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Drugs of the Future: Review

Cellular and molecular aspects of drugs of the future: oxaliplatin

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Abstract. Oxaliplatin (Eloxatine) is a third-generation platinum compound which has shown a wide antitumour effect both in vitro and in vivo, a better safety profile than cisplatin and a lack of cross-resistance with cisplatin and carboplatin. In this scenario, oxaliplatin may represent an innovative and challenging drug extending the antitumour activity in diseases such as gastrointestinal cancer that are not usually sensitive to these coordination complexes. Oxaliplatin has a non-hydrolysable diaminocyclohexane (DACH) carrier ligand which is maintained in the final cytotoxic metabolites of the drug. Like cisplatin, oxaliplatin targets DNA producing mainly 1,2-GG intrastrand cross-links. The cellular and molecular aspects of the mechanism of action of oxaliplatin have not yet been fully elucidated. However, the intrinsic chemical and steric characteristics of the DACH-platinum adducts appear to contribute to the lack of cross-resistance with cisplatin. To date, mismatch repair and replicative bypass appear to be the processes most likely involved in differentiating the molecular responses to these agents.

Key words. Oxaliplatin; DACH compounds; platinum resistance; cancer; replicative bypass; mismatch repair deficiency.

Introduction

Oxaliplatin is a platinum-based antitumour drug which has shown antitumour activity in a wide range of murine and human tumour cell lines in preclinical studies [1–3]. Interest in oxaliplatin was raised when the National Cancer Institute Anticancer Drug Screening Programme showed a lack of cross-resistance between oxaliplatin and both cisplatin or carboplatin in most of the colon cell lines tested in the study [4].
Oxaliplatin was first introduced into clinical trials by Mathé and colleagues in 1986 [5]. Molecular biology studies showed that oxaliplatin may be identified as a separate family of platinum-based compounds, differing in the mechanism of action and resistance from cisplatin and carboplatin and thus potentially effective in tumours with intrinsic or acquired resistance to both these drugs.
In vitro, oxaliplatin displayed a broad antiproliferative activity against mouse and human leukaemia, neuroblastoma, melanoma and non-small-cell lung, colon, ovarian, breast, gastric and bladder carcinoma cell lines [6, 7]. Interestingly, the cytotoxic effect of oxaliplatin is not affected in cisplatin-resistant cell lines. This observation provided the first evidence of the absence of cross-resistance between oxaliplatin and cisplatin/carboplatin [3].
Preclinical combinations in in vitro and in vivo studies showed additive or synergistic interactions of oxaliplatin with 5-fluorouracil, AG337, gemcitabine, irinotecan, paclitaxel, cisplatin and cyclophosphamide [8–13]. Notably, synergism between oxaliplatin and 5-fluorouracil is also maintained in 5-fluorouracil- and cisplatin-resistant

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cell lines, suggesting a therapeutic role in both first- and second-line treatment regimens [6, 8]. Phase I studies confirm that treatment with oxaliplatin is not associated with the nephrotoxicity and ototoxicity induced by cisplatin treatment [14]. Nausea and vomiting were frequently reported but the systematic use of antiemetics has significantly decreased the severity of this toxicity. Diarrhoea has been registered only in a small subset of patients receiving doses of 150 mg/m^2 or greater. A transient sensory neuropathy is the dose-limiting toxicity of oxaliplatin. This neuropathy is characterized by paresthesia and dysesthesia in hands, feet and the perioral area which is triggered or enhanced by cold [15, 16]. These symptoms arise during the infusion of the drug and increase with dose and the number of courses. Interestingly, the neurotoxicity appears to be cumulative although it generally reverses within 4–6 months after treatment discontinuation. A recent study by McKeage and colleagues [17] has suggested that the observed damage to the nucleolus of ganglionic sensory neurons in rats is probably related to the inhibition of rRNA synthesis and might be responsible for neurotoxicity.

Based on preclinical reports showing synergistic effects with several new anticancer agents including irinotecan, topotecan, capecitabine and paclitaxel, clinical trials have aimed to evaluate different combinations. These studies have been performed or are still ongoing in those malignancies in which oxaliplatin alone showed antitumour activity, such as ovarian, non-small-cell lung and breast cancer and non-Hodgkin lymphoma [18–21]. These preliminary results suggest that oxaliplatin alone or in combination with other antineoplastic drugs could be very attractive for future clinical trials in various tumours.

Platinum compounds

Since the fortuitous discovery of cisplatin by Rosenberg and colleagues in the late sixties [22], around 3000 cisplatin analogues have been synthesised with the aim to retain the wide antitumour spectrum of activity of cisplatin and to reduce its side toxicity. Cisplatin is clinically active in testicular carcinoma, small-cell lung carcinoma (SCLC), ovarian carcinoma and paediatric tumours. The main dose-limiting factors for the clinical outcome of cisplatin are the observed toxicity on the nervous system, nephrotoxicity, myelotoxicity, nausea and vomiting [23–26] and auditory impairment. The drug is also mutagenic [27, 28]. The development of resistance to the action of cisplatin during treatment represents also limits to its clinical use. Of the thousands of platinum complexes synthesised in the attempt to overcome these limiting factors, carboplatin showed the best compromise between antitumour activity and tolerable side toxicity with respect to cisplatin. Carboplatin (cis-diamino-1,1-cyclobutane dicarboxylato platinum II) has a bidentate cyclobutane di-carboxylato ligand replacing the two chloride groups of cisplatin and represents the second generation in the platinum complex history (fig. 1). This chemical structure confers a 17-fold increase in water solubility (w.sol.) of carboplatin over that of cisplatin (w.sol. 1 mg/ml) [29]. Unlike cisplatin, carboplatin reversibly binds to blood proteins in rats [30]. Furthermore, carboplatin is more stable and is excreted mainly unchanged in the urine [31]. These pharmacokinetic properties might explain the limited nephrotoxicity of carboplatin and its ability to pass more easily through the blood-brain barrier [32]. Like cisplatin, carboplatin targets DNA with the formation of DNA-intrastrand cross-links (ICLs) and protein-DNA cross-links (DPCs) being the major toxic path-

