7 of the guanine is very reactive and platinum forms cross-links between adjacent guanines on the same DNA strand. Adenine-guanine cruciate bonds are also rapidly formed.

These intra-strand cross-links are mainly 1,2-d(GpG) and to a lesser extent 1,2-d(ApG). 1,2-intra-strand cross-links structurally distort the DNA duplex and ultimately lead to genotoxicity and antitumor activity.

DNA complexes with cisplatin inhibit replication and transcription of DNA and lead to breakages and errors in coding. Cisplatin induces cell cycle arrest at the DNA synthesis phase, and as the time increases, accumulation of cells in sub-G1 phase of cell cycle is detected, although the effects due to the formation of cross-links are more marked during cellular phase S.

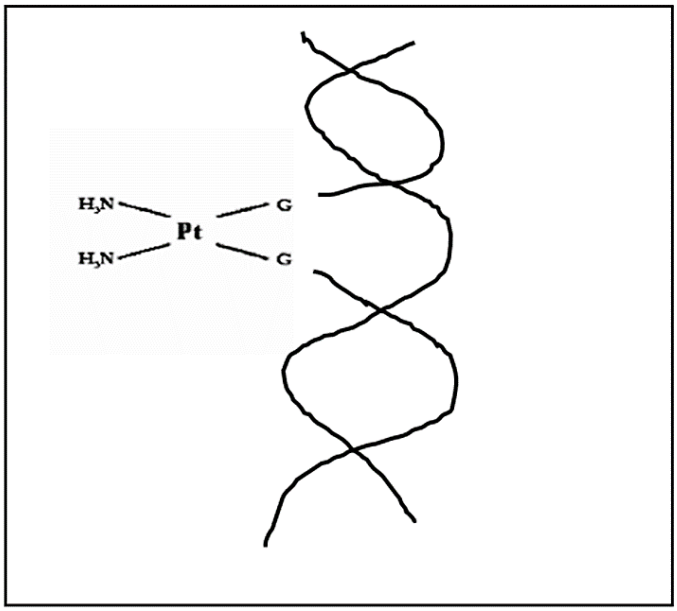

Figure 2. Interaction of cisplatin with DNA.

Cisplatin adducts can be repaired by the DNA repair system named NER (Nucleotide Excision Repair), which is an effective system for correcting a wide variety of lesions causing distortion of the double DNA helix and are caused by both physical and chemical agents [33]. In particular, the NER is required for the repair of frequent damage caused by ultraviolet radiation. Mutations in human genes coding for proteins in this system are associated with at least three different genetic diseases: Xeroderma pigmentoso, Cockaynie syndrome, and Tricothiodidystrophy, which have in common an extreme sensitivity of patients to sunlight. The NER system includes proteins with damage recognition, cutting, synthesis and binding activities. About 30 polypeptides that participate in this system have been identified, including the protein ERCC1 (*Excision Repair Cross Complementing group 1*), which plays a fundamental role in the process of incision of the filament, which is the limiting stage of the process of repairing.

The DNA structural alteration caused by cisplatin interferes with DNA replication and gene transcription, and thus, needs to be repaired correctly before permanent mutations or cell death occurs. DNA helix-distorting lesions can be recognized by NER surveillance proteins such as Xeroderma pigmentosum complementation group C (XPC) and HR23B protein. Once bound to damaged DNA, XPC recruits transcription factor II H (TFIIH), a 10-subunit protein complex consisting of two important DNA helicases, XPB and XPD. XPB and XPD, together with XPA and replication protein A (RPA), separate the two DNA strands around the damage site, creating a pre-incision DNA bubble that is recognized by repair endonucleases ERCC1-XPF and XPG.

Subsequently, two endonucleases, XPG and the XPF-ERCC1 complex, affect the damaged filament in the phosphodiester bond at a distance between 22 and 24 nucleotides from the 5' end of the lesion and following the action of the TFIIH complex, the fragment containing the lesion is eliminated and, through the activity of DNA polymerase enzymes, the DNA fragment is replaced. Since the cytotoxic effect of cisplatin is mainly determined by the formation of adducts with DNA, the removal of which is mediated by the NER system, it follows that the protein ERCC1 plays a crucial role in the response to this therapy. It has long been observed, in *in vitro* studies, that ERCC1 activity is essential for the repair of cisplatin adducts and the consequent sensitivity to this chemotherapy [34, 35].

