

## Review

# Cellular and Molecular Pharmacology of Oxaliplatin<sup>1</sup>

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### Abstract

**Oxaliplatin, a diaminocyclohexane-containing platinum, has a spectrum of activity and mechanisms of action and resistance that appear to be different from those of other platinum-containing compounds, notably cisplatin. The first part of this review describes the differences between oxaliplatin and cisplatin in terms of their spectrum of activity and adduct formation and then goes on to discuss molecular and cellular experimental data that potentially explain them. Particular emphasis is placed on the differential role of DNA repair mechanisms. In addition, the anticancer effects of oxaliplatin are optimized when it is administered in combination with other anticancer agents, such as 5-fluorouracil, gemcitabine, cisplatin, or carboplatin; topoisomerase I inhibitors; and taxanes. *In vitro* and preclinical combination data that could optimize oxaliplatin-based chemotherapy are also reviewed.**

### Introduction

Platinum-based drugs are among the most active anticancer agents and have been widely used in the treatment of a variety of human tumors. Over the last 30 years, a large number of platinum analogues has been synthesized to enlarge the spectrum of activity, overcome cellular resistance, and/or reduce the toxicity of both first (e.g., cisplatin) and second generation (e.g., carboplatin) platinum drugs (1). Of these platinum analogues, compounds containing a DACH<sup>3</sup> carrier ligand, such as oxaliplatin (Fig. 1; Refs. 2–4), have 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 (1, 5–7). The DACH-Pt complex of oxaliplatin can exist as three isomeric conformations that interact differently with DNA. Kidani *et al.* (8) showed that the *trans*-I(R,R) isomer of oxaliplatin was the most effective against cisplatin-sensitive and cisplatin-resistant cancer cell lines. Stability, formulation, solubility, and/or safety issues were more auspicious for oxaliplatin than for other DACH-Pt compounds initially selected for preclinical testing and evaluated in early clinical trials.

An example of primary resistance to first and second generation platinum compounds is found in colorectal cancers in which both cisplatin and carboplatin have been shown to be clinically inactive and have failed to increase time-related survival parameters, either as single agents or in combination with thymidylate synthase inhibitors (5-FU; Refs. 9–11). The primary resistance of most colon cancer cells to cisplatin and carboplatin remains poorly understood but is thought to be related to an intrinsically high expression level of different resistance mechanisms, including nonspecific inactivation and efflux at the cytoplasmic level, and specific DNA adduct repair mechanisms at the nuclear level. However, the activity of oxaliplatin against colorectal and other cancers has been recognized both *in vitro* and *in vivo*, as well as in clinical studies. Although differences between oxaliplatin and cisplatin in DNA binding, adduct formation, strand breaks, and apoptosis have been reported, the mechanisms behind the more potent cytotoxic activity of oxaliplatin compared with cisplatin against colon cancer cells have not yet been completely elucidated.

This review focuses on experimental pharmacology data that may shed some light on the differences in the antiproliferative effects of oxaliplatin and cisplatin. In the second part, combination experiments that might lead to optimal utilization of oxaliplatin in humans are considered.

### Oxaliplatin's Spectrum of Activity Differs from Other Platinum Compounds

Using the Drug Discovery program from the National Cancer Institute (COMPARE), DACH compounds, including oxaliplatin, were shown to have a markedly different spectrum of activity to cisplatin and carboplatin (12). Oxaliplatin has a cytotoxic effect in a broad range of cell lines, including colon, ovarian, and lung cancer, with IC<sub>50</sub> values ranging from 0.5 to 240 μM in colon (12, 13), 0.12 to 19.8 μM in ovarian (12, 14), and 2.6 to 6.1 μM in lung (15). In *in vivo* studies, oxaliplatin is active against breast, colon, and gastric cancer; renal cell carcinoma; and sarcoma (16). In addition, oxaliplatin has been tested *in vitro* and *in vivo* against cisplatin-resistant cell lines and tumor models, including human ovarian, lung, cervix, colon, and leukemia cell lines. Cisplatin resistance ranged from 8- to 80-fold in comparison with the parental lines. Direct comparison between oxaliplatin and cisplatin in

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<sup>3</sup> The abbreviations used are: DACH, diaminocyclohexane; oxaliplatin, [SP-4-2-(1R-*trans*)]-(1,2-cyclohexanediamine-*N,N'*)[ethane-diato(2)O, O']platinum; Pt, platinum; 5-FU, 5-fluorouracil; MMR, mismatch repair; JNK, c-Jun-NH<sub>2</sub>-terminal kinase; FA, folic acid.

