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MECHANISMS OF RESISTANCE TO PLATINUM CYTOTOXIC DRUGS

(DIPLOMA THESIS)

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MECHANIZMY VZNIKU REZISTENCE VŮČI PLATINOVÝM CYTOSTATIKŮM

(DIPLOMOVÁ PRÁCE)

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Petr Bouška

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1. Objective

The aim of this thesis is to summarize the most important mechanisms that can contribute to the development of a resistance to platinum drugs (i.e. clinically used cisplatin, carboplatin and oxaliplatin; this essay does not consider agents that are not in clinical use at this time).

This work focuses especially on the resistance caused by decreased influx and increased efflux of platinum drugs (diminished accumulation), detoxification by glutathione and metallothionein, improved repair of DNA lesions and enhanced tolerance to DNA-Pt adducts. For each of these intracellular mechanisms of resistance this essay tries to describe presumptive mechanism of action, importance in clinic and briefly assessed possibilities of circumventing resistance.

2. Introduction

Although the first platinum drug, **cisplatin** [*cis*-diamminedichlorplatinum (II)] (Figure 1) was initially described by M. Peyrone in 1845 [1], its biological activity was discovered more than 100 years later. In 1965, Rosenberg and co-workers reported that the cytostatic effect induced by electric fields on cultures of Escherichia Coli was due to the formation of cisplatin and its corresponding tetrachloroplatinum(IV) analog by electrochemical reactions on platinum electrodes [2]. First preclinical pharmacology studies in 1969 have approved cisplatin antitumor properties [3]. Cisplatin is now in widespread use for the treatment of variety of human malignancies. However, many tumors are intrinsically resistant, and the development of acquired resistance during the course of treatment of initially sensitive tumors is a common occurrence that constitutes a major obstacle to the curative use of this drug [4]. Another important obstacle is severe side effects of which nephrotoxicity and peripheral neurotoxicity are the most serious [5, 6] (Figure 2) and limit its curative potential. Nephrotoxicity is primarily due to uptake by the proximal tubule cells of the nephron, with uptake by other cells having a lesser effect [6]. Nephrotoxicity has largely been controlled by diuretics and pre-hydratation of patients, such that neurotoxicity has now become the dose-limiting side effect. These pharmacological disadvantages stimulated the search for other platinum analogues with improved pharmacological properties. A large number of platinum analogues have been synthesized since cisplatin cytostatic activity was discovered but only a few are in clinical use.

Carboplatin [*cis*-diammine-1, 1-cyclobutanedicarboxylate platinum(II)] (Figure 1) is a second generation analogue. It has the same mechanism of action as cisplatin, is cross-resistant and forms similar lesions on DNA. But in contrast to cisplatin dose-limiting side effects - nephrotoxicity, neurotoxicity and ototoxicity - carboplatin alone shows a low incidence of nephrotoxicity because of its slower rate of conversion to active platinum aquo species [7] and is also notably less neurotoxic than cisplatin at conventional doses (but with similar sensory neuropathy occurring in approximately 6% of patients). [8]. Its dose-limiting side effect is myelosuppression, specifically neutropenia and thrombocytopenia [9] (Figure 2). Both agents (cisplatin and carboplatin) are used for many types of cancer, including ovarian, cervical, head and neck, non-small cell lung and lymphoma, though carboplatin is supplanting the use of

cisplatin for most ovarian tumors and for treatment of non-small cell lung carcinoma [10].

Oxaliplatin [*trans*-L-1,2-diaminocyclohexaneoxalate platinum(II)] (Figure 1) is another clinically used platinum based analogue. It contains a DACH carrier ligand (diaminocyclohexane) and perhaps for this, oxaliplatin has consistently demonstrated antitumor activity in cell lines with acquired cisplatin resistance and appear to be active in tumor types that are intrinsically resistant to cisplatin and carboplatin [11-13]. Oxaliplatin's intrastrand cross-links are different and may account in part for its different spectrum of activity as reviewed in preclinical screen of National Cancer Institute [14]. Oxaliplatin was shown to have markedly different spectrum of activity to cisplatin and carboplatin [15]. In in vivo studies, oxaliplatin is active against breast, colon, and gastric cancer, renal cell carcinoma, and sarcoma [16]. It has also been tested against ovarian, lung, cervix, colon, and leukemia cell lines. Much like previous platinum drugs, likewise oxaliplatin has its side effects. The most common toxicity associated with oxaliplatin treatment is peripheral neuropathy, which ranges from acute and transient to a cumulative neuropathy [9]. Oxaliplatin is generally free of ototoxicity and nephrotoxicity, with only moderate isolated cases of neutropenia and thrombocytopenia [17].

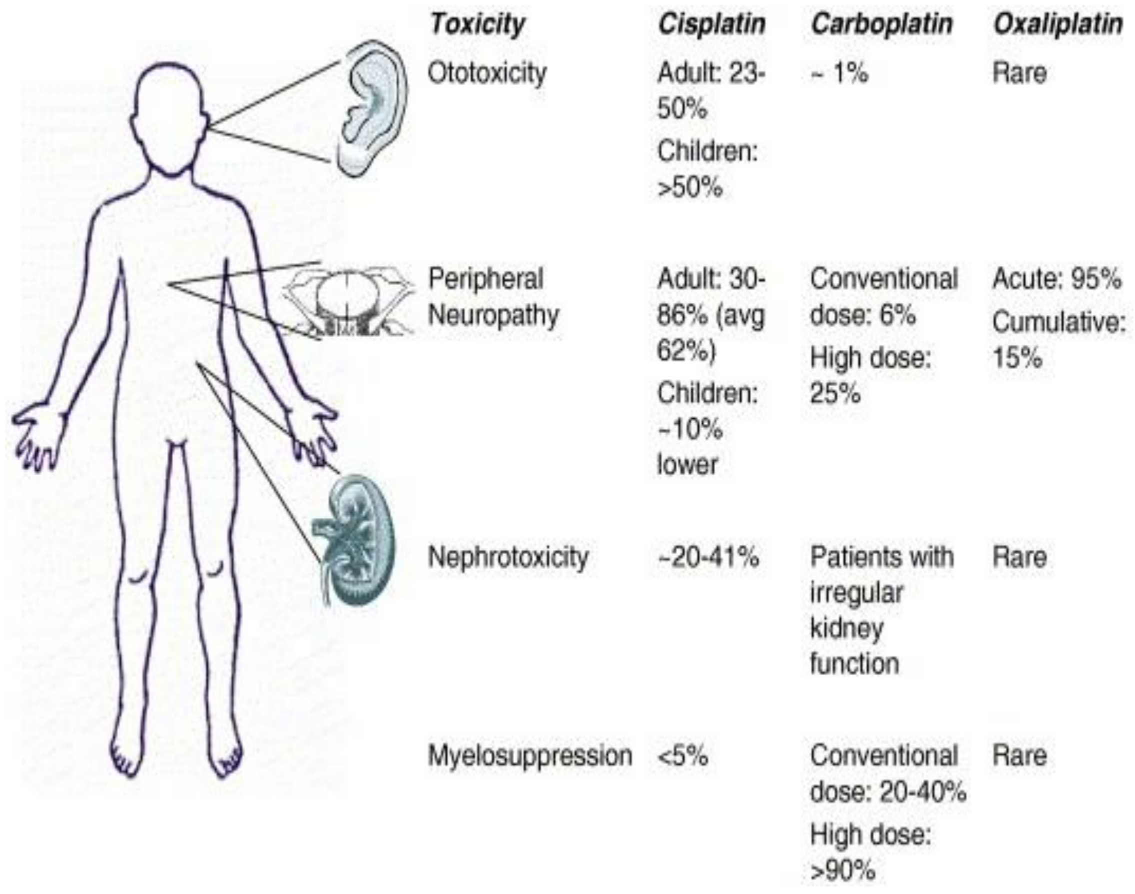
A number of analogues have failed over the years, some because unexpected toxicities (such as nephrotoxicity) and some because of no clear advantage over the remarkable toxicity reduction exemplified by carboplatin. Nevertheless, a number of platinum analogues (e.g. satraplatin, transplatin, tetraplatin) and some formulations are currently undergoing study. How these new compounds differ mechanistically as well as pharmacologically from currently available platinum drugs should be the key to their future development [18].

cis-DDP, cisplatin

Carboplatin

Oxaliplatin

Figure 1. The chemical structures of cisplatin, carboplatin and oxaliplatin.



Toxicity	*Cisplatin*	*Carboplatin*	*Oxaliplatin*
Ototoxicity	Adult: 23-50% Children: >50%	~ 1%	Rare
Peripheral Neuropathy	Adult: 30-86% (avg 62%) Children: ~10% lower	Conventional dose: 6% High dose: 25%	Acute: 95% Cumulative: 15%
Nephrotoxicity	~20-41%	Patients with irregular kidney function	Rare
Myelosuppression	<5%	Conventional dose: 20-40% High dose: >90%	Rare

Figure 2. Toxicities associated with the treatment with platinum drugs. The most common side effects associated with cisplatin treatment are ototoxicity, peripheral neuropathy, myelosuppression, and nephrotoxicity. Ototoxicity is notably higher in pediatric patients, while neuropathy is relatively more common in adult patients. The most common toxicity associated with carboplatin is myelosuppression, with rare cases of neurotoxicity and nephrotoxicity. Oxaliplatin most commonly causes neurotoxicity (Adopted from [9]).

3. Platinum drugs resistance

The development of resistance to platinum-based chemotherapy in the clinic is a major challenge for cancer chemotherapy [19]. Although the phenomenon of multidrug resistance against natural product drugs exemplified by the ATP-dependent efflux pump P-glycoprotein and other transporters is well characterized [20], the cellular responses that confer resistance to platinum complexes are multifactorial and less well understood [21, 22]. The generally accepted intracellular mechanisms by which cells acquire resistance to cisplatin and its congeners are (i) increased detoxification of drug by the thiols glutathione and metallothionein; (ii) improved repair of, and tolerance to, nuclear lesions, leading to a concomitant reduction in apoptosis; and (iii) diminished accumulation of platinum complexes [22, 23]. Overall, several factors may contribute to resistance (Table 1).