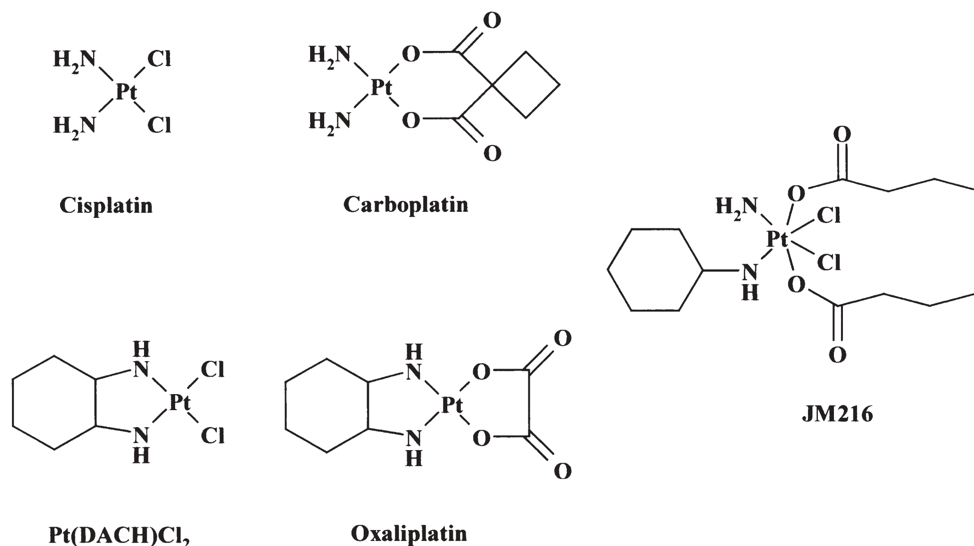

Figure 1. Chemical structures of platinated compounds.

ways [33]. However, carboplatin is a less efficient DNA-damaging agent than cisplatin, producing DNA adducts more slowly, and it is also 45 times less active in L1210 cells than cisplatin [33]. The main clinical achievement obtained with carboplatin was the reduced nephrotoxicity compared with cisplatin, although myelotoxicity became the most relevant toxicity during treatment with the drug. For this reason, carboplatin had a limited use in combination with other myelosuppressive agents. Furthermore, the drug failed in overcoming cisplatin-acquired resistance.

To overcome the limitations encountered with carboplatin, many cisplatin analogues were made. Of these, the simple substitution of the two amino groups of cisplatin with the diaminocyclohexane (DACH) group afforded agents with good antitumour activity and a lack of cross-resistance with cisplatin. Furthermore, the antitumour potency of the (DACH)Pt compounds was shown to depend on the absolute configuration of the DACH ligand. The latter can exist as two geometrical isomers, the trans and the cis complexes. Of these, the trans-DACH can be further separated in two enantiomers, (1R,2R-DACH)Pt and (1S,2S-DACH)Pt, whereas the cis-DACH is the diastereoisomer (1R,2S-DACH)Pt (fig. 2). Interestingly, cis-DACH showed lower antitumour activity than the trans forms [34–38]. Furthermore, cytotoxicity assays showed that the trans-R,R isomer (form 'l') was more potent than the trans-S,S isomer (form 'd') [38, 39] suggesting a carrier-ligand specificity in the recognition process of the DNA adducts by the repair machinery. In a study by Pendyala et al. [38], based on the three isomers of the DACH-Pt compound oxaliplatin, the superior cytotoxicity of the trans-l isomer was shown to correlate with increased cellular accumulation and DNA binding. However, the same did not hold true for the other two isomers. The authors found that the trans-d form, although more cytotoxic than the cis-form, showed the lowest cellular uptake and DNA binding, suggesting that the three isomers may have different access to the DNA major groove and the transport proteins. Alternatively, they may form different types and/or amounts of DNA adducts or undergo differential repair [38]. In a detailed study on the DNA-binding efficiency of the three DACH isomers, Boudny and colleagues [37] showed that the cis-DACH complex bound most slowly to DNA. Moreover, the same isomer also showed the lowest transformation kinetic from monofunctional to bifunctional DNA adducts (e.g. intrastrand cross-links). The authors proposed that these differences might be related to the differences in the orientation of the cis- and trans-DACH adducts. For example, the trans-DACH group is almost perpendicular to the DNA helix and appears easily accommodated into the major grove, whereas a different situation is observed with the cis counterpart, (1R,2S-DACH)Pt [40]. This isomer can exist as two configurations which are mutually interchangeable by inversion of the cyclohexane ring [37]. Unlike the trans-complex, the two (1R,2S-DACH)Pt forms have the DACH ligand lying almost parallel to the DNA helix. Although one of these two forms does not appear to generate high steric hindrance, the other has been shown to produce more dramatic conformational alterations in the DNA region surrounding the bifunctional adduct. The overall conclusion from this study was that the cis-DACH-DNA adduct would produce more extensive conformational changes in the DNA structure than the trans-complex.

Although the DACH-complexes achieved the important goal of bypassing cisplatin resistance while retaining anti-tumour activity, their low water solubility was a limiting factor in their clinical development as antitumour drugs. Therefore, other chemical modifications of the starting structure focused on the replacement of the chloride atoms with more anionic leaving groups. The best result from these attempts was oxaliplatin, which has an oxalate ligand as leaving group and the DACH ligand in the trans-l form (fig. 2). Oxaliplatin (trans-l-diaminocyclohexane oxalatoplatinum) is a third-generation platinum compound [29, 41] which was selected for further investigations based on its water solubility (about eight-fold more soluble than cisplatin [29]), promising antitumour activity on the L1210 cell line and lack of cross-resistance with cisplatin. Overall, oxaliplatin appears to retain the antitumour properties of cisplatin without its clinical toxicity whereas some doubts have been raised about its mutagenic potential [1, 29, 42–45].