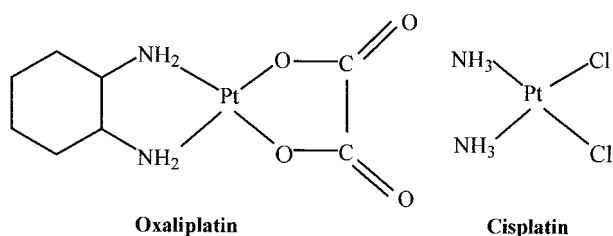


Fig. 1. Chemical structures of oxaliplatin and cisplatin.

cisplatin-resistant cells was not systematically performed in several studies. However, in cisplatin-resistant cell lines, the cross-resistance with oxaliplatin appeared to be of a lower magnitude, with  $IC_{50}$  values ranging from 0.19 to 14.3  $\mu M$  (Table 1). Studies indicate that oxaliplatin activity is maintained in cisplatin-resistant cell lines (12, 17, 18), as well as in a 5-FU- and a doxorubicin-resistant cell line (19).

The extent of *in vitro* activity of many anticancer drugs is often difficult to correlate with the objective response rate in the clinic. Human tumor models, such as cell lines, only partially reflect the complexity of human cancers and are usually artificially selected for their sensitivity to anticancer drugs. The attempt to correlate *in vitro* findings and tumor models to the clinical observations is further complicated by parameters, such as drug distribution in overall genomic instability of tumors in patients. Therefore, it is not surprising that the activity of cytotoxic drugs in models overestimates the drug's potential in the clinical setting. The development of the human tumor cloning assay was developed to reflect more realistically the cytotoxic effects of a broad variety of unselected human cancers. Interestingly, a study using the human clonogenic assay in cisplatin-resistant tumors showed that oxaliplatin concentrations  $>5 \mu g/ml$  have activity in several cisplatin-unresponsive tumors (16). As with other platinum complexes, *in vitro*, oxaliplatin cytotoxicity depends markedly on the duration of exposure, the drug being more effective with longer exposure, e.g., in the A2780 ovarian cell line, the cytotoxicity ( $IC_{50}$ ) of oxaliplatin in a 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide assay was 0.25 and 19.8  $\mu M$  when the drug was given as a 72- and 24-h exposure, respectively (13, 14).

### Oxaliplatin Forms Fewer DNA Adducts than Cisplatin

It is commonly accepted for all platinum drugs that DNA damage is largely responsible for their cytotoxic properties. According to this central paradigm, differences between oxaliplatin and cisplatin are likely to be principally derived from interactions at the DNA level. Given that oxaliplatin is at least equally as cytotoxic and frequently more cytotoxic than cisplatin, oxaliplatin-induced DNA damage should be more cytotoxic than cisplatin-induced damage. Surprisingly, oxaliplatin shows lower reactivity than cisplatin with naked DNA and with cellular DNA (20). Experiments have demonstrated that in some cell lines, levels of total Pt-DNA adducts, intrastrand cross-links, and DNA protein cross-links were sig-

nificantly lower for oxaliplatin than for cisplatin at equimolar and/or equicytotoxic concentrations (Table 2; Refs. 20–22).