Table 1 - Mechanisms of resistance to platinum drugs (Adopted from [24])

Mechanism	Contributing factor		
Impaired blood flow/drug delivery	↑ Tissue pressure	↑ Plasma fibrinogen[b]	↑ Blood viscosity[b]
	↓ Blood pressure[e]	↓ RBC deformability[b]	
Extracellular matrix/other factors	↑ Tissue pressure/↓ diffusion	↑ γ-Glutamyltransferase	
	↑ Fibronectin	↑ Type IV collagen	↑ Laminin
Decreased drug uptake	↑ Cell membrane rigidity	↑ Sphingomyelin	↑ Cholesterol
	↑ NaClc	↑ KCl[c]	↑ Mannitol[c]
	↑ Extracellular pH	↑ Protein binding	
	↓ Copper transporter CTR1	↑ Copper	↓ $CaCl_2$[c]
	↓ Uptake concurrently of several factors	Concurrent ↓ expression several transporters	↓ γ-Catenin
	Defective endocytosis/formation of endocytic recycling compartment	↓ Small GTPases (rab5, rac1, rhoA) which regulate endocytosis	
Increased efflux	↑ Cu transporters ATP7A, -7B	↓ CuCl2c	↑ Intracellular pH
	↑ MRP2/cMOAT/GSH-X pump[d]	↑ MRP1[d]	↑ p-Glycoprotein[d]
	↑ MVP/LRP[d]	Abnormal sorting into exosomal pathway	↑ Sequestration intracelluarly
Increased detoxification	↑ GSH	↑ GSTd	↑ GST-pi/GST-pi SNPs[e]
	↑ γ-Glutamylcysteine synthase	↑ γ-Glutamyltransferase	↑ GSH peroxidase
	↑ Glutamate cysteine ligase	↑ GSH reductase	↑ Catalase
	↑ Dihydrodiol dehydrogenase	↑ Superoxide dismutase	↑ Metallothioneins[d,e]
Decreased drug binding	↑ Proton pumps	↑ Intracellular pH	↑ Extracellular pH
	↑ In cell cycle G1/ ↓ in G2/M	↑ Histone methylation	
Increased DNA repair	↑ Nucleotide excision repair system (ERCC1 and XPF)	↑ XPAe	↑ BRCA1[e]
	Host ERCC1/XPD SNPs[e],	↑ Topoisomerase-II	↑ REV1
	↑ Base excision repair (DNA polymerase-β[d], -zeta, and -eta)	↑ Homologous recombination repair	↑ DDB2 (damaged-DNA -binding-protein-2
	↑ DNA damage recognition protein HMG1		
Increased tolerance of DNA damage	↓ DNA postreplicational mismatch repair	↓ hMLH1, hMSH2, hMSH6d	↓ Non-homologous end-joining repair
Decreased pro-apoptotic factors	Down-regulation/↓ expression (p53, p53-binding-protein-2, Bax, Fas, caspases 8, 9, other)	↓ Activation (Fas, caspase 9)	P53 mutation (with overexpression of a non-functional protein)[d,e]
	Mitochondrial abnormalities	P53 deletion	
Increased apoptosis inhibitors	↑ Bcl-2[d,e]	↑ Bcl-xL[d,e]	↑ Bfl-1/A1
	↑ Survivin	Hypoxia (via ↑ Bcl-xL)	↑ FLIP
	↑ Xiap	↑ IAP-2	↑ COX-2[d,e]

Altered mitochondria	↑ Fatty acid use for O_2 consumption	↑Mitochondrial-uncoupling-protein-2	↑ No. mitochondria
	↓ Membrane potential		
Increased chaperones	↑ HSP27d	↑ HSP90-β	↑ HSP70
	↓ GRP78		
Altered cell signaling pathways	↑ E-cadherin	↑ EGF/EGFR	Catenins: ↑ α & β / ↓ γ
	↑ Heregulin/ ↑ p21WAF1/CIP1	↑ Her-2/neud	PTEN loss
	↑ PI3K	↑ AKT	↑ mTOR
	↑ MAPK signaling cascade[d]	↑ p110α	↑ Hyaluronan-CD44
	↑ c-Myc/c-Fos/c-Jun activation	↑ /Mutated ras	↑ c-cot
	↑ STAT1/STAT3/JAK2	↑ PDE2	↑ PKC-iota
	↑ Protein phosphatases 2A & 4	SRPK1 inactivation	↓ SAPK/JNK activation
	↓ p38 kinase activation	↓ IP3R1	↓ HGF
Transcription factors, cell cycle related factors, checkpoint kinases, etc.	↑ YB-1	↑ CTF2	↑ ATF4
	↑ ZNF143	↑ mtTFA	↑ Ets-1
	↑ Zipper transcriptional factor	↑ AP-2	↑ SKP2
	↑ NF-kappaBd	↑ Cyclin D1	
	↓ Chk1	↓ Chk2	
	↓ Telomerase mRNA expression	↓ Telomere length	↓ Telomerase activity
Gene arrays: differential expression	↑ FN1	↑ TOP2A	↑ LBR
	↑ ASS	↑ COL3A1	↑ STK6
	↑ SGPP1	↑ ITGAE	↑ PCNA
	↑ MDR1	↑ MRP1	↑ MRP2
	↑ CD55	↑ PGK1	↓ Caveolin 1
Proteomic analyses: differential expression	↑ HSP60/HSP90/heat-shock cognate 71 kDa protein	↑ Calmodulin	↑ Calumenin
	↑ Peroxiredoxins PRX 2/PRX 6	↑ GST	↑14-3-3
	↑ Voltage-dependent anion-selective channel-1		
Miscellaneous	↑ Ribosomal proteins RPS13, RPL23	Altered sphingolipid pathway	Altered ganglioside expression
	Chromosomal abnormalities	↑ Splicing factor SPF45	↑ Serum LDH[d,e]
	↑ Glucose utilization[f]	↑ Lactate production[f]	↑ LDH-5[e,f]
	↑ Golgi apparatus	↓ Microsatellite D6S1581	↓ Pyruvate kinase M2

a Paradoxically associated with improved cisplatin efficacy and patient survival.

b Thought to be important for drugs in general, but not directly tested with platinum drugs.

c Alter platinum cellular uptake and efficacy when added in vitro.

d Effect not seen consistently across all studies, or opposite effect seen in some studies.

e Demonstrated in clinical studies.

f Despite cells with low intracellular and extracellular pH having decreased platinum efflux and increased platinum uptake, binding and efficacy.

3.1. Resistance due to decreased blood flow and drug delivery

As reviewed by Stewart [24], delivery of chemotherapeutic drugs and oxygen varies with blood flow. Hypoxia reduces efficacy of many agents, but has a little impact on platinum drugs [25]. With respect to drug delivery, tissue drug concentrations conform to either flow-limited models (varying with blood flow) [26] or to membrane-limited models (not proportional to flow) [27, 28]. In contrast to a flow-limited model for cisplatin, concentrations are as high in necrotic as in viable human tumors [29] and cisplatin concentrations in human autopsy tissues do not correlate with organ blood flow rates [30]. Human tumor cisplatin concentrations do vary with pulse and blood pressure, with metastatic site, and with tumor type [24, 29].

Since blood flow autoregulation is defective in tumors, blood pressure fluctuations have greater impact on flow to tumors than to normal tissues [31], and agents that alter blood pressure may selectively alter tumor blood flow/drug delivery [31-33]. Decreased red blood cell deformability, high fibrinogen levels, etc. may reduce tumor blood flow by increasing blood viscosity [34, 35], while agents that reduce blood viscosity (e.g., pentoxifylline, mannitol or fibrinolytics [34, 36-38]) might increase tumor blood flow and drug delivery. While both blood flow [39] and drug diffusion through interstitium from vessel to tumor cell [40] may be impeded by the abnormally high tissue pressures in tumors, higher interstitial fluid pressures may nevertheless be associated with increased platinum drugs efficacy and prolonged patient survival [41]. Overall, it is unknown to what extent tissue pressure and tumor blood flow affect platinum drugs activity clinically.

3.2. Resistance due to diminished accumulation

Studies reported over the past 30 years have analyzed the ability of Pt-containing drugs to accumulate in cancer cell lines, measured the ability of new compounds to accumulate in resistant cells and have consistently demonstrated that accumulation of drug is a determinant of cellular resistance/sensitivity [42, 43]. Johnson et al. reported a strong correlation (r = 0.98) between cisplatin accumulation and relative cisplatin resistance for a series of increasingly resistant lines derived from the BEL7404 human hepatoma cell line [44]. Koga et al. examined seven primary bladder cancer cell lines derived from untreated transitional cell cancer of the urinary bladder and found a positive correlation between cisplatin accumulation and sensitivity (r = -0.778) among the intrinsically resistant cell lines [45].

It is important to note that the correlation between diminished accumulation of drug and increased cells resistance is not unique to cisplatin, and has been observed for the clinical analogs carboplatin [46] and oxaliplatin [47] and compounds in clinical trials as well [48, 49]. In addition, cross-resistance among platinum drugs was observed at an early stage of platinum drug research [50], and in some cases reflected reduced accumulation of these compounds (although there are exceptions) [46]. Stewart et al. examined platinum concentrations in human autopsy samples and demonstrated that patients whose tumors responded to Pt-containing therapies had higher tumor platinum concentrations than those that failed to response, seeming to indicate that platinum accumulation is an important factor for clinical efficacy [51].

It is presumed that accumulation of platinum drugs occurs by a variety of mechanisms, including passive diffusion and facilitated transport by multiple transport systems. To reduce drug accumulation to a significant extent, or to confer cross-resistance to multiple cytotoxic Pt-containing drugs, cells must simultaneously inactivate more than one of these transport systems [19].

3.2.1. Diminished accumulation caused by influence of extracellular environment

3.2.1.1. Plasma and interstitium proteins

Binding of platinum drugs to plasma and interstitium proteins may contribute to resistance. Platinum that is bound to protein is much less cytotoxic than is free [52, 53], with substantially reduced uptake into cell [53] and tissues [54]. There is a general resemblance between the distribution patterns of cisplatin and carboplatin, although in vitro incubation with mouse plasma showed that number of interactions is higher for cisplatin [55]. The influence of protein-binding on oxaliplatin cytotoxicity has not been evaluated separately.