Several studies have shown that the presence of a high expression of ERCC1 in ovarian and gastric tumor tissues is associated with increased resistance to

chemotherapy containing cisplatin. Similarly, using gene expression analysis with real-time PCR, was observed a correlation between the mRNA levels of ERCC1 and TS in tissues and the response to chemotherapy with oxaliplatin and 5-fluorouracil in 50 patients suffering from colon cancer [36].

In the study of Lord *et al*. 56 patients with advanced stage pulmonary carcinoma (IIIb or IV) treated with gemcitabine 1250 mg/m^2 (days 1 and 8) and cisplatin 100 mg/m^2 (day 1 every 3 weeks) showed that overall average survival is significantly higher in patients with lower ERCC1 expression [37]. In the study of Olaussen *et al*., the expression of ERCC1 was determined by immunohistochemical analysis on 761 patients with non-small cell lung cancer treated with cisplatin: 44% of patients presented a higher expression of the gene compared to 56% of the samples that had a reduced expression of ERCC1. Treatment with cisplatin was found to be of significant benefit to patients with no ERCC1; such chemotherapy treatment allowed for significant life expectancy in patients with ERCC1-negative tumors but not in those with ERCC1-positive tumors. However, it has also been observed that, among patients not undergoing chemotherapy, individuals with ERCC1-positive tumours live longer than those with ERCC1-negative tumours [38]. The study published by Rosell, in which 100 patients with lung cancer treated with cisplatin and gemcitabine were enrolled, came to the same conclusions [39].

Results show that patients with low levels of expression of ERCC1 and ribonucleotide reductase subunit RRM1, a fundamental enzyme for the synthesis of deoxyribonucleotides involved in the mechanism of action of gemcitabine, have a statistically significant advantage in terms of median survival.

Based on these findings, Rosell himself conducted a trial, whose preliminary findings, obtained from 264 patients, were presented during the ASCO 2005 meeting; patients were initially randomized in the control branch receiving docetaxel and cisplatin independently of the mRNA level of ERCC1, or in the experimental branch receiving docetaxel and cisplatin in case of low mRNA levels of ERCC1 or docetaxel and gemcitabine in the case of high mRNA levels of ERCC1 [40, 41].

The percentage of objective responses in patients with low m level was 56.6%, while in the control branch the percentage was 40.4%: conducting a sub-group analysis in this branch, it was observed that patients with low mRNA levels have a response rate of 47.3% while in patients with high mRNA levels the rate is reduced to 26.1%. The logistical regression model for tumor progression indicates a significant benefit for randomized docetaxel and cisplatin patients based on low ERCC1 levels. Despite the fact that the results were preliminary, overall survival as well as median time to tumor progression were significantly longer for patients with low mRNA levels of ERCC1.

It has been speculated that single nucleotide polymorphisms (SNPs) in DNA repair genes may change gene expression and activity, and hence influence the effectiveness of cancer treatment. A SNP involving at codon 118 has been identified (Esone 4): this is a silent mutation C>T that converts the codon AAC in codon AAT, both coding for the amino acid asparagine, although the second is used less frequently. Such polymorphism was initially studied in two cellular lines of ovarian carcinoma equally resistant to cisplatin but genotypically divergent. This polymorphism resulted in a triplet code for the same amino acid, asparagine. However, the TT genotype resulted in a 50% reduction in codon usage in MCAS (cisplatin-resistant mucinous cystadenocarcinoma) cell lines when compared to A2780/CP70 (also cisplatin-resistant) ovarian cancer cell lines. This reduction translated to decreased ERCC1 mRNA expression and reduced cisplatin–DNA adduct repair.

In MCAS cell line, the lower capacity of repair of DNA lesions caused by cisplatin, was however compensated by the increased activity of the cytosolic inactivation mechanisms of the drug [42]. In the Ryu study, 109 non-small cell lung cancer (NSCLC) patients were enlisted and treated with chemotherapy regimen containing cisplatin [43].

As for survival rate with respect to the polymorphism of codon 118 in ERCC1, the results showed a median survival time in patients showing C/C genotype longer than that of patients with the variant genotype (T/T or C/T) ($P = 0.0058$). Therefore, the C/C genotype in codon 118 of ERCC1 was considered a surrogate marker for predicting better survival in non-small-cell lung cancer patients treated with cisplatin.