Like cisplatin, oxaliplatin reacts with DNA, forming primary lesions that block DNA replication and transcription. Furthermore, oxaliplatin forms DACH-Pt DNA adducts with the same sites at the nucleotide level and with the same regional interactions as cisplatin, and both drugs show a preference for nuclear DNA over mitochondrial DNA (22). Lesions may be intrastrand, interstrand, or DNA protein cross-links. Intrastrand cross-links with adjacent guanines and occasionally adenines are the largely prevailing DNA lesions for both cisplatin and oxaliplatin (Table 2; Ref. 20). Both drugs form interstrand and DNA protein cross-links. Estimates of the frequencies of these lesions suggest that both interstrand and DNA protein cross-links constitute a minor fraction of total adducts for both cisplatin and oxaliplatin (21), although it is important to note that interstrand cross-links are difficult to detect with oxaliplatin adducts. In addition, the proportion of DNA single-strand breaks is remarkably higher with oxaliplatin compared with cisplatin. It is unknown whether the DNA strand breaks are induced as a result of primary DNA lesions or are attributable to rapid induction of apoptosis.

The lower level of adduct formation with oxaliplatin is unlikely to be attributable to differences in cellular uptake of the two drugs, because essentially the same differences in DNA lesion formation are seen when intact nuclei are incubated with oxaliplatin and cisplatin. Moreover, these differences are unlikely to be attributable solely to an effect of the DACH carrier ligand, because oxaliplatin also forms fewer adducts in cultured cells than equimolar concentrations of  $Pt(DACH)Cl_2$ , a DACH-Pt analogue of cisplatin (23). Thus, the lower levels of platinum adduct formation with oxaliplatin most likely reflect the slow dissociation rate of the oxalate ligand under physiological conditions (Fig. 2; Refs. 24 and 25). The intracellular mechanism for removal of the oxalate ligand is currently unknown but is unlikely to involve only the intermediate conversion of oxaliplatin to  $Pt(DACH)Cl_2$  (23).

Recent data suggest that despite quantitatively lower levels of oxaliplatin-DNA adducts compared with cisplatin, these DACH-Pt adducts may induce cell death more efficiently than cisplatin-DNA adducts in cultured cancer cells, e.g., in A2780 ovarian cells, both oxaliplatin and cisplatin induce early and persistent strand breaks with inhibition of DNA synthesis and induction of apoptosis (26). However, the amount of platinum bound to cellular DNA is significantly less for oxaliplatin compared with cisplatin at equitoxic concentrations, correlating with the extent of platinum-DNA adducts formed for these two agents (Table 2).

From a chemical standpoint, the differential resistance of cisplatin and oxaliplatin appears to be, at least in part, linked to the DACH carrier ligand, which is present in oxaliplatin and not in cisplatin. This DACH ligand may induce DNA lesions, which are poorly recognized by DNA recognition and repair pathways (27–29). Although the DACH-Pt-DNA adducts formed by oxaliplatin and the *cis*-diamine-Pt-DNA adducts formed by cisplatin are similar in structure (30), the bulky DACH moiety that protrudes into the minor groove appears to furnish DACH-containing adducts with different biological properties, notably that equivalent cytotoxicity is seen with

Table 1 Cytotoxicity of single agent oxaliplatin in cisplatin-resistant cell lines

Cell line		Extent of resistance	Origin	Assay	Duration of exposure	Oxaliplatin IC <sub>50</sub> (μM)	References
Parental	Resistant						
Cervix							
KB 3-1	KBCP	Cisplatin × 20	Human	MTT	96 h	2.27 ± 1.2	12
Ovarian							
A2780	A2780E	Cisplatin × 80	Human	MTT	96 h	4.7 ± 0.9	12
	A2780/CP	Cisplatin × 18	Human	MTT	72 h	0.33	13
	A2780/CP	Cisplatin × 20	Human	Cell count	72 h	25.4 ± 5.2	27
	A2780C13	Cisplatin × 13	Human	Cell count	48 h	13.5 ± 4.6	19
	A2780C10	Cisplatin × 8	Human	MTT	72 h	0.19 ± 0.09	100
	A2780C25	Cisplatin × 12	Human	MTT	72 h	2.2 ± 0.14	100
	A2780C70	Cisplatin × 32	Human	Cell count	72 h	6.7 ± 0.2	27
Lung							
PC-9	PC9/CDDP	Cisplatin × 11	Human	Cell count	72 h	13.3 ± 0.7	18
PC-14	PC14/CDDP	Cisplatin × 10	Human	Cell count	72 h	14.3 ± 2	18
Ovarian							
A2780	A2780/LOHP	Oxaliplatin × 4	Human	MTT	72 h	0.9	13
Leukemia							
L1210	L1210/CP	Cisplatin × 11	Murine	MTT	72 h	0.1	13
	L1210/DACH	Oxaliplatin	Murine	MTT	72 h	14	13
Colon							
HT29	HT29-5-FU	5-FU × 6	Human	Cell count	48 h	1.7 ± 0.8	19
Breast							
MCF-7	MCF-7/mdr	Doxorubicin × 2	Human	Cell count	48 h	12.2 ± 9.1	19