Stewart reported [24] that cisplatin induced apoptosis is reduced in the presence of extracellular matrix proteins fibronectin, type IV collagen and laminin that may bind tumor cells [56]. Extracellular gamma-glutamyltransferase that may cleave glutathione to render thiol groups that bind and inactivate cisplatin and other electrophilic drugs also reduces apoptosis [57]. However, the impact of these factors in the clinic remains unknown.

3.2.1.2. Extracellular pH

Contrary of the tumor intracellular pH (that is neutral to alkaline), tumor extracellular pH is often acidic [58, 59]. It is because of anaerobic glycolysis that occurs in tumor cells. Lowering extracellular pH favors uptake of weak acids [58], markedly increases uptake of cisplatin and its DNA binding and also lowers intracellular pH [60]. In vivo, extracellular pH is lowered by intravenous glucose administration [61], while oral bicarbonate administration raises it [62].

Other agents could also potentially have an impact on… [24]. For instance mannitol and NaCl, both used to reduce cisplatin nephrotoxicity, decrease cisplatin uptake and

cytotoxicity in vitro, as does KCl, while $CaCl_2$ and $CuCl_2$ may increase net cisplatin accumulation and cytotoxicity [63].

In summary, there is substantial preclinical evidence suggesting that reduced extracellular and intracellular pH may be associated with platinum drugs uptake, binding and cytotoxicity, while increased extracellular pH may be associated with platinum drugs resistance. However, its clinical importance has not been adequately assessed yet [24].

3.2.2. Decreased drug uptake

3.2.2.1. Passive diffusion

It has long been presumed that cisplatin and carboplatin are taken up passively by the cell, as uptake is not saturable, nor it is inhibited by structural analogs [64]. Also oxaliplatin uptake was meant to be most likely passive, as a correlation between hydrophobicity and uptake has been shown [65]. Andrews demonstrated that Pt compounds could not lower accumulation by competitive inhibiton as would be expected if a unique active transporter were at play, whereas compounds that compromised membrane integrity increased accumulation [66].

The relative amount of drug entering a cell by passive diffusion is dependent on the concentration of drug: at low drug concentrations, active transporter(s) may mediate the uptake of the majority of drug, but at high concentrations most uptake would be via passive diffusion. Collectively, there is evidence that drug can enter a cell by passive diffusion; however, given that resistant cells demonstrate only small changes (if any) in their membrane composition and biophysics, passive diffusion is not the key to decreased drug accumulation and the lowered accumulation must be due to other alterations (i.e. alterations of active mechanisms of uptake-see below) [19]. By reason that decreased drug uptake is thought to be dependent on alterations of active mechanisms, ability of drugs to enter cells by passive diffusion led to the design of lipophilic Pt-complexes that are capable of circumventing cisplatin resistance through increased accumulation in resistant cells, in a way independent of any active component of uptake [65, 67, 68].

3.2.2.2. Na^+,K^+-ATPase and Na^+ gradient

Na^+,K^+-ATPase primarily maintains the sodium gradient across the cell membrane (pumps sodium out and potassium into the cell). During the search for active uptake component Andrews et al. noted that preincubation with the Na^+,K^+-ATPase-specific

inhibitor oubain reduced cisplatin accumulation in both the sensitive and the resistant cells by up to 50% [69, 70]. Short-term accumulation experiments showed that drug accumulation was immediately reduced on exposure, suggesting Na^+,K^+-ATPase inhibition affects influx; replacement of sodium with choline in cell growth media reduced cisplatin uptake by a similar amount as oubain; and cisplatin uptake increases with extracellular sodium concentration, suggesting it is the sodium gradient dissipation rather than Na^+,K^+-ATPase inhibition that lowers cisplatin uptake [69]. Interestingly, tissues subject to cisplatin toxicity, such as kidney and the inner ear, do express high levels of Na^+,K^+-ATPase [69, 71]. Hall et al. reported that cisplatin is not a substrate for Na^+,K^+-ATPase, and the inhibition of Na^+,K^+-ATPase by oubain reduces the Na^+ gradient across the cell membrane that affects active/facilitated transport of cisplatin, although the specific transporters that rely on this sodium gradient have not been identified [19].

3.2.2.3. Gated channel Aquaporin 9

Gately and Howell proposed that cisplatin accumulation could be facilitated via a gated channel, but cisplatin's minimum cross-section (3.97 Å by 6.92 Å) is greater than that of biggest channel pores, with the possible exception of aquaporin 9, recently reported to have a pore size of approximately 7 Å by 12 Å [72]. Aquaporin 9 has been shown to permit the passage of neutral molecules, such as glycerol, urea, purines, and pyrimidines [73], and its expression correlates with As_2O_3 accumulation in myeloid and lymphoid leukemia lines [74].

Although the ability for the aquaporins to transport Pt drugs has not been directly demonstrated, it is important to note that Pt-resistant lines have reduced expression of aquaporin 9, presenting a potential new Pt-drug transport family and new possibility that can contribute to Pt-drug resistance [19].

3.2.2.4. Copper transporter CTR1

Major inroads have been made recently in defining the role of transport of platinum drugs via copper transporter 1 (CTR1, SLC31A1) (Figure 3) into the cell as another determinant of drug sensitivity and resistance [75]. It has become clear that platinum drugs utilizes the copper transporters (and exporters), as well as other cation transporter [76]. CTR1 is an evolutionarily conserved copper influx transporter present in plant, yeast, and mammals, and is the main copper importer in mammalian cells. The human version, hCTR1, is expressed in all tissues and is a key player in the exquisite homeostatic regulation of intracellular copper levels [19, 77].

Cisplatin, at plasma concentrations, not only prevents copper from being transported by CTR1 (cisplatin and copper are competitive inhibitors for the transport of the other molecule into the cell [75]) but also down-regulates protein expression of CTR1 in human ovarian carcinoma cell lines [78]. Larson et al. demonstrated that exposure to cisplatin triggered the rapid degradation of mammalian CTR1, suggesting that its contribution to influx was likely to be on initial phase of drug entry. Loss of CTR1 decreased the initial binding of cisplatin to cells and reduced influx. Loss of CTR1 also almost completely eliminated the initial influx of carboplatin and reduced the initial uptake of oxaliplatin [79]. Re-expression restored both cisplatin uptake and cytotoxicity [79]. It is not surprising that in comparing sensitive and resistant cell line pairs, the resistant small cell lung carcinoma (SCLC) line SR2 expresses less than half of CTR1 protein than its sensitive counterpart SCLC [80]. As expected SR2 cells take up much less cisplatin and carboplatin than SCLC cells [80]. Expression of transfected CTR1 protein in SR2 cells results in an increase in the uptake rate of cisplatin [80].

Although hCTR1 has been implicated in transport of cisplatin, carboplatin and oxaliplatin, it has been proposed to be important predominantly for the import of cisplatin and carboplatin into the cells [80, 81]. The results publicized by Holzer et al. [82] indicate that CTR1 regulates the cellular accumulation of all three drugs at concentrations attainable in humans but that at 5-fold higher concentrations, although accumulation of cisplatin and carboplatin is still CTR1-dependent, oxaliplatin accumulation becomes CTR1-independent, indicating that it is a substrate for another

cell-entry mechanism – a feature consistent with its different clinical spectrum of activity.

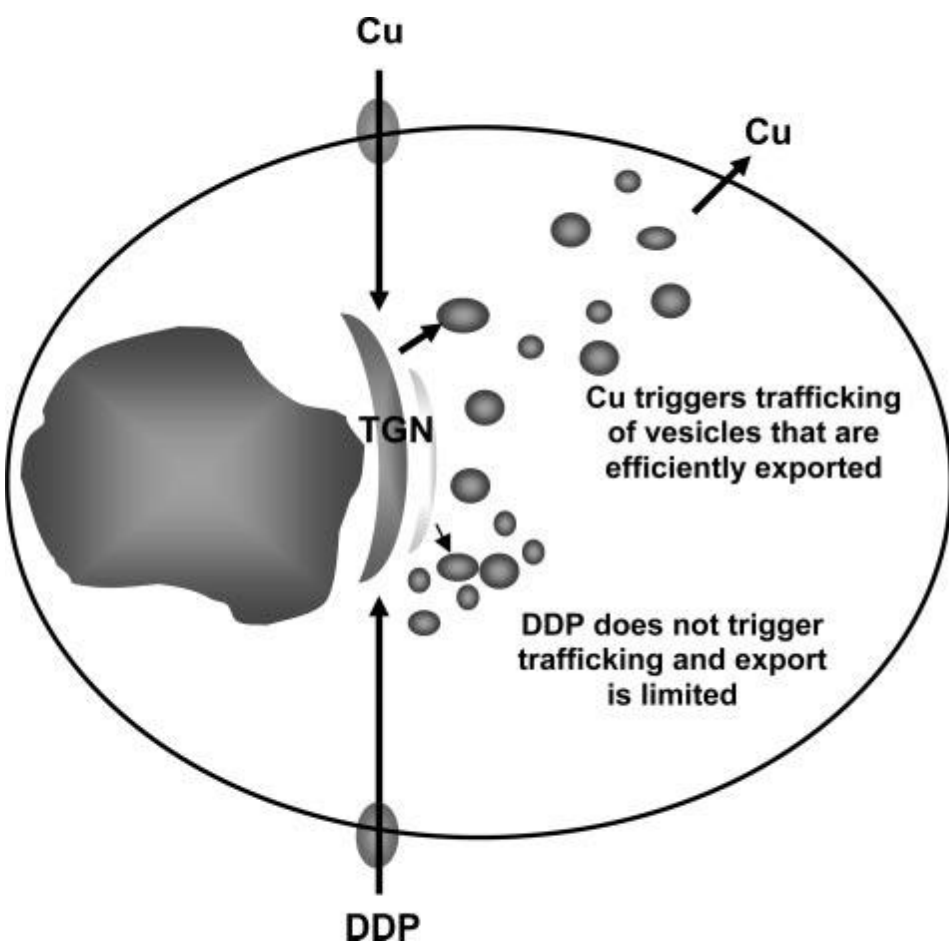


Figure 3. Schematic diagram of hypothesized differences in copper (Cu) and platinum (Pt) drug vesicular sequestration, trafficking, and export pathways. Cu and the Pt drugs enter predominantly via hCTR1 and are sequestered by ATP7A/B into the trans-Golgi network (TGN) and vesicles of the secretory pathway. Cu triggers relocalization of vesicles that are then efficiently exported. The Pt drugs fail to trigger relocalization of vesicles into which they are sequestered by ATP7A, and the export of these vesicles appears to be limited (Adopted from [83]).