Biotransformation and active metabolites of oxaliplatin

Although passive diffusion is believed to play an important role in cisplatin transport, the exact mechanism responsible for cisplatin accumulation in cells is not fully understood [46–50]. With most of the DACH complexes, including oxaliplatin, a linear correlation between hydrophobicity and cellular uptake has been reported [51]. Once inside the cell, all cisplatin analogues undergo biotransformation processes which appear responsible for the production of the ultimate cytotoxic form of the drugs. For example, once entered into the cell, cisplatin undergoes hydrolysis of the two chloride ions, producing a cis-diaminodiaquo complex that is involved in DNA adduct formation and cytotoxicity (fig. 3).

As a general consideration, the biotransformation process of oxaliplatin is based on its chemical properties, with the non-leaving DACH ligand being chemically inert and the bidentate oxalate moiety working as a hydrolysable carrier whose major role is to confer water solubility on the molecule. These characteristics would explain why most of the products of oxaliplatin metabolism show a

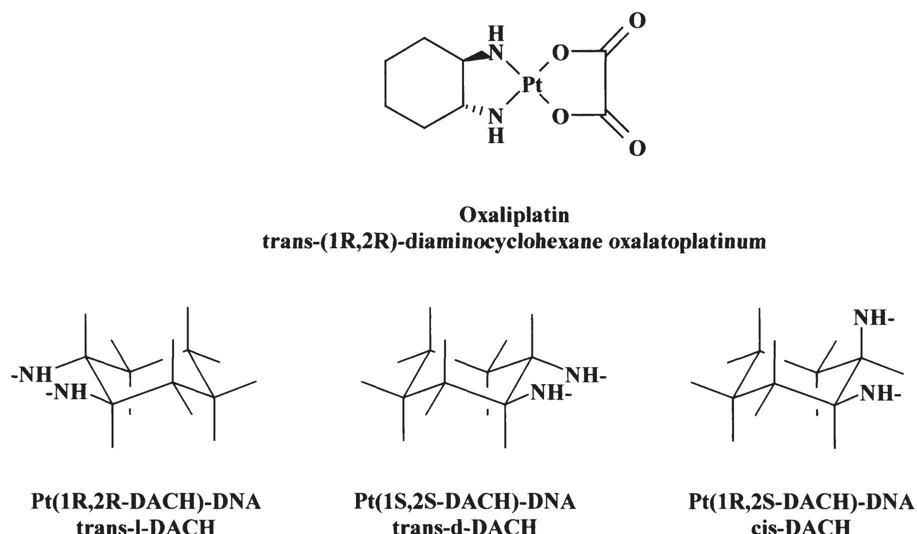

Figure 2. Stereochemistry of DACH compounds.

Pt(DACH) unit. The main biotransformation products of oxaliplatin are thought to derive from replacement of the bidentate oxalate moiety with chloride ions followed by hydrolysis. The latter process can take place through the interaction with HCO_3^- and $H_2PO_4^-$ at physiological concentrations. These reactions appeared to be the main biotransformation processes of DACH platinum compounds in the L1210 cell line and likely represent the main activation pathway for oxaliplatin [52].

Grolleau et al. [53] suggest a possible role for the bidentate oxalate metabolite in the neural damage observed after treatment of cockroach dorsal unpaired median neurons with the drug. Chelation of calcium ions through the bidentate oxalate moiety was proposed to block the voltage-gated sodium channels, producing acute toxicity and, in the long term, oxaliplatin-induced neuropathy.

Similar to cisplatin, the ultimate species produced from oxaliplatin is the diaquo complex which may react with proteins such as globulins and gammaglobulins and which is also the active form in the covalent binding to DNA (fig. 3). Together with the activated aquo complexes, the biotransformation of oxaliplatin leads to chemically unreactive species. Among them are the amino acid complexes $Pt(DACH)(Cys)_2$ and Pt(DACH) Met coming from the interaction with L-cysteine and L-methionine, respectively, whereas reaction with glutathione (GSH) gives rise to $Pt(DACH)(GSH)_2$. Free DACH is also a further unreactive metabolite observed during the biotransformation of the drug (fig. 3).

A study by Luo et al. [51] on the metabolism of oxaliplatin in rat blood in vitro showed that the binding of the drug to plasma and red blood cells was very rapid and reached equilibrium by 4 h from incubation with the drug. Furthermore, only chemically unreactive products accumulated in the cytosol of red blood cells by this time. On the other hand, $Pt(DACH)Cl_2$ and unchanged drug were only observed at early times. Under equilibrium conditions, only 12% of total Pt-DACH was found in the plasma ultrafiltrate, whereas about 35% was bound to plasma proteins and 53% was detected in the red blood cells, where it was mainly associated with cytosolic proteins and cellular membrane. A different study from Pendyala and Creaven [3] showed that the DACH-Pt found in red blood cells was not exchangeable with the plasma, leading to the conclusion that erythrocytes do not constitute a reservoir for the cytotoxic drug. Furthermore, both oxaliplatin and the DACH complexes were observed to be generally taken up by the cells better than cisplatin and carboplatin. Of the total 35% of Pt found in the plasma, the major complexes were again those with methionine, cysteine and GSH, whereas the dichloro-Pt complex was only transiently observed. In a different study, Luo et al. [54] suggested that the potency of the drug was mainly determined by the plasma concentration of the parent compound.