lower levels of DNA adducts induced by oxaliplatin compared with cisplatin.

### Why Does Cell Sensitivity to Oxaliplatin Differ from Cisplatin? The DNA Repair Hypothesis

Oxaliplatin-induced DNA damage appears to be more potent than that of cisplatin in eliciting cytotoxic effects (26). Resistance to platinum anticancer agents can result from decreased accumulation, increased inactivation by glutathione, or an increased ability of the cells to tolerate Pt-DNA adducts. Decreased accumulation does not explain the difference in sensitivity of cells to oxaliplatin as compared with cisplatin, because cisplatin-resistant cell lines with decreased accumulation of cisplatin also show decreased accumulation of carboplatin, ormaplatin, and oxaliplatin (17). Likewise, the level of resistance to cisplatin and oxaliplatin appears similar in a number of cell lines that are known to use glutathione as a mechanism of resistance to platinum compounds, while showing limited cross-resistance with compounds designed to circumvent such resistance, such as ZD0473 (31).

The ability of cells to repair platinum-induced DNA lesions is known to be an important factor in cisplatin cytotoxicity (32), and this section of the review focuses on the potential differences between cisplatin and oxaliplatin in this context (33, 34). By analogy to what is known concerning mechanisms of cisplatin resistance, we consider the following possible reasons for differences in cell sensitivity to oxaliplatin compared with cisplatin: (a) nucleotide excision repair; (b) MMR; (c) downstream responses that contribute to distinct mechanisms of cell death; (d) postreplicative mechanisms, such as inhibition of DNA chain elongation and replicative bypass; and (e) other targets.

**Nucleotide Excision Repair Mechanisms.** Interestingly, plasmid reactivation experiments have shown that oxaliplatin-induced DNA damage is no more difficult to repair than cisplatin-induced damage (30). Plasmid reactivation effects are likely to mainly reflect excision repair processes needed for the removal of both primary drug adducts and secondary DNA lesions. This is consistent with *in vitro* data showing that both cisplatin and oxaliplatin adducts are removed to a similar extent by the excision repair system mechanism (28). With the genome being exposed to a wide variety of DNA-damaging agents, nucleotide excision repair has an extremely broad specificity. Thus, it is not surprising that nucleotide excision repair does not appear to discriminate between oxaliplatin and cisplatin-DNA adducts (28).

**Role of MMR Mechanisms in Platinum Resistance.** Resistance to platinum compounds is affected by the presence and functionality of damage recognition proteins that bind to Pt-DNA adducts. Studies have shown that microsatellite instability is frequently involved as a mechanism of carcinogenesis and in resistance to anticancer drugs, especially in colorectal cancer (35). Inherited defects in the DNA MMR genes *MSH2* and *MLH1* are common in such cancers (36–40), and microsatellite instability is more often observed in advanced stages of colorectal cancer when methylation can lead to simultaneous silencing of multiple genes (41). In a study including 46 cases of sporadic colorectal cancers with microsatellite instability (42), silencing of the *MLH1* gene was common and was largely attributed to promoter hypermethylation. Functional loss of *MLH1* through promoter hypermethylation leading to microsatellite instability has also been reported in many other advanced solid tumors, such as breast, ovarian, lung, prostate, and endometrial cancers (43–47). This may be the consequence of tumoral progression