Another possible molecular determinant of cisplatin chemotherapy response is represented by the XPD protein, originally called ERCC2, which acts as a helicase within the TFIIH complex. Studies have demonstrated the presence of different polymorphisms of XPD that can have a role both in etiopathogenesis and in the response to chemotherapy of several cancers [44].

Regarding the study of such polymorphisms in patients with NSCLC, the homozygous mutated genotypic variants of Snps XPD A751C and G312A have been associated with a suboptimal ability to repair DNA damage, in contrast to wild-genotypestype which showed increased repair capacity for both Snps [45]. These findings led authors to believe that in clinical trials patients with wild-type genotype could show an advantage in terms of objective responses and survival. In the first clinical studies, where a correlation between the polymorphisms of XPD and the outcome of chemotherapy treatment containing cisplatin was studied, a statistically significant difference between the different genotypes, activity and effectiveness of the chemotherapy was not highlighted [43].

Recently, a large number of studies suggested that ERCC1 and XPD polymorphisms can predict the therapeutic response to antineoplastic treatment and the prognosis in human cancer but further studies are needed to clarify their definitive role.

Pharmacoresistance in cisplatin therapy

Cancer cells can show innate or acquired resistance. If the tumor does not respond to the first treatment with the drug, it is called innate resistance. For example, some cancers can have innate resistance to cisplatin, such as some forms of colorectal carcinoma, prostate and lung cancers [46]. If there is a subset of cells in the tumor that has developed a mutation that can partially or completely block the action of the drug, they will be able to survive the first round of chemotherapy. The tumor will then be formed only by the surviving cells or their mutated forms and will thus acquire resistance to the drug [47, 48]. Subsequent treatment cycles will therefore have less efficacy and greater doses of the drug will be needed. In addition to drug resistance, some types of cancer develop resistance to drugs of the same chemical family (cross-resistance) or to multiple chemotherapeutic drugs with different structures and functional activity (multi-drug resistance MDR) [49-54].

The onset of acquired resistance after the first treatment is the major limitation of the clinical use of cisplatin. Pharmacoresistance is due to a multiplicity of mechanisms making an approach that can overcome them all, difficult. With regard to cisplatin, five main mechanisms have been identified with which the cancer cell becomes resistant to the effect of the drug:

1. Decreased entry of cisplatin into the cell. The expression of Ctr1 is decreased and this leads to a decrease in the amount of cisplatin entering the cell [55].
2. Increased cisplatin cell efflux. There is an increase in the expression of proteins that function as efflux pumps such as Multidrug resistance protein (MRP-2) and the trans-membrane protein ATP7B that regulates copper efflux [56, 57]. This leads to a lower accumulation of cisplatin in the cell.
3. Increased deactivation by molecules with thiolic groups. There is an increased amount of molecules with thiolic groups that are able to deactivate cisplatin by formation of complexes. The main one is glutathione (GSH), but thiodixin-1 and thiodixin-1 reductase also appear to be implicated in cisplatin resistance [58, 59].
4. Increased DNA repair capacity. DNA repair mechanisms such as mismatch repair (MMR) and excision of nucleotides or excision nucleotides repair (NER) (in particular ERCC-1) are enhanced. This leads to increased removal of cisplatin-DNA adducts without induction of apoptosis in the tumor cell [4].
5. Increased tolerance to DNA damage and inhibition of apoptosis. A higher tolerance to DNA damage can be given by an over-expression of β polymerase which, unlike other human polymerases, continues during the process of DNA replication without recognizing adducts with cisplatin. Inhibition of the apoptosis process in resistant cells may be due to high levels of apoptosis inhibitors or low levels of apoptosis promoters [60-62]. Depending on the type of tumor, these mechanisms may be present individually or at the same time.

CONCLUSIONS

In recent years, cisplatin has found widespread use in the treatment of several cancers of both adults and children. Currently, cisplatin is one of the most used drugs in North America and Europe. Its use may, however, be limited by the appearance of resistance of cancer cells. Knowledge of the mechanisms of cytotoxicity and resistance can be useful for the daily practice of clinicians engaged in the treatment of cancer.

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CONFLICT OF INTEREST STATEMENT

The authors certify that they have NO affiliations with or involvement in any organization or entity with any financial interest or non-financial interest in the subject matter or materials discussed in this manuscript.

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