Mechanism of cytotoxicity

Similar to cisplatin and carboplatin, oxaliplatin forms DNA bis-adducts. The main cytotoxic pathway is characterised by the formation of ICLs with either two adjacent guanines (GG) or with guanine-adenine bases (AG). Potentially high lethal DNA interstrand cross-links constitute a small portion of the total adducts [55]. The major effect of the formation of these DNA adducts is the inhibition of DNA replication, resulting in cell cycle arrest and apoptosis [56, 57]. Interestingly, in mouse leukaemia L1210 cells, oxaliplatin also appeared to interfere with RNA synthesis

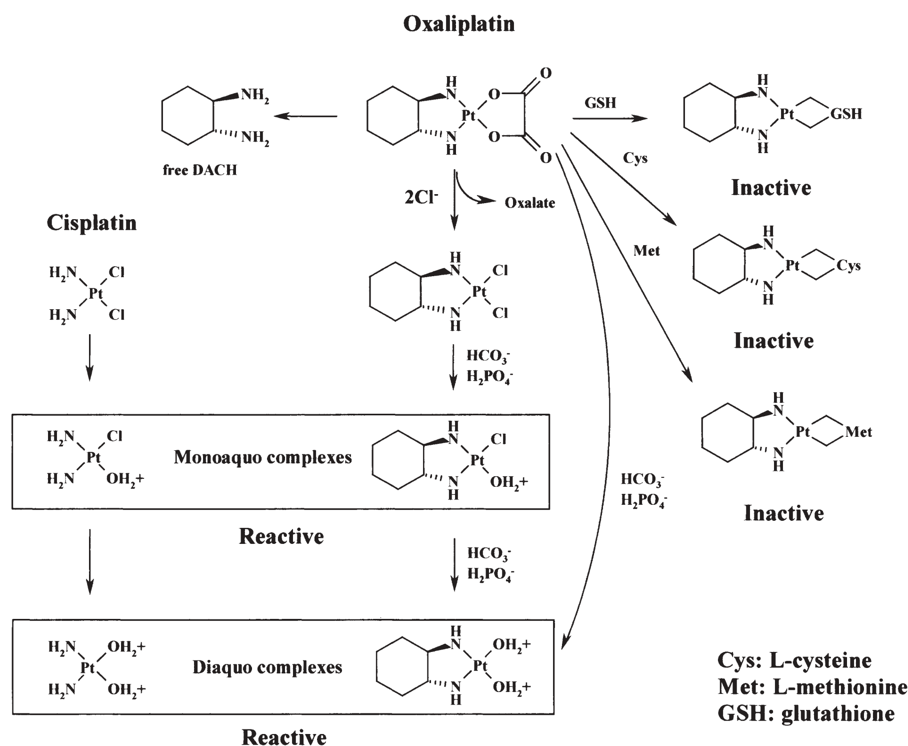

Figure 3. Major oxaliplatin and cisplatin metabolites.

whereas cisplatin did not [1]. Although oxaliplatin appears to produce DNA covalent adducts in a similar fashion to cisplatin, the total amount of ICLs and DPCs induced by equimolar concentrations of oxaliplatin is significantly lower, showing about two- to sixfold less DNA adducts than cisplatin in the same region of DNA [55].

The formation of these bis-adducts proceeds through a two-step mechanism. Substitution of one of the two chlorides with water affords a monoaquochloro complex which rapidly reacts with the N(7) of guanine to generate a monoadduct. A similar reaction occurs on the other leaving chloride affording a diaquo complex which is the same final species from both cisplatin and carboplatin (fig. 3). This aquo complex in turn reacts with a second guanine (or adenine) taking the platination process to completion. Although the sites of DNA platination are similar for oxaliplatin, cisplatin and carboplatin, the kinetics of this two-step process appear to be highly dependent on the chemistry of the carrier ligands in the platinum complex. With oxaliplatin, DNA adduct formation in vitro appears to be slower than that for cisplatin [58, 59] although the DACH complexes showed completion of the two-step process in about 15 min [60]. The slower reaction observed with oxaliplatin may be related to the constraint shown by the N-Pt-N bond of the DACH complex. In addition, the DACH-Pt adducts of oxaliplatin generate a hydrophobic site which is also bulkier than the cis-diamine-Pt adducts of both cisplatin and carboplatin. As a result, although showing a lower reactivity towards DNA, oxaliplatin produces a more efficient DNA synthesis inhibition resulting in enhanced cytotoxicity with respect to cis-diamino-Pt adducts [4, 61, 62].

More information about the differences in the response to oxaliplatin and cisplatin derive from a study by Scheeff et al. [63] based on the molecular mechanic minimisation and restrained molecular dynamics of the known crystal structure of DNA-cisplatin. Using the latter model as a guide, the authors showed that covalent binding of oxaliplatin produced cisplatin-like effects on DNA conformation. In both cases, a bend towards the major grove and the concomitant flattening and broadening of the minor grove (A-DNA-like conformation) was observed. However, though the square-planar geometry exhibited by oxaliplatin was similar to that of cisplatin, the cyclohexane group of the DACH ligand (in the chair conformation) was responsible for further narrowing the major groove. By protruding into the major grove (3.7 Å further than with cisplatin) and by exhibiting a non-covalent bond with the DNA strands, the DACH ligand appears to produce a more A-DNA-like helix. These differences in the steric properties between cisplatin- and oxaliplatin-DNA complexes may therefore produce different conformational distortions in the DNA region around the adducts. This aspect together with the less polar character of the DACH-Pt complexes has been proposed to contribute to the failure to detect the DNA-oxaliplatin adducts by the

recognition proteins of the mismatch repair pathway (MMR) [63].

Resistance to platinum-based compounds

The main attractive clinical gains of oxaliplatin over cisplatin were the better safety profile and the antitumour activity in cisplatin-resistant tumours. The latter aspect represents the most striking difference between the two drugs, although their mechanisms of action appear to be similar. Why does oxaliplatin exhibit a lack of cross-resistance with cisplatin and carboplatin? Some insights into the molecular bases of oxaliplatin action may be obtained from many studies focused on the mechanisms of cisplatin resistance. At least six different ways for a cell to become resistant to the action of platinum agents have been proposed (fig. 4). For example, a decrease in cellular uptake and increase in drug efflux will reduce the actual amount of the intracellular drug available for cytotoxic action. On the other hand, while accumulating into the cell, the compound undergoes side reactions which may result in its inactivation/trapping (e.g. reaction with the sulphydril group of GSH) before a cytotoxic action can be expressed. A further way to impair cisplatin action may derive from quenching of the DNA monoadducts through reaction with GSH or other metallothioneins. Finally, resistance to cisplatin may arise from increased repair of drug-DNA adducts [through nuclear excision repair (NER)] and/or an increased tolerance to platinated DNA. The latter situation can be observed through the increase in the replication bypass process past the DNA-platinum adducts and/or a defective MMR [64].