3.2.2.5. Organic cation transporters (OCTs)

The organic cation transporters (OCTs) are the other key proteins involved in platinum drugs transporters [18]. The organic cation transporters (OCT) 1 (SCL22A1), 2 (SLC22A2) and 3 (SLC22A3) are in the class of plasma membrane transporters belonging to the SLC22A family [84, 85]. The OCTs mediate intracellular uptake of a broad range of structurally diverse organic cations. Substrates of OCTs include endogenous compounds, such as choline, creatinine, and monoamine neurotransmitters, and variety of xenobiotics, and clinically used drugs, such as metformin, cimetidine, and amantadine [84]. In humans, OCT1 is primarily expressed in the liver [85-87] and less so in the intestine [88], whereas OCT2 is predominantly expressed in the proximal tubules of the kidney [84, 85]. OCT3 is expressed in many tissues, including placenta, heart, liver, and skeletal muscle [89, 90]. The expression of the OCTs has also been detected in several human cancer cell lines [91].

The interactions of cisplatin with human OCTs has been investigated, and the results are discordant (Table 2) [92, 93]. In the recent study reported by Zhang et al. was observed that the influx transporters OCT1 and OCT2 play a critical role in the cellular uptake and consequently cytotoxicity of oxaliplatin. In contrast, the two transporters were relatively unimportant in mediating the uptake and cytotoxicity of cisplatin and carboplatin.

Overexpression of OCT1 and, more strikingly, OCT2 in transfected cells not only increased the rate of cellular platinum drugs accumulation but also elevated the level of platinum-DNA adducts after oxaliplatin exposure [94]. Structure-activity relationship studies suggest that the 1, 2-diaminocyclohexane (DACH) moiety of oxaliplatin is an important pharmacophore for its interaction with the OCTs and that an organic on the nonleaving portion of the platinum complexes is essential. The greater affinity of oxaliplatin for these transporters, and their presence in colon cancer may be an explanation for its usefulness in this disease in contrast to other platinum drugs against which are colon cancer cells resistant [18, 95, 96].

In contrast to OCT1 and OCT2, overexpression of human OCT3 did not affect the cytotoxicity or resistance of any of the platinum drugs [94].

Table 2 – Observations on cisplatin, carboplatin, and oxaliplatin substrate specificity for OCT1, OCT2, and OCT3 influx transporters (rat and human) (Adopted from [19]).

Cisplatin	**OCT1**	**OCT2**	**OCT3**
Pan et al. [97]	---	Yes	---
Yonezawa et al. [98]	No	Yes	---
Briz et al. [92]	No	No	No
Ciarimboli et al. [93]	No	Yes	---
Yonezawa et al. [99]	Yes(weak)	Yes	No
[illegible]4]	Yes(weak)	No	No

Carboplatin	**OCT1**	**OCT2**	**OCT3**
Ciarimboli et al. [93]	---	No	---
Yonezawa et al. [99]	No	No	No
Zhang et al. [94]	Yes(weak)	No	No

Oxaliplatin	**OCT1**	**OCT2**	**OCT3**
Ciarimboli et al. [93]	---	No	---
Yonezawa et al. [99]	No	Yes	Yes(weak)
Zhang et al. [94]	Yes	Yes	No

3.2.3. Increased drug efflux

3.2.3.1. Copper efflux transporters ATP7A and ATP7B

Resistance may also be associated with increased platinum drugs efflux from cells [100] or from nucleus into cytoplasm [101]. The copper-transporting P-type adenosine triphosphatases ATP7A and ATP7B are implicated in platinum drugs efflux and resistance [83, 102-112] (Figure 3). As reviewed by Rabik et al. [9], ATP7A/B are primarily responsible for the export of copper from the cell. Under normal (Cu replete) conditions, ATP7A/ATP7B reside in the trans-Golgi network, where they receive Cu from the chaperone Atox1 and translocate it to the luminal side for incorporation into enzymes. When excess Cu exists in the cell, ATP7A/B are trafficked to the cell surface to directly efflux Cu from the cell [77].

ATP7B was first proposed to be involved in cisplatin resistance when Komatsu et al. overexpressed this transporter in human epidermoid carcinoma cells and observed that these cells gained resistance to cisplatin as a result of ATP7B overexpression [113]. Cisplatin accumulation in ATP7B transfected cells was only 60% of that observed in cells transfected with empty vector. It was due to ability of ATP7B to sequester cisplatin into vesicles and transport it similar to copper out of the cell. Additionally, eighty-two primary ovarian carcinomas were profiled for expression of several known resistance genes – including ATP7B, MDR1, MRP1, MRP2, LRP, and BCRP [114]. With the exception of ATP7B, none were indicators for resistance of ovarian cancer to cisplatin.

Patients whose carcinomas express high levels of ATP7B have a significantly poorer prognosis than patients with tumors that express low level of ATP7B [103]. ATP7B overexpression is associated with poor outcome in cisplatin-treated patients with ovarian carcinoma [103], esophageal cancer [108], squamous cell cancer of the head and neck [110] or human endometrial carcinoma [105], human solid carcinoma [112], human non-small cell lung cancer [109], human hepatocellular carcinoma [106].

In accordance with Samimi et al. [83] ATP7A is able to sequester into vesicles not only cisplatin, but also both carboplatin and oxaliplatin. Increased expression of ATP7A in the 2008 cell line of ovarian carcinoma leads to increased resistance to all three of the clinically available Pt drugs; interestingly, overexpression leads to increased sequestration of platinum drugs and not to decreased total accumulation [83]. This seems to suggest that simple overexpression of ATP7A alone may be sufficient to compartmentalize and deactivate Pt, but not be enough to lower accumulation. ATP7A is not relocalized to the plasma membrane for Pt efflux as it is for Cu efflux, suggesting the Cu sensing domain associated with trafficking cannot sense Pt [115].

In contrast with these outcomes, Rabik et al. presented data demonstrating that the expression of both ATP7A and ATP7B provide increased cellular resistance to cisplatin, while cells become hypersensitive to oxaliplatin [81]. This is in agreement with outcomes presented by Samimi et al. [116] and also by Plasencia et al. [117] who reported that oxaliplatin resistant colon cancer cells exhibited low basal expression levels of ATP7A (and ATP7B) compared to parent, non-resistant, cells.

Overall, the role of ATP7A and ATP7B transporters in platinum drug sensitivity has not been completely elucidated. Some results contradict each other. The answer to these discrepancies could be found in tissue specificity. The potential tissue specificity should be analyzed through both overexpression and knockdown experiments in tumor cell lines to determinate the role of these transporters in clinical resistance and sensitivity to platinum drugs. In some tumor types, these transporters may be vital in providing resistance to platinum drugs, and this can be targeted in developing new therapies to modulate platinum drugs treatment.

3.2.3.2. Drug efflux transporters

The chelation of Pt drugs by glutathione is generally accepted to be a deactivation pathway (see below). Once the Pt drug is chelated by glutathione, the glutathione-Pt complex is effluxed from the cell in an ATP-dependent way by a transporter family termed MRP (ABCC) drug efflux transporters [118]. MRP drug efflux transporters are

ATP-dependent organic anion transporters. The first identified member of the family was the multidrug resistance-associated protein 1 (MRP1, ABCC1), a glycoprotein capable of effluxing a range of glutathione-conjugated molecules, and a member of the ABC (ATP-binding cassette) family of drug efflux transporters [119]. Seven more MRP1 homologues have subsequently been indentified (MRP2-MRP8) but their importance in platinum drugs resistance remains unclear. Glutathione-conjugated Pt is already deactivated, so efflux by the MRP transporters is not necessarily a part of the accumulation-resistance phenotype; however, there is evidence in specific cases that their expression is associated with clinical resistance to cisplatin, but more work needs to be done [19].

Other drug efflux transporters that play important role in regulation of intracellular drug concentrations and thereby determining cell sensitivity to chemotherapeutic agents are ATP-binding cassette (ABC) drug efflux transporters ABCB1 (P-glycoprotein, MDR1) and ABCG2 (Breast cancer resistance protein, BCRP).

As discussed in the work of Ceckova et al. [120] the influence of ABCG2 on cisplatin resistance is unclear and there are remarkable discrepancies in current literature evaluating its importance. Several studies demonstrated no correlation between expression of ABCG2 and cisplatin resistance [121, 122]. However, dramatic up-regulation of mRNA expression of ABCG2 has been recently reported in oxaliplatin-resistant colorectal cancer cell line [123] and a correlation between ABCG2 positivity of tumor cells and low response rate to platinum-based chemotherapy has been described in patients with advanced non-small cell lung cancer (NSCLC), suggesting the ABCG2 efflux transporter to be responsible for drug resistance in patients with NSCLC [124].

ABCB1 seems to be unimportant in platinum drugs resistance as several studies demonstrated no correlation between its expression and cisplatin resistance [114, 125].

3.3. Drug detoxification

3.3.1. Glutathione and Glutathione-S-transferase

In the cytoplasm, platinum drugs become aquated, which then enables them to react with thiol-containing molecules, including glutathione (GSH) and metallothioneins. Increased concentrations of these compounds are known to induce resistance against cisplatin [126-129]. However, few studies have described the role of these enzymes in oxaliplatin detoxification [117].Glutathione itself acts as an antioxidant of the cell, it helps to maintain the redox environment while maintaining reduced sulfhydryl groups. The resistance induced by GSH may be caused by binding/inactivating cisplatin, enhancing DNA repair, or reducing cisplatin-induced oxidative stress [126].