Table 2 Platinum-induced lesions

8-h drug exposure (25 $\mu$ M)	Monoadducts	Lesions (per Mbp)		DNA strand breaks
		Interstrand cross-links	Protein cross-links	
Oxaliplatin	118	0	2	44
Cisplatin	371	23	4	0

J. Woynaroski, unpublished data.

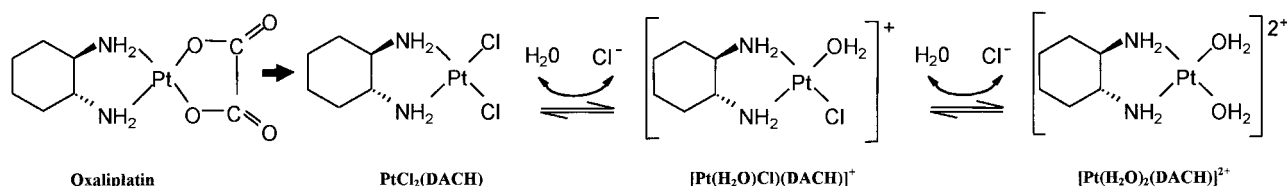


Fig. 2. Biotransformation pathway of oxaliplatin.

and/or cell population selection through cytotoxic treatment (48).

Experimental evidence has identified links between MMR deficiency and cytotoxic drug resistance for alkylating agents (49–51). Studies of DNA repair mechanisms after exposure to cisplatin showed that cisplatin adducts are recognized by the MMR complex (52–54). This presumably gives rise to futile rounds of DNA synthesis on single-strand breaks and could trigger apoptosis (27). Selection for cisplatin resistance in several human cancer cell lines gives cells with loss of expression of the MMR proteins hMLH1 and hMSH2 in most (90%), implicating the MMR system in cisplatin activity (50). Cisplatin-sensitive cell lines and human biopsies are hypermethylated in the promoter of only one *hMLH1* allele, whereas resistant cell lines all exhibit hypermethylation of the promoters of both *hMLH1* alleles (55, 56). Full methylation of all sites tested was invariably found to be associated with loss of hMLH1 expression, whereas a partial increase in methylation appears compatible with either loss or maintenance of expression. Treatment of resistant cell lines with 5-azacytidine, a known inhibitor of methylation, results in re-expression of hMLH1 and increased sensitivity to cisplatin (51). This suggests that methylation of the hMLH1 promoter may be a common mechanism for loss of hMLH1 expression, and possibly for cisplatin resistance, in some cancers at later stages.

Whereas MMR is clearly involved in cisplatin activity, *in vitro* and preclinical experiments have shown that MLH1-, MSH2-, and MSH6-deficient cells, which are resistant to cisplatin, are nonetheless susceptible to oxaliplatin (27, 57, 58) and that defects in MMR are associated with a modest to moderate level of resistance to cisplatin but not to oxaliplatin (54, 58, 59). The mechanism of action downstream of MMR leading to cisplatin cytotoxicity is not known with certainty; however, it has been proposed that the binding of the MMR complex to the Pt-DNA adducts may directly initiate a signal transduction pathway leading to cell cycle arrest and/or apoptosis (54, 58). Interestingly, the MMR protein hMutS $\alpha$  binds to cisplatin adducts but not to oxaliplatin adducts (54). Thus, although MMR is a crucial element in the repair of

cisplatin-induced DNA damage, this system does not appear to recognize DACH-Pt DNA adducts.

**Cellular Responses and Apoptotic Effects of Oxaliplatin and Cisplatin.** It has long been known that cisplatin activates known components of damage-response pathways, such as JNK and c-Abl kinases, but only in MMR-proficient cells (60). Consequently, cisplatin depends on an intact MMR system for its maximal cytotoxicity for signaling apoptosis via the JNK-mediated pathway. In contrast, oxaliplatin adducts are poorly recognized by the MMR protein complex (54) and do not activate JNK and c-Abl (60), thus providing oxaliplatin with a means to retain cytotoxicity in both MMR-proficient and -deficient cells (27, 58, 60). Thus, differences in the ability of oxaliplatin and cisplatin adducts to activate signal transduction pathways ultimately leading to apoptotic DNA fragmentation (26) are likely to contribute to differences in their ability to induce cellular death.