A comparative study by Hector et al. [65] on oxaliplatin- and cisplatin-mediated resistance in the human ovarian carcinoma cells A2780 indicated the existence of similar mechanisms of reduced drug accumulation and DNA adduct formation between the two drugs.

In a different study, El-Akawi et al. [66] showed that resistance to oxaliplatin in human ovarian carcinoma sub-

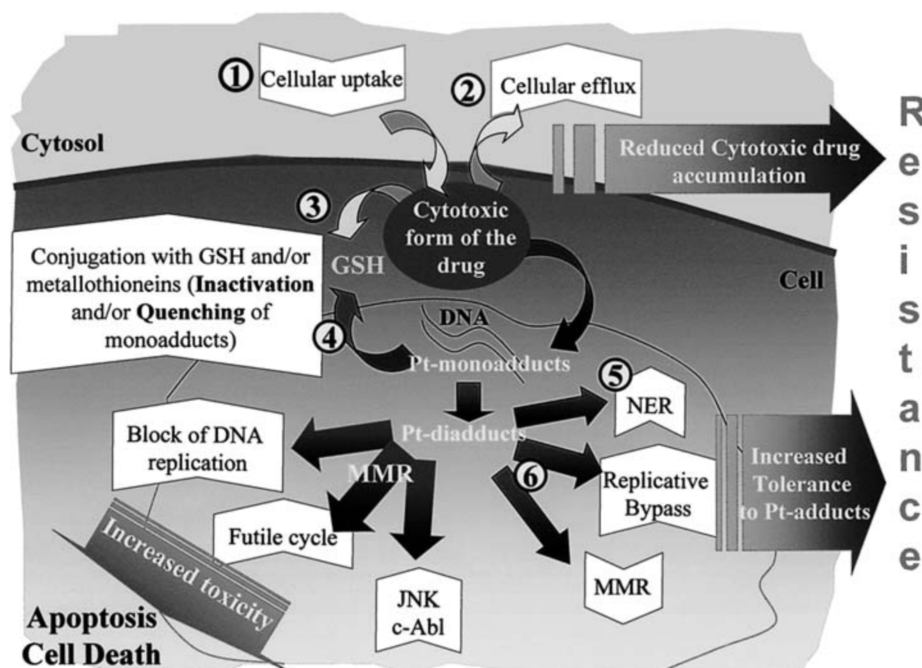

Figure 4. Schematic view of resistance processes to platinum compounds (e.g. cisplatin). Toxicity of platinum compounds is due to direct damage on DNA resulting in cell death (bottom left-hand side of the picture). Cisplatin MMR-mediated toxicity is proposed to play different roles: mismatch repair proteins can directly recognise the adducts and trigger signalling cascades (through increase of c-ABL and JNK) leading to cell death and apoptosis; MMR proteins can contribute to the 'futile cycle' of continuous damage removal from the newly synthesised strand and DNA resynthesis (replicative bypass), ending in strand break production and ultimate cell toxicity; MMR proteins can selectively bind to cisplatin over oxaliplatin adducts and shield them from simple replication, repair and/or translesion synthesis processes. Alternatively, this protein-adduct interaction may trigger a signalling cascade leading to replicative bypass inhibition. On the other hand, resistance to cisplatin may arise from either an increase in the intracellular concentration of cytotoxic compounds or increased tolerance to their toxicity. A decrease in cellular uptake ① and/or increase in cellular efflux ② would both reduce the level of drug available to express a toxic action. Before interaction with DNA can take place, cisplatin may be sequestered by GSH or metallothioneins that contribute to its inactivation ③. However, these types of reaction may also take place on the monoadducts, resulting in their quenching ④. Once the bis-adducts have been produced, increase in their repair through the nuclear excision repair (NER) pathway ⑤ may contribute to enhanced tolerance to the action of cisplatin. The same outcome is observed as a result of either an increase in replicative bypass or a decrease in MMR processes ⑥.

lines from A2780 was associated with an increase in cellular GSH. GSH-mediated drug resistance is usually due to alterations in the main GSH biosynthetic route which is catalysed by γ-glutamyl cysteine synthetase (γ-GCS). However, the authors found that γ-GCS activity was unchanged in the oxaliplatin-resistant subline, whereas both the mRNA levels and the enzyme activity of γ-glutamyl transpeptidase (γ-GT), the 'salvage' pathway enzyme of GSH metabolism, were elevated [66]. Similarly, cisplatin was shown to induce an elevation in the mRNA levels of γ-GT, although to a lower degree than oxaliplatin [67].
The general lack of evidence of any significant differences in drug uptake/efflux and inactivation/quenching of cisplatin- over oxaliplatin-DNA adducts has suggested that the molecular bases of the discrimination between the two drugs might be found in the mechanisms of DNA repair.