Rabik et al. [9] reviewed that in bladder carcinoma cell lines that are known to be resistant to cisplatin, exposure to buthiomine sulfoximine (BSO), which significantly depletes cellular glutathione concentration, resulted in a significant enhancement in cisplatin cytotoxicity [129]. Additionally, the NSAID (non-steroidal anti-inflamatory drug) indomethacin significantly decreases cellular concentrations of GSH and sensitizes bladder carcinoma cells to cisplatin treatment [129]. However, neither of these treatments sensitizes these cells to the level of their parent sensitive strain, indicating either that glutathione levels are only one component of cisplatin resistance [129], or that the NSAID may have other effects in the cell that prevent complete sensitization [9].

Glutathione-S-transferase (GST), particularly GST-π [130-136] or specific GST-π polymorphisms [136], may augment resistance by catalyzing GSH-drug binding. Colon, lung adenocarcinoma, and glioblastoma tumor cell lines [137], and ovarian [135, 138, 139] and head and neck clinical samples [140] do exhibit a correlation between high GST-π levels and cisplatin resistance. However, in other studies of ovarian, cervical, and lung carcinoma, no relationship was evident [141-144]. Another study has shown that ovarian cancers with high expression of GST-π typically have lower survival and

less favorable response to cisplatin. Much of the GSH/GST data is conflicting, leading to question about its importance. While it may have some role in certain types of cancers, it does not appear to be a global indicator of cisplatin resistance [9].

3.3.2. Metallothioneins

Metallothioneins (MT) are very low molecular weight proteins comprised of several cysteine and aromatic amino acid residues. They are thought to be involved in controlling levels of copper and zinc, as well as protecting cells from oxidative stress and toxicities associated with heavy metals, including copper, cadmium, and zinc [145, 146]. Elevated levels of metallothionein II have been described in cisplatin-resistant cell lines. Human bladder cancer xenografts [146] and esophageal [147] and transitional cell primary carcinomas [148] that express high levels of MT exhibit less of a clinical response to cisplatin. In head and neck cancers, cisplatin induces metallothionein expression [149], while in germ cell and testicular tumors, no relationship between MT and cisplatin was observed [150]. The association of MT levels with cisplatin resistance may be tissue specific and may play a minor role depending on the cellular environment [9].

3.4. Platinum drugs and DNA

3.4.1. Drug binding and DNA lesions

Upon entering a cell, all platinum drugs become aquated, losing chloride or oxalate ions, and gaining two water molecules. This positively charged molecule is then able to interact with nucleophilic molecules within the cell, including DNA, RNA, and proteins [9], although it is generally agreed that DNA is the preferential and cytotoxic target for cisplatin and other platinum drugs [151]. Platinum drugs cytotoxicity and DNA binding are highest with cell exposure during G1 and lowest during G2/M [152]. Reducing histone methylation relaxes condensed chromatin, increases cisplatin access to DNA, increases DNA-platinum adduct formation, and augments platinum drugs efficacy [153]. Rabik et al. reviewed that when binding to DNA, platinum drugs favor the N7 atoms of the imidazole rings of guanosine and adenosine. Three different types of lesions can form on purine bases of DNA: monoadducts, intrastrand crosslinks, and interstrand crosslinks (Figure 4). Monoadducts are first formed as one molecule of water is lost from aquated platinum drugs; however, greater than 90% of monoadducts then react to form crosslinks. Almost all of these crosslinks are intrastrand, with the majority being 1,2-d(GpG) crosslinks (for cisplatin 60-65%) and 1,2-d(ApG) crosslinks (for cisplatin 20-25%). Oxaliplatin forms fewer crosslinks than cisplatin at equimolar concentrations; however, it is equally as potent at these concentrations [154, 155] and is able to induce similar numbers of single-strand and double-strand breaks on DNA [156].

All crosslinks result in contortion of DNA [157]. Cisplatin and carboplatin intrastrand crosslinks bend the double helix by 32-35° toward the major groove, whereas oxaliplatin treatment bends the helix even further [65]. Both 1,2-d(GpG) and 1,2-d(ApG) intrastrand crosslinks unwind DNA by 13°, while the 1,3-d(GpXpG) intrastrand lesion unwinds DNA by 34°. Interstrand lesions induce even more steric changes in DNA, with extrusion of the cytosines at the crosslinked d(GpC)d(GpC) sites, bending of the double helix toward the minor groove by 20-40°, and extensive DNA unwinding of

up to 80°. Oxaliplatin adducts are bulkier and more hydrophobic than those formed from cisplatin or carboplatin, leading to different effects in the cell [17].

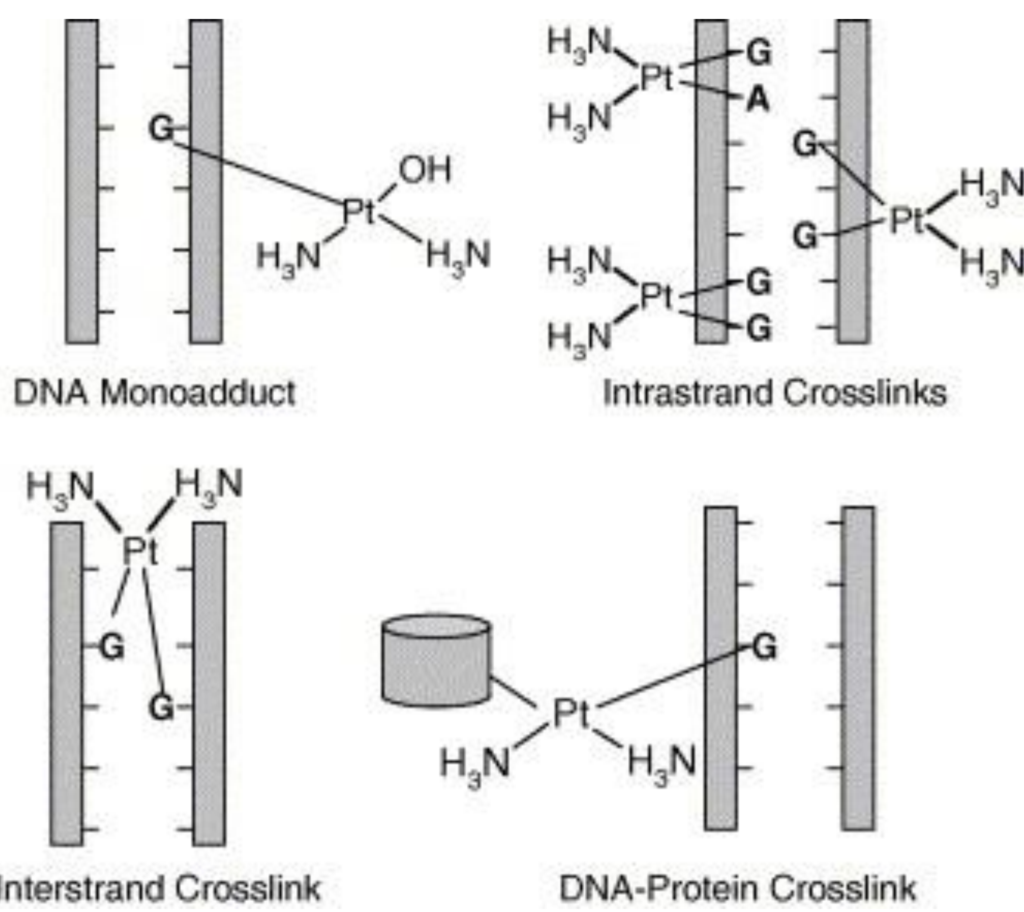


Figure 4. Platinum drugs adducts on DNA. Platinum drugs are able to react with DNA to form monoadducts, intrastrand crosslinks (1,2-d(GpG), 1,2-d(ApG), 1,3-d(GpXpGp)), interstrand crosslinks (G-G), and DNA–protein crosslinks (Adopted from [9]).

There are different theories as to which lesion is responsible for cytotoxicity. Some believe that the interstrand crosslink is cytotoxic because of the level of distortion in the DNA; however, most believe that the predominant 1,2-intrastrand crosslinks are the cytotoxic lesion. It is because of comparisons with the biologically inactive *trans* isomer of cisplatin, *trans*-diamminedichlorplatinum (II) (trans-DDP). Due to steric reasons, *trans*-DDP is unable to form 1,2-intrastrand adducts between two adjacent purines in the same DNA strand, so *trans*-DDP mainly forms 1,3-intrastrand and interstrand crosslinks [158]. It is generally accepted that 1,2-intrastrand DNA adduct is responsible for the anticancer activity of cisplatin (although the possible importance of other adducts should not be ruled out).

This assumption is supported by the discovery that some High mobility group (HMG) proteins specifically recognize this type of DNA adduct and therefore may regulate the processing of 1,2-d(GpG) intrastrand crosslinks formed by cisplatin [159]. Their presence is thought to be crucial also for sensitivity to carboplatin. The binding of HMG-1 to cisplatin helps in preventing replicative bypass (see below) [160] and HMG proteins such as SRY, UBF, and LEF-1 have been shown to block nucleotide excision repair (NER; see below) components from repairing the lesion via a “shielding mechanism” [161]. The cisplatin-DNA-HMG-1 ternary complex is also able to block transcription factors, thus preventing both transcription and replication. This block in cellular processes may be responsible for sending out DNA damage signals that result in initiation of apoptosis [126]. HMG has a much lower affinity for oxaliplatin crosslinks on DNA than it does for cisplatin or carboplatin adducts [160]. The molecular geometry of the oxaliplatin adduct , with a narrower major groove and correspondingly wider minor groove, is thought to be responsible for this observation [9].

3.4.2. Repair of DNA lesions

3.4.2.1. Nucleotide excision repair

The ability of cells to repair DNA damage appears to be a critical determinant of resistance or sensitivity to platinum drugs. Nucleotide excision repair (NER) pathway is one of the major pathways involved in repair of DNA damage caused by platinum drugs. NER includes the recognition of DNA damage and demarcation of the specific area affected, followed by the formation of a complex to unwind the damaged portion and excise it. Finally, the excised area is resynthesized and ligated to maintain the DNA molecule [162] (Figure 5).