**Postreplicative Bypass Mechanisms.** Postreplicative bypass discriminates between cisplatin and oxaliplatin adducts. The mechanism by which replicative bypass discriminates between cisplatin and oxaliplatin adducts is likely to be complex. Human pol  $\beta$ , yeast pol  $\zeta$ , human pol  $\gamma$  (29), and human pol  $\eta$  have all been shown to replicate past oxaliplatin-GG adducts more efficiently than cisplatin-GG adducts (45). Of these polymerases, pol  $\beta$ , pol  $\zeta$ , and/or pol  $\eta$  could, in theory, participate in the replicative bypass of Pt-DNA adducts *in vivo*. However, the specificity of these translesion polymerases for bypass of cisplatin and oxaliplatin adducts is different to the specificity of replicative bypass that is seen in cisplatin-resistant cell lines. Thus, whereas the level of expression of translesion polymerases, such as pol  $\beta$ , pol  $\zeta$ , or pol  $\eta$ , may determine the extent of replicative bypass, the specificity of replicative bypass is likely to be influenced by other factors, e.g., the increased replicative bypass of cisplatin adducts, but not of oxaliplatin adducts seen in cell lines with MMR defects (27), is thought to be attributable to “futile cycles” of translesion synthesis followed by MMR removal of the newly synthesized strand. This would result in the presence of persistent gaps in the DNA that would directly or indirectly lead to cell death. Thus, loss of MMR

activity leads to increased net replicative bypass of those adducts that are recognized by the MMR complex. Because cisplatin adducts are preferentially recognized by the MMR complex, loss of MMR leads to preferential bypass of cisplatin adducts. Alternatively, the MMR complex may prevent net replicative bypass of the adducts, thus leading to the presence of persistent gaps in the DNA that serve as signals for apoptosis. Blocking replicative bypass in MMR-deficient cells increases their sensitivity to cisplatin (61). It has also been suggested that MMR proteins modulate the levels of recombination-dependent replicative bypass and, as such, can modulate cisplatin activity (62). Finally, Pt-DNA damage recognition proteins have also been shown to block translesion synthesis past Pt-DNA adducts (29). Thus, those Pt-DNA damage recognition proteins, which bind to cisplatin and oxaliplatin DNA adducts with different efficiencies, could also impart specificity to the process of replicative bypass (29).

**Other Potential DNA Damage Targets.** Over 25 cellular proteins that bind to Pt-DNA adducts have been described. All of these Pt-DNA damage recognition proteins are proteins that bind to bendable DNA and normally play important roles in chromatin structure, transcription, repair, recombination, and/or damage recognition. The role of these proteins in determining the response to platinum anticancer agents has not been determined, but they have been proposed to block nucleotide excision repair of the adducts, block replicative bypass of the adducts (29), hijack essential transcription factors, and/or act as “damage sensors” that initiate signal transduction pathways leading to cell cycle arrest or apoptosis. It has already been shown that Pt-DNA damage recognition proteins bind with significantly different affinities to cisplatin and oxaliplatin adducts (29). Additional studies need to be carried out to evaluate the potential roles that these proteins may play in determining the differential activity of oxaliplatin and other platinum-containing agents.

**Role of Platinum-binding Targets Other than DNA.** One aspect of platinum drugs, which has been very little studied, pertains to the possible contribution of targets other than DNA to the cytotoxic effects of such drugs. Interestingly, only 5–10% of covalently bound cell-associated cisplatin is found in the DNA fraction, whereas 75–85% of the drug binds to proteins (63, 64). The binding of platinum drugs to cellular proteins via sulfur atoms in the cysteine and/or methionine residues may affect the activity of enzymes, receptors, and other proteins. The resulting functional protein damage is likely to contribute to apoptosis induction (65) and may differ between platinum drugs. *Ex vivo* and *in vivo* pharmacokinetics in A2780 cells show that the rate of protein binding is high for oxaliplatin (13). It has been postulated that the hydrophobic DACH moiety in oxaliplatin may direct drug reactivity toward cellular proteins with sulfhydryl groups in hydrophobic pockets that may be poorly reactive with cisplatin, which is polarized (21). Thus, protein binding might also be a factor contributing to apoptosis in oxaliplatin-treated cells that is disproportionately profound compared with the modest levels of DNA lesions (26, 66).