Oxaliplatin and repair pathways

Nuclear excision repair

Although multifactorial, resistance to cisplatin (either acquired or intrinsic) appears to correlate with an enhancement in the NER pathway (fig. 4) [68–70]. NER targets damaged or inappropriate bases within DNA. The human NER comprises at least six repair factors involved in the first important step of damage recognition and removal [71–73] that is also the rate-determining step of the overall repair pathway. XPA and RPA are the repair factors involved in the formation of a damage recognition subunit [71, 72]. This preliminary step triggers the recruitment of endonucleases (XPG and XPF · ERCC1) responsible for a dual incision at both 3′ and 5′ sites to the lesion [71, 72]. This step produces the excision of damage-containing oligomers of 22–32 nucleotides in length. The final steps are DNA resynthesis and then ligation of the new strand to the parental DNA to fill in the gap left during damage removal.
NER plays a major role in the repair of cisplatin-DNA adducts, although the most abundant 1,2-dipurinyl ICLs appear to be poorer substrates for the repair machinery than the 1,3-GG cross-links [74–76]. Cells from xeroderma pigmentosum patients that are defective in the NER pathway [77, 78] consistently showed a hypersensitivity to cisplatin whereas cells made resistant to the drug showed increased NER expression. These observations have assigned the NER pathway a role as a protective mechanism against platinum toxicity [74, 75].
Reardon and colleagues [79] showed that the initial kinetic and the overall rate of the NER process were comparable for three platinum compounds, cisplatin, oxaliplatin and bis-aceto-amine-dichloro-cyclohexyl-amine-platinum (IV) (JM216) (fig. 1). The rate-determining step of the overall process is the recognition mode of damaged DNA that takes place when the interactions between drug and DNA produce a local distortion followed by base pair denaturation [80]. This observation is in disagreement with the fact that oxaliplatin induces a greater DNA helical distortion than cisplatin and JM216 [81]. The overall conclusion from these and other studies is a consistent indication that NER is not responsible for the different cellular responses to cisplatin and oxaliplatin. Mainly for this reason, other mechanisms of resistance to the platinum compounds have been studied in more detail in the hope of gaining some insight into this unanswered question.

Tolerance to platinum damage

More recently, many studies have focused on both MMR and post-replication repair. The latter is not a true 'repair' process, because it refers to the ability to replicate DNA past a bulky adduct without introducing further gaps or discontinuities into the nascent strand [82–85]. Thus, the adducts are not removed from the DNA and for this reason the whole process has been better defined as translesion synthesis or replicative bypass. Evidence is increasing that either a decrease in MMR or an enhancement in replicative bypass play a role in discriminating between cisplatin and oxaliplatin cytotoxicity [86]. For example, loss of MMR activity correlates with acquired resistance to cisplatin or carboplatin but not to oxaliplatin [87–89]. On the other hand, in some ovarian cancer cells, resistance to cisplatin, and to a lesser extent to oxaliplatin, appears to correlate linearly with the increase in the replicative bypass of platinum-DNA adducts [90]. The rationale behind these findings may be that, although multifactorial, resistance to platinum compounds may be enhanced by increased tolerance to the damage incurred by the DNA. Nonetheless, the ability of certain cells to tolerate the damage (becoming resistant) rather than to prevent it may be able to discriminate cisplatin from oxaliplatin, likely because of the differences in the chemical and geometrical properties of the platinum-DNA adducts induced by the two drugs.

MMR and platinum adducts

MMR is a post-replication repair pathway which corrects base mispairs and looped intermediates of one to three bases arising during replication. Human MMR is associated with six genes called hMLH1, hMLH2, hPMS2, hMSH2, hMSH3 and hMSH6 (GT-binding protein or p160). One of two mismatch complexes with partially overlapping action appears to be involved in the recognition of mispaired bases and/or deletions-insertions in the newly synthesised strand: hMutSα (heterodimer of hMSH2 and hMSH6, also called GTBP [91, 92]) and hMutSβ (heterodimer of hMSH2 and hMSH3 [93]). During the repair process, these hMutS complexes are

thought to recruit the hMutLα heterodimer where hMLH1 is partnered with hPMS2. Many proteins are required to take the whole process to completion through the excision of the damaged site from the newly synthesised strand followed by DNA resynthesis to fill in the gap left by removal of the lesion [85, 94–97]. Loss of MMR results in an increased spontaneous mutation rate and microsatellite instability [98–100] other than defects in transcription-associated repair [101]. Resistance to cisplatin has been shown to correlate primarily with defects in the hMLH1 subunit of the hMutLα complex [85, 94–96]. However, in cells never previously exposed to the drug, enhancement in the resistance to cisplatin resulted from the loss of either hMLH1 or PMS2 subunits in the hMutLα complex and/or defects of the hMSH2 subunit in the hMutSα complex [89, 102, 103]. Yamada et al. [104] showed that mismatch repair attempts are likely to take place following replication of cisplatin-adducted DNA. The authors showed that the hMutSα complex interacts favourably with 1,2-GG cross-links paired to CT in the complementary strand. This finding is in agreement with the evidence that replicative bypass of platinum-DNA adducts was active in either cell extracts or intact cells [82, 105].

As a general consideration, a loss of MMR ability is usually associated with a relatively small decrease (about twofold) in the cellular sensitivity to cisplatin or carboplatin. Nonetheless, this small resistance to the drugs appears sufficient to account for treatment failure in human tumour xenografts [95, 96, 102, 106].

Other than being a post-replicative repair pathway, MMR is involved in the cellular response to DNA damage where it plays a role in the signal transduction pathway, cell cycle regulation and apoptosis [107–109]. A study by Nehmé et al. [109] on the signalling cascades triggered by the interaction of cisplatin with DNA in MMR-proficient and -deficient cell lines showed a positive correlation between MMR expression and kinase activation. The authors found that the drug activates both JNK (c-Jun NH_2-terminal kinase, a serine/threonine kinase) and c-Abl (a non-receptor nuclear tyrosine kinase) and this activation was higher in MMR-proficient versus -deficient cells. Interestingly, in the same study, oxaliplatin did not appear to activate the apoptotic process, regardless of the mismatch repair status of the cells [109]. A different study showed that cisplatin-induced apoptosis was triggered by an increase in p73 through the activation of c-Abl in an MMR-dependent manner [110]. p73 is a p53-related gene belonging to the family of tumour suppressor proteins. Unlike p53, p73 is not directly induced by DNA damage but it requires binding and phosphorylation with the non-receptor tyrosine kinase c-Abl [111, 112]. c-Abl is localised in the nucleus and in the cytoplasm of both resting and actively dividing cells and is involved in cell cycle regulation [113]. Thus, c-Abl overexpression has been observed in response to ionising radiation and DNA-damaging agents such as cisplatin and mitomycin C, resulting in cell cycle arrest in G_1 [114–116].