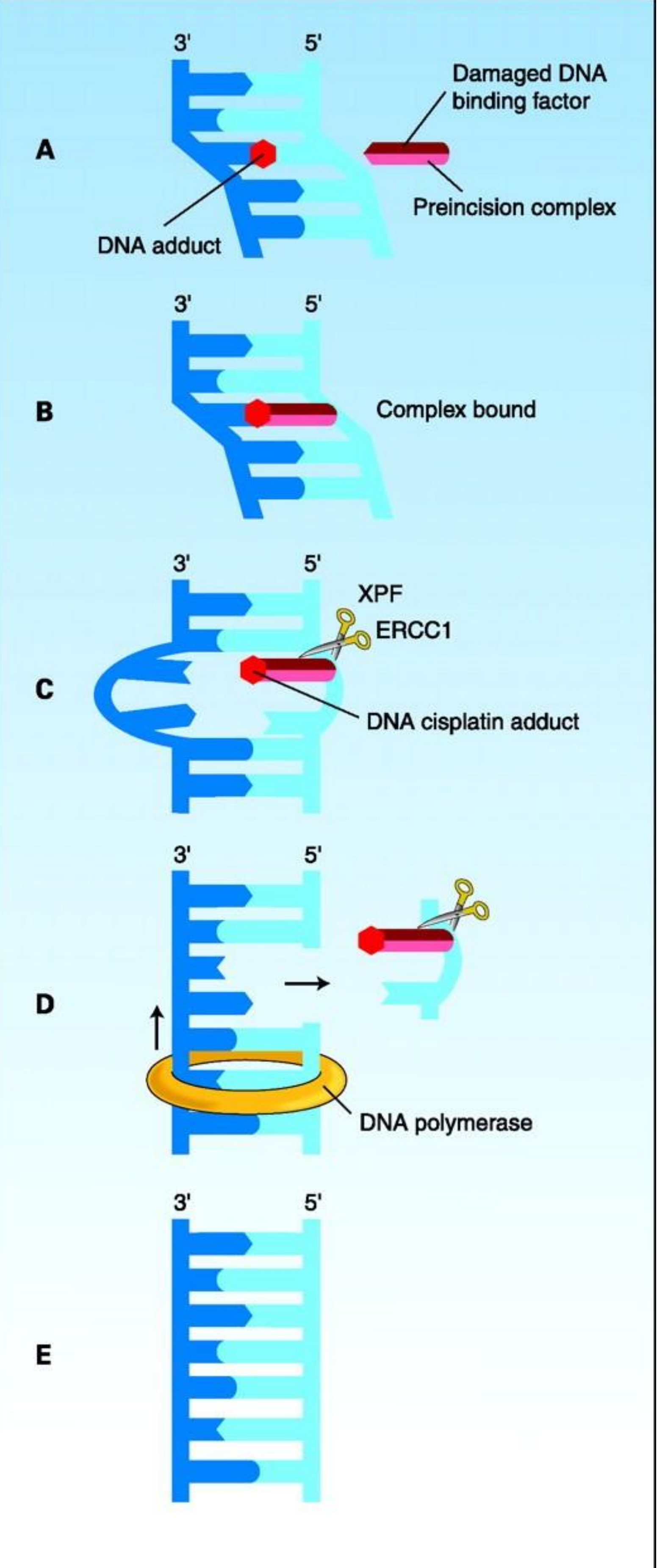
While NER recognizes all three types of intrastrand crosslinks (1,2-d(ApG), 1,2-d(GpG), and 1,3-d(GpNpG)), the 1,2 intrastrand crosslinks are repaired less efficiently than the 1,3 intrastrand crosslinks, supporting the hypothesis that the 1,2 intrastrand crosslinks are the cytotoxic lesion [163, 164]. High levels of specific proteins of this pathway are expressed in cisplatin resistant cells [165, 166]. Rabik et al. reviewed that in ovarian cancer, XPA and ERCC1 (excision repair-complementation group)/XPF (xeroderma pigmentosum complementation group F) were shown to have increased expression in tumors of patients resistant to platinum drugs treatment [167, 168]. In primary ovarian tumors, levels of XPB transcripts were significantly higher in tumors resistant to cisplatin than in tumor samples from patients who responded well to platinum drugs treatment [169]. Similarly, gastric cancer showed a correlation between cisplatin resistance and ERCC1/XPF mRNA levels [170]. In addition, cell lines that developed resistance in vitro after exposure to cisplatin chemotherapy were found to have increased expression of ERCC1 [171].

Koberle reported that testis tumor cell lines, generally very responsive to cisplatin, has low levels of XPA and ERCC1/XPF. This is sufficient to explain their poor ability to remove cisplatin adducts from DNA and is providing further correlative evidence for the importance of NER in cisplatin resistance [172, 173]. Oxaliplatin treatment also induces the expression of NER genes and the rate and kinetics of NER are similar to

cisplatin [174]. In HT29 colon cancer cells, expression of ERCC1 and XPD was significantly higher in cells treated with oxaliplatin compared to untreated control cells [117] and also the median overall survival was significantly longer in patients without ERCC1 expression [175].

NER may be inhibited by the presence of nucleosomes along the DNA. Previous studies have indicated that the presence of nucleosome on DNA is able to inhibit NER in cells treated with DNA damaging agents, including cisplatin [176, 177]. Comparison of the extent of repair by mammalian cell extracts of free and nucleosomal DNA containing the same platinum-DNA adduct reveals that the nucleosome significantly inhibits nucleotide excision repair. With the d(GpTpG) DNA substrate, the nucleosome inhibits excision to about 10% of the level observed with free DNA, whereas with the less efficient d(GpG) DNA substrate the nucleosome inhibited excision to about 30% of the level observed with free DNA [177]. Nucleosome induced NER inhibition may be overcome by the activity of the SWI/SNF chromatin remodeling complex, which is activated upon damage recognition by the NER factors XPA and SPC [178]. Although SWI/SNF was not shown to be crucial for platinum drugs resistance/sensitivity, inhibition of this pathway may lead to a targeted therapy for sensitization of tumors to cisplatin and other DNA damaging agents.

Figure 5. A, NER-nucleotide excision repair begins when a DNA adduct is formed and causes a change in the shape of the DNA helix. B, damaged DNA binding factor binds to preincision complex and this protein complex localizes to the damaged area of DNA. C, the DNA helix is unwound and the damaged portion is excised by ERCC1/XPF. D, DNA polymerase then resynthesizes the absent portion of DNA. E, when NER-nucleotide excision repair is complete, the DNA is repaired and resumes its normal helical shape. In the setting of ERCC1deficiency,the DNA cannot be repaired, and the altered DNA is unable to replicate, or perform its normal function, leading to cell death (Adopted from [162].



3.4.2.2. Mismatch repair

As reviewed by Topping, the mismatch repair (MMR) system of proteins plays roles in diverse cellular processes, perhaps most notably in preserving genomic integrity by recognizing and facilitating the repair of post-DNA replication base pairing errors. Recognition of these errors and recruitment of repair machinery is performed by the MutSα complex (consisting of the MMR proteins MSH2 and MSH6) or MutSβ complex (consisting of MSH2 and MSH3) [179] (Figure 6). When MMR is deficient, unrepaired areas of DNA accumulate, resulting in microsatellite instability (MSI) [180]. This accumulation occurs when unrepaired base pair mismatches are replicated during DNA synthesis [162].

In addition to their role in DNA repair, MMR proteins also play a role in cytotoxicity induced by specific types of DNA damaging chemotherapeutic drugs, such as cisplatin. MutSα recognizes multiple types of DNA damage, including 1,2-intrastrand cisplatin adducts. Cisplatin adducts interfere with normal MMR activity, prevent a repair from being completed, and therefore treatment with cisplatin results in MMR protein-dependent cell cycle arrest and cell death [179, 181-184]. MMR proteins thus serve to detect the DNA damage caused by platinum drugs and generate an injury signal that eventually contributes to the triggering of the apoptic reaction that destroys the cell [4]. MMR mediates cisplatin and carboplatin induced apoptosis [185-187] but there is no difference in sensitivity between MMR-proficient and MMR-deficient cells for oxaliplatin [4].

The MMR protein-dependent cytotoxic response to cisplatin is largely unknown. Previous studies reported that only the p53-related transactivator protein p73 and the c-Abl kinase were clearly implicated as potential mediators of cisplatin/MMR protein-dependent cell death in human cells [188, 189]. However, recent studies show that cisplatin induced MMR protein-dependent cytotoxic response is independent of p53 signaling and demonstrate a MMR protein-dependent pro-death signaling pathway in cells treated with cisplatin [179].

Pro-death members of the Bcl-2 family, such as Bax and Bak, target the outer mitochondrial membrane and cause the cytosolic release of pro-death factors residing

within the mitochondria of unstressed cell [190]. Predominant among these factors is cytochrome c, whose cytoplasmatic localization results in the formation of caspase-activating platform (caspase-Cysteine Aspartate Specific proteinASE) known as the apoptosome [191]. This complex includes the adaptor protein Apaf-1, and when formed the apoptosome promotes the cleavage and activation of caspase-9 [192, 193]. Once activated, this apical caspase proceeds to cleave and activate caspase-3, the predominant effector protease of apoptosis [179]. Cleaved caspase-3 can cleave a number of substrates, including poly(ADP-ribose) polymerase (PARP).

MMR seems to be important for cisplatin and carboplatin cytotoxicity. It is connected with triggering of apoptosis and therefore MMR deficiency or inhibition of apoptosis (see below) can cause cisplatin and carboplatin resistance; however, deficiency of MMR does not affect cytotoxicity of oxaliplatin. This is particularly important in the usage of oxaliplatin to treat mismatch repair deficient tumors (e.g. colorectal cancer) that are resistant to cisplatin and carboplatin.