### Optimization of Oxaliplatin Activity Based on Combination Therapy

Despite the advantages of oxaliplatin’s cellular resistance, its clinical activity is somewhat limited. To improve its efficacy, oxaliplatin is frequently used in combination regimens. The pharmacokinetics/pharmacodynamics of oxaliplatin along with its excellent safety profile (67), with little or no hematological toxicity at recommended doses and relatively late cumulative (but usually reversible) neurotoxicity, make it amenable for association with other anticancer agents (68). Thus, the potential optimal clinical efficacy of oxaliplatin relies on its ability to be safely and synergistically combined with a variety of other cytotoxic drugs. Over past years, oxaliplatin combinations have been explored preclinically and clinically, mainly with thymidylate synthase inhibitors and other platinum compounds, such as cisplatin or carboplatin, but also with other agents, such as topoisomerase I inhibitors and taxanes. The preclinical data on its association with other agents are reviewed below. Most of the preclinical associations studied have already been explored and/or confirmed clinically (68, 69).

**Thymidylate Synthase Inhibitors.** Combination of oxaliplatin with a variety of thymidylate synthase inhibitors, including 5-FU, AG337, and UFT, *in vitro* and/or *in vivo* shows additive or synergistic effects. *In vitro*, simultaneous administration of oxaliplatin and 5-FU yielded cytotoxic synergy in colon, breast, and ovarian cancer cells (19). 5-FU potentiated oxaliplatin antitumor activity against HT29 cell lines and xenografts *in vitro* and *in vivo* (19). The *in vivo* effect was seen both in parental and 5-FU-resistant HT29 cells. Given that thymidylate synthase overexpression and/or MMR deficiencies have been correlated with 5-FU resistance (70, 71), it is interesting to speculate on the mechanistic basis for the synergy. The demonstration that sequential administration of oxaliplatin followed by 5-FU results in a significant decrease in thymidylate synthase gene expression (72) opens up the possibility of reacquired 5-FU sensitivity. Although experiments in colon cancer cells showed synergy and no sequence dependency with these two agents, cytotoxicity was significantly different depending on the type of 5-FU exposure (short > mixed > continuous exposure). FA significantly increased the cytotoxicity of the 5-FU/oxaliplatin combination regimen *in vitro* (73, 74). In a mouse leukemia model, oxaliplatin was shown to have synergistic activity when combined with 5-FU and cyclophosphamide (75).

Likewise, combination of oxaliplatin with AG337 displayed synergistic effects both *in vitro* in the 2008 ovarian cell line and *in vivo* against GR murine mammary tumors (19, 76). *In vivo*, oral UFT/FA/oxaliplatin treatment had a higher antitumor activity compared with oxaliplatin or UFT/FA alone (77). An additive effect has been demonstrated in an *in vitro* and *in vivo* study of oxaliplatin and raltitrexed in human colon cell lines and a murine tumor model (78).

**Gemcitabine.** The recently introduced antimetabolite gemcitabine displayed synergistic effects with oxaliplatin in two different colon cancer cell lines (HCT116 and Colo 320 DM). The cytotoxic effect was sequence dependent, gemcitabine followed by oxaliplatin being more cytotoxic than the reverse sequence (79). In the same experimental setting,

gemcitabine followed by oxaliplatin was more effective than gemcitabine followed by cisplatin, in both MMR-deficient and -proficient cells. The molecular mechanisms that explain the synergy between oxaliplatin/cisplatin and gemcitabine are not perfectly understood. Loss of MMR rendered cisplatin-treated cells unable to sense the presence of cisplatin-DNA adducts yet capable of generating clones resistant to gemcitabine (80). Interestingly, loss of MMR is associated with resistance to gemcitabine only in p53-deficient cells. Although no experimental evidence has yet been provided, it is tempting to hypothesize that unlike cisplatin-DNA adducts, oxaliplatin-DNA adducts are unable to generate resistance to gemcitabine.