The rationale behind the MMR-dependent activation of signalling pathways is that the DNA mismatch repair system may work as a DNA damage detector rather than a post-replication repair machinery. In this model, the mismatch repair system would initiate the cellular responses to the damage through the recognition of DNA adducts, leading to cell cycle arrest and cell death by apoptosis. In addition, cisplatin toxicity in MMR-proficient cells has been proposed to be due to an incomplete repair of DNA adducts similar to the model proposed for O6-methylguanine-induced DNA lesions [101]. Accordingly to this hypothesis, a futile cycle would take place in which the synthesis beyond the DNA lesion is followed by recognition and removal of the newly synthesised strand by mismatch proteins and retention of the parental strand containing the adduct, resulting in the ultimate accumulation of lethal strand breaks [85, 87, 97, 104, 117, 118]. Furthermore, the binding of MMR recognition proteins to platinum-DNA adducts has been proposed to contribute to the enhancement of cisplatin toxicity by prolonging the life of the lesions. This would take place by shielding the DNA lesions from repair and, on the other side, by impairing the efficacy of the replication bypass system [101]. These findings are in agreement with all the studies reporting on the correlation between an MMR-defective pathway and resistance to cisplatin (fig. 4) [85, 89, 94–96, 103].

As MMR deficient cells have shown a differential sensitivity to oxaliplatin and cisplatin, a selective recognition of the platinum-DNA adducts by mismatch proteins has been proposed to represent the key step for the discrimination between the cellular responses to the two drugs [119]. In this model, unlike the cis-diamine groups of cisplatin and carboplatin, the bulkier and more hydrophobic DACH ligand of oxaliplatin would not be recognised by the proteins of the MMR system or other damage recognition proteins (e.g. high-mobility-group proteins, such as HMG-1). Thus, if this hypothesis held true, the carrier ligand of the platinum analogues might be able to determine the ultimate blocking potential of the lesion [120]. Zdraveski et al. [119] have shown that MutS (the bacterial analogue of the human MMR proteins) can recognise cisplatin-DNA adducts with a twofold higher affinity than oxaliplatin-DNA adducts. Consistent with the previous model, the authors proposed that the intrinsic characteristics of the DACH adducts may be responsible for the unfavourable interactions with MutS [121, 122]. Furthermore, addition of ADP was found to increase MutS affinity for cisplatin-DNA adducts but not for oxaliplatin-DNA. The actual role of ADP binding on the functions of MutS in both mammals and prokaryotes is still unclear. However, the

couple ADP/ATP has been proposed to serve as molecular switch to downstream components belonging to the repair machinery and/or to the apoptotic pathway [123, 124]. Specific ADP modulation of the binding of MutS to cisplatin-DNA has been proposed to underlie a damage-recognition signal pathway [119].

Replicative bypass of oxaliplatin-induced lesions

Replicative bypass of DNA lesions may determine tolerance to the toxic action of drugs and/or radiation [125]. In this context, replicative bypass and translesion synthesis can be used interchangeably to indicate the ability of certain polymerases to carry on DNA synthesis despite the presence of bulky DNA adducts on the leading strand of DNA which are ignored by the replicating machinery that carries on through them [82–85]. Thus, platinum-DNA adducts do not represent an absolute block of DNA replication, although the local helical distortions produced by the covalent binding of the drugs on DNA may affect the action of polymerases. Many in vitro studies have shown that DNA lesions (e.g. UV-induced dimers, cisplatin- and oxaliplatin-DNA cross-links) can be overcome by the action of certain polymerases whose efficiency and accuracy has been associated with both the fidelity of the enzyme involved and the type of damage encountered [126]. Furthermore, in vitro studies on human DNA polymerase η by Vaisman and colleagues [126] have suggested that the fidelity of translesion synthesis in the presence of platinum-DNA lesions may be determined by the structures of both the adduct and the DNA polymerase active site beyond their mutual interactions. This implies that those polymerases involved in replicative bypass may be able to discriminate between the carrier ligands of cisplatin- and oxaliplatin-DNA adducts [82, 90, 117]. The replicative bypass process specificity for platinum ligands has been suggested to contribute to the lack of cross-resistance to oxaliplatin observed in certain cisplatin-resistant cell lines [82, 90].

Although the polymerases involved in the translesion synthesis of platinum adducts in human cells in vivo are still unknown, many in vitro studies have started to gain some insights into the process. Vaisman et al. [120, 126] reported on the ability of yeast DNA polymerase ξ and human polymerases β, γ and η to pass through oxaliplatin-DNA better than through cisplatin adducts (fig. 5). Thus, the differential resistance to the two drugs observed in cell lines characterised by an enhanced translesion synthesis process [2, 90] may be explained by a selective action of certain DNA polymerases enzymes on the adducts. However, this specificity for the carrier ligand of platinum-DNA adducts is not universal since HIV-RT (a viral polymerase from the transcriptase family) does not appear to discriminate in vitro between cisplatin and oxaliplatin [120].