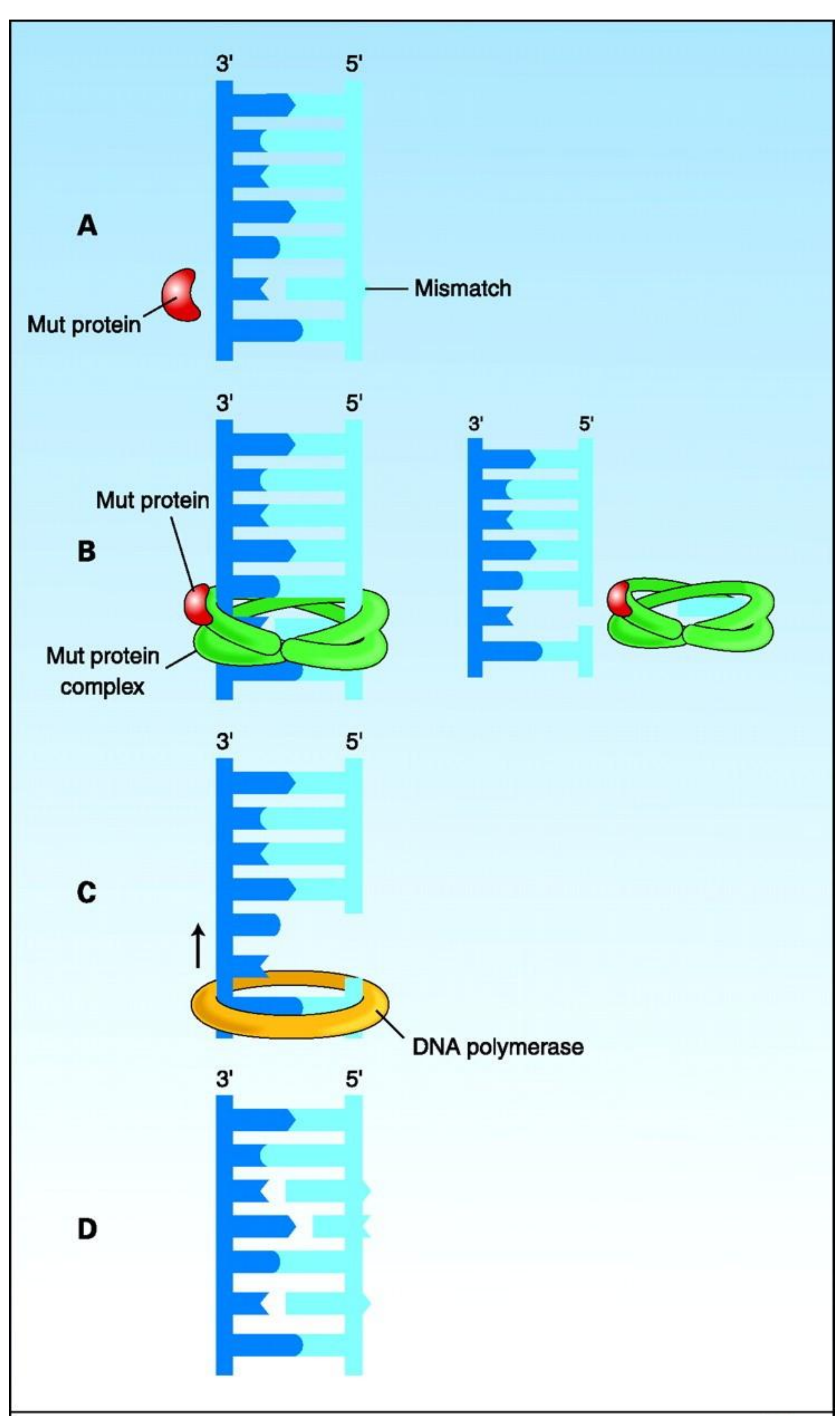


Figure 6. A, MMR occurs when the Mut protein recognizes a mismatch or insertion/deletion loop. B, theMut protein orders the assembly of a protein complex which localizes to the affected area on the DNA molecule and excises it. C, DNA polymerase then resynthesizes the missing portion of DNA. D, accumulation of insertion deletion loops on a strand of DNA in the petting of MMR deficiency (Adopted from [162]).

3.4.2.3. Homologous recombination

Homologous recombination has been proposed to play a role in repairing double strand brakes resulting from cisplatin-induced interstrand DNA adducts [194]. This can be caused by NER components XPF and ERCC1 [195, 196] and by proteins BRCA1 and BRCA2 (breast cancer type 1,2 susceptibility proteins). As reviewed by Powell and Kachnic BRCA2 has been shown to play a direct role in the repair of DNA by homologous recombination, by interacting with the Rad51 protein and facilitating the formation of Rad51 aggregates at the site of DNA damage. In the absence of BRCA2, the cell is more dependent on residual repair via Rad52, which makes Rad52 a target for therapy in BRCA-deficient tumors. BRCA1 plays a role in sensing DNA damage and replication stress and mediating the signaling responses [197].

Powell and Kachnic demonstrated that the defect in homologous recombination changes the drug sensitivity profile, rendering the BRCA-deficient breast cancers sensitive to cisplatin and other drugs that produce complex double-stranded lesions in DNA [197]. Additionally, Tassone et al. demonstrated increased sensitivity of human breast cancer xenografts to platinum drugs in BRCA1-defective cells [198]. These results suggest the influence of BRCA1 and BRCA2 on resistance to platinum drugs and good prognosis of patients with BRCA-deficient tumors.

3.4.3. Adduct tolerance

3.4.3.1. Replicative bypass

According to Rabik and Dolan [9], platinum drugs tolerance can be achieved without the need for DNA repair. In order for platinated DNA to be replicated and tolerance to form, DNA polymerase must skip the platinum adduct, which is most commonly an intrastrand lesion. The classic DNA replication polymerases – α, θ, and ε – cannot bypass the lesion; however, several polymerases have been shown to bypass intrastrand crosslinks by translesion synthesis – namely, β, η, ζ, and τ [199-203].

Dong et al. [204] examined expression of pol-β in patients with esophageal cancer, and found that pol-β expression in tumor tissue was higher than in the corresponding normal tissue. Iwatuski et al. [205] in recent study demonstrated that pol-β suppression increases cisplatin sensitivity, but interestingly, does not affect oxaliplatin-mediated cytotoxicity. This is consistent with previous studies that showed dependence of cisplatin resistance on pol-β overexpression [206, 207].

Pol-ζ has been shown in MMR deficient cells to play a role in DNA tolerance and bypass of lesions [208]. Recent experiments with pol-η null and expressing variant of human fibroblast cells have shown significance of pol-η in platinum drugs resistance and have demonstrated that the absence of pol-η results in a statistically significant enhancement in cisplatin, carboplatin as well as in oxaliplatin sensitivity [209]. However, bypassing oxaliplatin adducts is caused in particular by pol-ζ and pol-γ without evident influence of pol-β which can be an additional clue for the difference between mechanisms of action of cisplatin, carboplatin and oxaliplatin [160].

3.4.3.2. Reduced apoptic response

Several genes regulating DNA damage, apoptosis and survival signaling may contribute to resistance [210]. Platinum drugs may induce apoptosis through the Fas-receptor/Fas-ligand signaling complex (with activation of caspase-8, then caspase-3, -6,

-7), by mitochondrial cytochrome-c release [159], or by defective apoptic pathway (i.e., p53) [211].

As reviewed by Stewart [24], cells with p53 deletions or mutations are often resistant to cisplatin [212, 213]. Cisplatin resistance has been associated with p53 mutation in vitro in ovarian carcinoma [214], head and neck squamous cell carcinoma [215], and clinically in germ cell tumors [216] and advanced laryngeal carcinoma [217].

Caspases-3, -8 and -9 are important in cisplatin-induced apoptosis [126]. A cisplatin resistant lines have global downregulation of caspase and Bax expression, but increased Bcl-2 [218]. Decreased Fas expression or pathway activation after cisplatin may lead to inhibition of activation of caspase-3 and -8 [126], and was associated with resistance in germ cell tumors [219] and ovarian carcinoma cells [220]. Loss of caspase-8 pathway was associated with resistance in a human laryngeal squamous cell carcinoma cell lines [221]. Decreased cisplatin caspase-9 activation was noted in cells with normal mitochondrial cytochrome-c release and normal Bcl-2 and Bcl-xL expression [222]. Cisplatin-resistant cells have also been reported with abnormal mitochondrial membrane potential, intracellular distribution, or structure, and with up-regulation of cytochrome-c in the mitochondria in response to cisplatin rather than release into the cytoplasm [223].

3.4.3.3. Apoptosis inhibitors

Apoptosis can be inhibited by overexpression of X-linked inhibitor of apoptosis proteins (Xiap), an intracellular anti-apoptic proteins, that plays a key role in cell survival by modulating death signaling pathways, including modulation of the PI3-K/Akt pathway [224]. Overexpression of Xiap and other anti-apoptic proteins (incl. IAP-2 (inhibitor of apoptosis protein) and survivin) correlated with cisplatin resistance in cisplatin-resistant prostate cancer cells [225]. Similar, Xiap down-regulation increased cisplatin sensitivity, caspase-3 activity and apoptosis in resistant ovarian carcinoma [226] and prostate cancer cells [227]. Transfection with hRFI (ring finger domain highly

homologous to XIAP) induced cisplatin resistance and inactivation of caspase-3 in colorectal cancer cells (where it is naturally preferentially expressed) [228].

Overexpression of Bcl-2 and Bcl-xL genes (with marked downregulation of caspase-3 expression [229]) may contribute to apoptic inhibition and the development of cisplatin-resistance in human ovarian cancer [230, 231]. Bcl-xL is also up-regulated in cells adapted to hypoxic stress and contributes to their resistance to cisplatin treatment [232]. In addition, nitric oxide (NO) induces Bcl-2 S-nitrosylation, inhibits its ubiquitination and upregulates Bcl-2 expression. NO synthase activity and NO production correlate with resistance in NSCLC cells [233].

Overexpression of ribosomal proteins (RP) S13 and RPL23 can promote cisplatin resistance in gastric cancer cells by suppressing drug-induced apoptosis (through increased Bcl-2 expression and the Bcl-2/Bax ratio) and increasing GST activity and intracellular GSH content [234]. Resistant cells may also exhibit overexpression of the Bcl-2 related protein Bfl-1/A1, mediated by nuclear factor-kappaB (NF-kappaB) [235].