**Other Platinum Compounds.** On the basis of the National Cancer Institute COMPARE program results, classical platinum compounds, including cisplatin and carboplatin, were combined with DACH-Pt compounds, and synergy was observed both *in vitro* and *in vivo* in cisplatin-sensitive and -resistant cell lines (12). *In vitro*, the combination of oxaliplatin and cisplatin showed at least additive and possibly synergistic effects (12). *In vivo*, simultaneous injection of oxaliplatin and carboplatin resulted in synergistic antitumor activity against cisplatin-resistant murine leukemia L1210, with 70% of animals cured (81, 82).

**Topoisomerase I Inhibitors.** Oxaliplatin has been combined with SN38, the active metabolite of irinotecan (CPT11), *in vitro* and with irinotecan itself *in vivo*. *In vitro*, SN38 showed synergistic effects when combined with oxaliplatin in the HT29 colon cancer cell line. The cytotoxicity of this combination was sequence dependent, oxaliplatin followed by SN38 being more cytotoxic than either the reverse sequence or simultaneous administration. The supra-additive toxicity observed with oxaliplatin and SN38 was associated with evidence of reciprocal interactions: prior exposure to oxaliplatin enhanced the toxic effects of SN38, with a more pronounced S phase block. A high rate of DNA fragmentation was detectable in cells at 48 h, confirming that S phase-arrested cells were undergoing apoptosis. DNA and RNA synthesis inhibition after topoisomerase I-mediated DNA damage may also slow the reversion of oxaliplatin-induced interstrand cross-links (83, 84). The oxaliplatin/CPT11 combination was also active *in vivo* against the GR1 mouse mammary tumor (84). However, the activity of this combination was not superior to the activity of oxaliplatin alone in a model of osteogenic tumor (85, 86).

**Taxanes.** Very little *in vitro* or molecular pharmacological data using oxaliplatin/taxane combinations are currently available. Data have been published showing that when oxaliplatin is combined with paclitaxel in the MV522 lung cancer model, at least additive efficacy is induced (87). Addition of the chemosensitizing agent tirapazamine to the oxaliplatin/paclitaxel combination produced an additive effect and was well tolerated in the same xenograft model (88). Recently exciting results showed clinical activity with the oxaliplatin/paclitaxel combination in platinum-pretreated ovarian cancer patients (89). Molecular mechanism(s) that could explain the synergism between oxaliplatin and paclitaxel in the clinic deserves additional research.

## Conclusion

Laboratory data typically indicate that oxaliplatin is at least as potent as cisplatin in cancer cells that are sensitive to platinum agents. Furthermore, it is able to retain activity in a variety of cancer cells that are either primary or secondary cisplatin resistant, an activity which is best exemplified for primary resistance by clinical trials in colorectal cancer patients. Research to date shows that these differences can, at least in part, be attributed to MMR, replicative bypass, downstream transcription pathways, and Pt-DNA damage recognition proteins, all of which have a role in discrimination between cisplatin and oxaliplatin DNA adducts. In addition, the extent and specificity of replicative bypass is likely to be determined by translesion DNA polymerase(s), MMR activity, and Pt-DNA damage recognition proteins. Research in coming years should focus on evaluating the relative importance of these proteins in determining the overall cellular response to cisplatin and oxaliplatin. Hopefully, this information can be used to identify molecular markers that predict the relative efficacy of cisplatin and oxaliplatin chemotherapy (16).

Preclinical studies showing marked synergistic effects with most of the commercially available thymidylate synthase and topoisomerase I inhibitors encourage clinical oxaliplatin-based combination chemotherapy. To date, preclinical studies showing the synergy of oxaliplatin/5-FU have been confirmed in Phase III clinical trials (90, 91). On the basis of the preclinical studies described above, clinical trials investigating the effects of oxaliplatin with raltitrexed (92), irinotecan (93–96), topotecan (97), and taxanes (89, 98, 99) have been completed, and many more are ongoing.

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