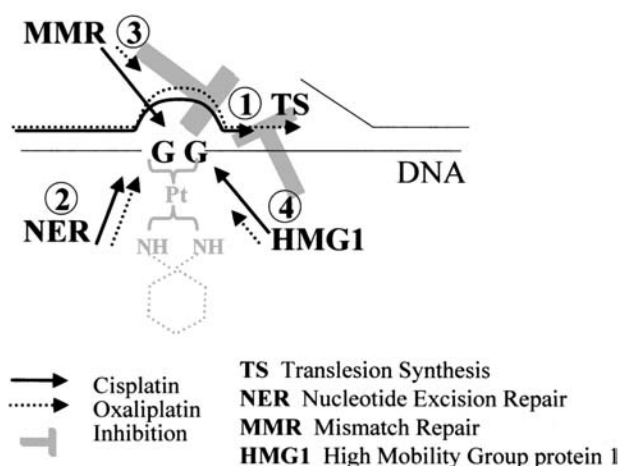

Figure 5. Molecular differences between oxaliplatin and cisplatin. Schematic comparative view of the major sites of interactions between cisplatin- and oxaliplatin-DNA adducts and damage recognition processes that are thought to contribute to discriminating between the two drugs. The arrows indicate the sites of interaction with oxaliplatin (dotted line) and cisplatin (continuous line). Arrows length is linearly correlated with the relative intensity of the interaction. ① Translesion synthesis (TS; error prone, potentially mutagenic). Its efficacy depends on the specificity of DNA polymerase, damage recognition proteins and/or MMR-mediated processes. TS may be inhibited by the selective recognition of platinated DNA by either MMR proteins or HMG-1 which will shield the adducts from the replication machinery. In MMR-proficient cells, TS is usually more efficient for DACH-Pt over cisplatin adducts, whereas in MMR-defective cells, it is enhanced for cisplatin and lower for oxaliplatin adducts. ② NER. This is the major repair process involved in the removal of platinated DNA. No differences have been observed in the repair processes of oxaliplatin and cisplatin adducts. ③ MMR (hMutLα and hMutSα) recognises cisplatin-DNA. Poor recognition has been shown for oxaliplatin-adducts. ④ Selective recognition of cisplatin over oxaliplatin adducts by HGM-1 proteins that may result in a specific block of TS and/or other repair pathways.

Vaisman et al. [126] found that polymerase η is more efficient than other polymerases (such as polymerase β) at catalysing in vitro translesion synthesis past platinum d-GG adducts although it shows a lower fidelity. These results suggest that in vivo replicative bypass of platinum adducts may be mutagenic by promoting misincorporation of nucleotides and that the grade of mutagenicity may increase when the enzyme involved in the process is human polymerase η rather than polymerase β. This mutagenic potential of the translesion synthesis process is due to the ability of certain polymerases to carry on an error-prone rather than error-free DNA replication with the insertion of incorrect nucleotides.

The specificity of the translesion synthesis process for the carrier ligand of the platinum-DNA adducts may also be due to differential binding of recognition proteins (e.g. HGM-1 and MMR) to the adducts in addition to being

due to the type of DNA polymerase involved in the process. Binding of recognition proteins to the platinum adducts may shield the lesions from the interaction with polymerases and repair machineries, impairing the translesion synthesis process. For example, the HMG-1 protein is able to block in vitro replicative bypass past cisplatin-DNA adducts [127]. Furthermore, the preferential binding of HGM-1 to cisplatin- over oxaliplatin- and JM216-DNA adducts has been proposed to affect the specificity of the translesion synthesis process through the platinum adducts. Thus, HGM-1 was able to block translesion synthesis past cisplatin with 2.5-fold higher efficiency than past JM216 or oxaliplatin [120]. Differences in the structural features of platinum-DNA adducts (e.g. minor-groove width) and enhanced bulk and/or rigidity of DACH and aminecyclohexylamine carriers with respect to the cis-ammine group of cisplatin (fig. 1) may explain the differential affinity of HMG-1 binding to platinated DNA [81, 128, 129]. However, due to the similar distribution of HGM-1 in cisplatin-sensitive and -resistant cell lines [130], Vaisman et al. [120] pointed out that HGM-1 is probably not involved in the increased replicative bypass observed in cisplatin-resistant cells. The authors leave open the question whether other recognition proteins expressed either in a tissue-specific manner [131] or at different levels in cisplatin-resistant or -sensitive cell lines [132, 133] may be able to discriminate between different platinum carrier ligands and to influence the ultimate cytotoxicity of the drugs [120].

Different models have been proposed in the attempt to understand the association of translesion synthesis and MMR processes. For example, one model indicates that continuous operation of futile cycles of replicative bypass and MMR-based removal of platinum-DNA adducts may produce lethal strand breaks (see above). Vaisman et al. [117] have shown that defects in the hMutSα/hMutLα system (involved in the recognition and removal of cisplatin but not oxaliplatin adducts) but not in hMSH3 allow completion of translesion synthesis resulting in increased tolerance to the toxic action of the drug.

Alternatively, the binding of MMR proteins to platinum adducts has been proposed to produce either a direct inhibition of the translesion synthesis process or its inhibition through a signalling cascade pathway [95, 117]. These models of the possible interactions between the two processes represent a further attempt to draw a more complete picture of the whole process.

The preference of MMR proteins for cisplatin over oxaliplatin adducts is in agreement with the finding that defective MMR results in enhanced replicative bypass of the former compared to the latter adducts [117]. These findings would suggest that tumours with defective MMR and enhanced replication bypass may be more sensitive to the action of oxaliplatin over cisplatin treatment.

Conclusions

Resistance to platinum compounds appears to represent the main source of discrimination between cisplatin and oxaliplatin. The bulky and hydrophobic DACH ligand of oxaliplatin plays an important role in producing molecular and cellular differences to cisplatin. The current hypothesis of a damage recognition system able to selectively recognise cisplatin over oxaliplatin DNA adducts is in agreement with the evidence that, unlike cisplatin, oxaliplatin-bound DNA is a poor substrate for MMR proteins and for those polymerases involved in the replication bypass of platinated DNA. Thus, an increase in translesion synthesis and/or decrease in MMR would lead to resistance to cisplatin but not oxaliplatin, suggesting great clinical potential for this drug. However, to date, the molecular bases of oxaliplatin toxicity and its ability to overcome resistance to cisplatin have not yet been fully elucidated. Overall, oxaliplatin represents an interesting and successful example of a mechanism-based drug (e.g. targeting DNA) rationally designed to overcome some of the limits of cisplatin treatment. Furthermore, accumulating evidence on the mechanism of action of oxaliplatin may allow us to gain more insights into the complicated processes of toxicity/tolerance induced by DNA-platinum adducts.

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