3.5. Newer molecular factors linked to platinum drugs resistance

3.5.1. Cyclooxygenase-2 (COX-2)

As reviewed by Stewart [24], in preclinical studies, cisplatin treatment augmented tumor cell Cyclooxygenase-2 (COX-2) expression [236] and cisplatin resistance was induced by COX-2 overexpression. Clinically, high COX-2 expression was associated with reduced platinum-based therapy efficacy in esophageal [237, 238], bladder [239], cervical [240], and ovarian [241] cancers. The fact that a link is seen between therapy efficacy and COX-2 expression clinically makes the assessment of COX-2 inhibitors a particularly interesting focus for further research.

3.5.2. Heat shock proteins

Heat shock protein (HSP) HSP27 overexpression or gene transfection [242, 243] as well as overexpression of HSP-90β [244] and HSP70 also may augment cisplatin resistance, and cisplatin treatment increases HSP70 expression in vitro [245]. HSP inhibitors are currently undergoing clinical trials, but little is known regarding the role of HSP in clinical resistance, and it remains unknown whether HSP inhibitors will prove useful.

3.5.3. cAMP-phosphodiesterase-2

The gene PDE2, encoding cAMP-phosphodiesterase-2, may induce resistance by increasing tolerance of cisplatin-induced DNA lesions [246].

3.5.4. Cell cycle related factors

S-phase-kinase-associated-protein-2 (SKP2) controls stability of cell cycle related proteins. SKP2 overexpression reduced expression of p27Kip1, cyclin e, and p21Cip1, increased S-phase cells, and increased cisplatin resistance, while SKP2 downregulation increased sensitivity in vitro [247]. Cyclin D1 overexpression augmented pancreatic cancer cell chemoresistance both by promoting cell proliferation and by inhibiting drug-induced apoptosis in association with upregulation of NF-kappaB activity [248].

3.5.5. NF-kappaB

Up-regulation of expression of antiapoptic factors by NF-kappaB may antagonize cisplatin-induced apoptosis [249], and cisplatin significantly increases NF-kappaB DNA binding activity [250, 251]. NF-kappaB inhibitors augment platinum drugs activity against some cancer cell lines [251-254] and tumor xenograft models [250], but not against normal cells [251, 252] nor against some other cancer cell lines [251, 254].

3.5.6. Chromosomal alterations

Platinum drugs-resistant cells may have several chromosomal abnormalities [255, 256]. Telomere length, telomerase activity, and telomerase mRNA expression were reduced in cisplatin resistant ovarian carcinoma cell lines [257], and ovarian carcinomas with a loss of microsatelite D6S1581 were cisplatin-resistant [258].

4. Conclusion

This thesis evaluated possible ways of development of platinum drugs resistance. A variety of mechanisms has been described; however, none of them seems to be crucial in all resistant tumors. Moreover, none of them seems to be essential for all platinum drugs. The mechanisms by which cells acquire resistance to platinum drugs are mainly: (i) diminished accumulation of platinum complexes, (ii) increased detoxification of drug by the thiols glutathione and metallothionein, and (iii) improved repair of nuclear lesions and tolerance to them, leading to a reduction in apoptosis.

Platinum drugs probably enter cells by a number of influx transporters (especially by gated channel Aquaporine 9, Copper transporter CTR1 and Organic cation transporters OCT1 and OCT2) along with passive diffusion, and they can be extruded via the Cu efflux systems (including copper efflux transporters ATP7A and ATP7B) or possibly via drug efflux transporters (ABCG2). It is clear from a review of the literature that disagreement exists about the relative importance of each of these transport pathways to platinum drugs accumulation. Studies of sensitive and resistant cell lines have not been able to identify a single transporter whose decreased presence on the plasma membrane significantly contributes to a reduction in accumulation of platinum drugs. It seems that to reduce drug accumulation to a significant extent, or to confer cross-resistance to multiple cytotoxic platinum drugs, cells must simultaneously inactivate more than one of these transport systems.

Cell antioxidants glutathione and metallothionein also seem to play a role in platinum drugs resistance. They contain thiol molecules that can bind/inactivate platinum drugs and they can also contribute to enhancement of DNA repair and reduction of cisplatin-induced oxidative stress. However, much of the data is conflicting leading to question about their importance. They may have some role in certain types of cancers, but they do not appear to be a global indicator of cisplatin resistance.

As the cytotoxic effects of platinum drugs are caused by binding to DNA and formation of intrastrand and interstrand crosslinks, the most important mechanisms that contribute to platinum drugs resistance is improved repair of nuclear lesions and enhanced tolerance to DNA adducts. Nucleotide excision repair pathway, one of the

major pathways involved in repair of DNA damage, recognizes DNA damage, forms a complex that unwinds the damage portion and excises it. Finally, it re-synthesizes and ligates the excised area to maintain the DNA molecule. The importance of this pathway was approved by overexpression of specific proteins of this pathway in cells of many Pt-resistant tumors (e.g. ovarian, gastric, testis cancer, etc.). Mismatch repair system of proteins, another determinant of platinum drugs sensitivity/resistance, plays a role in preserving genomic integrity by facilitating the repair of post-DNA replication base pairing errors. Platinum drugs prevent a repair from being completed and therefore treatment with platinum drugs results in MMR dependent triggering of apoptosis. Another one DNA repair mechanism is the homologous recombination. It is proposed to play a role in repairing double strand brakes resulting from platinum drugs-induced interstrand DNA adducts.

Enhanced DNA adduct tolerance and reduction in apoptosis can appear if DNA polymerases skip the platinum adduct by translesion synthesis. This bypass can be caused by polymerase β, η, ζ, τ and is in common called Replicative bypass. Other cause for adduct tolerance is reduced apoptic response that can appear due to inhibition of pro-apoptic factors (caspase-3, -8, Fas, …) or due to overexpression of apoptosis inhibitors (Xiap, Bcl-2, Bcl-xL).

There are also several new molecular factors that have been linked to platinum drugs resistance (e.g. COX-2, HSP, cAMP-phosphodiesterase-2, NF-kappaB and other). With only a few exceptions, the effect on platinum drugs efficacy has been assessed only in vitro to date, with little information on their impact on resistance in xenograft models or in clinical use.

Overall, this thesis summarized most of yet known mechanisms that contribute to platinum drugs resistance. Some of them seem to be crucial in determining platinum drugs efficacy, but importance of most of them remains unclear. Therefore more work needs to be done to determine to which extent these mechanisms influence resistance to platinum drugs and influence their efficacy in the pharmacotherapy.

5. Abstract

Although the first platinum drug cisplatin was initially described in 1845, its biological activity was discovered more than 100 years later. Since then are cisplatin and its clinically used analogues carboplatin and oxaliplatin in widespread use for the treatment of variety of human cancers, including ovarian, cervical, head and neck tumors, non-small cell lung, breast, colon, gastric and renal cell carcinoma, sarcoma and relapsed lymphoma. However, the treatment is often accompanied by severe side effects of which nephrotoxicity, peripheral neurotoxicity and myelosuppression are the most serious. Another important obstacle in their clinical use is drug resistance. This thesis evaluates possible mechanisms of the development of platinum drugs resistance. There is a variety of them and they include (i) diminished accumulation of platinum drugs affected by influx transporters (Aquaporin 9, CTR1, OCT1, OCT2) and by efflux transporters (ATP7A, ATP7B,ABCG2); (ii) increased detoxification of drug by thiols glutathione and metallothionein; (iii) improved repair of nuclear lesions affected by NER, MMR, Homologous recombination, and enhanced tolerance to nuclear lesions caused by Replicative bypass, inhibition of pro-apoptic factors (including caspase-3, -8, Fas and other), or by overexpression of apoptosis inhibitors (Xiap, Bcl-2, Bcl-xL). Some of them seem to be crucial in determining platinum drugs efficacy, but importance of most of them remains unclear. Therefore more work needs to be done to determine to which extent these mechanisms influence resistance to platinum drugs and influence their efficacy in the pharmacotherapy.

6. Abstrakt

Ačkoliv první platinová sloučenina, cisplatina, byla poprvé popsána již v roce 1845, její biologická aktivita byla objevena o více než 100 let později. Od té doby je cisplatina a její klinicky využívané analogy karboplatina a oxaliplatina široce používána pro léčbu mnoha lidských karcinomů. Mezi ně patří zejména ovariální, cervikální, renální a nemalobuněčný plicní karcinom, karcinom hlavy a krku, prsu, tlustého střeva, žaludku, sarkom a lymfom. Bohužel je léčba často doprovázena vážnými nežádoucími účinky, ze kterých nefrotoxicita, periferní neurotoxicita a útlum kostní dřeně patří mezi ty nejzávažnější. Další významnou překážkou v jejich klinickém využití je vznik lékové rezistence. Tato práce hodnotí možné mechanismy vzniku rezistence na platinové sloučeniny. Jsou rozmanité a zahrnují (i) sníženou akumulaci platinových sloučenin způsobenou influxními (Aquaporin 9, CTR1, OCT1, OCT2) a efluxními (ATP7A, ATP7B, ABCG2) transportéry; (ii) zvýšenou detoxifikaci pomocí thiolových sloučenin glutathionu a metallothioneinu; (iii) zvýšenou schopnost opravovat jaderná poškození (NER, MMR a homologní rekombinace), zvýšenou toleranci k jaderným poškozením (Replicative bypass), inhibici pro-apoptických faktorů (kaspáza-3, -8, Fas a další) a nebo zvýšenou expresi inhibitorů apoptózy (Xiap, Bcl-2, Bcl-xL). Některé z těchto mechanismů se zdají být pro vznik rezistence na platinová cytostatika klíčové, avšak význam většiny z nich je nejasný. Pro objasnění toho, jakou měrou se podílejí na rezistenci nádorů vůči platinovým cytostatikům a jak ovlivňují účinnost těchto léčiv v protinádorové terapii musí být provedeny další studie.

7. References

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