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Resistance to cisplatin and analogues: mechanisms and potential clinical implications

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Summary. In view of the important role of cisplatin (CDDP) in cancer chemotherapy, the frequent occurrence of resistance to the drug is a major clinical problem. The main cause for unresponsiveness of a tumor to CDDP is thought to be cellular drug resistance, which may be caused by (1) a decreased uptake of CDDP, (2) an increase in metallothioneins, (3) an increase in glutathione and/or glutathione-S-transferase, (4) increased DNA repair, or (5) increased tolerance to unrepaired lesions in DNA. Several mechanisms may be concomitantly operative. However, almost all data on CDDP resistance are derived from cell lines or experimental animal systems, and it is uncertain whether they are relevant for human tumors. Possible methods for overcoming CDDP resistance in cancer patients include the use of high-dose CDDP or carboplatin or of different formulations of platinum derivatives, the regional administration of CDDP, the inducement of hyperthermia, the depletion of glutathione by buthionine-S-R-sulfoximine (BSO), or the use of platinum analogues. The development of methods to detect and classify CDDP resistance in human tumor samples is urgently required for the development of modalities to overcome resistance.

Introduction

The discovery of cisplatin [*cis*-diamminedichloroplatinum(II), CDDP] (Fig. 1) marked a major advance in the chemotherapy of solid tumors, such as testicular and ovarian cancer, as well as of bladder, head and neck, and cervical cancer [54]. Unfortunately, unresponsiveness to this agent occurs even in the more sensitive tumor types (primary resistance); an initial response to a CDDP-containing regimen is often followed by the development of secondary (acquired) resistance.

A variety of strategies for overcoming CDDP resistance has been proposed, and some of these have met with moderate clinical success. The latter include: (1) regional administration, such as by the i.p. route in ovarian cancer patients with minimal residual disease [89]; (2) switching to CDDP analogues to which the tumor is not (or only partially) cross-resistant [13]; and (3) the use of high doses of CDDP [63] or carboplatin [*cis*-diamminecyclobutane-1,1-dicarboxylatoplatinum(II), JM8, CBDCA] (Fig. 1) [64].

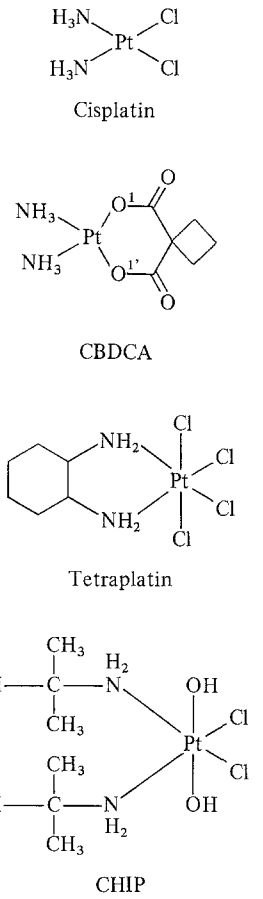

Fig. 1. The molecular structures of cisplatin and some platinum analogues

Clinical drug resistance may be a result of pharmacokinetic factors leading to inadequate drug delivery to the tumor cells [85]. Even after optimization of the dose, route of administration, and treatment schedule, the drug may not reach the tumor cells in adequate concentrations due to impaired blood circulation [56] or localization of the target cells in "sanctuaries," e.g., the central nervous system or the testes [33]. A tumor may be insensitive because of cell kinetic factors, e.g., the presence of a large fraction of growth-arrested cells [20]. The major cause for unre-

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sponsiveness of a tumor, however, is thought to be cellular drug resistance.

Cellular drug resistance may arise by a variety of mechanisms that can usually be classified in one of the following categories [18]: (a) decreased transport of the drug into the cell, (b) defective metabolism of the drug to its active species, (c) altered intracellular nucleotide pools (mainly pertinent in the case of antimetabolites), (d) increased drug inactivation, (e) enhanced cellular repair processes, and (f) altered target molecules. At times, these alterations may be associated with distinct genetic abnormalities, such as spontaneous or drug-induced mutations, or gene amplifications [41]. Obviously, acquired resistance may be a result of selection for intrinsically resistant tumor subclones, a result of de novo changes, or both. It appears likely that primary and acquired drug resistance may share at least some of their mechanisms and that several mechanisms may be concomitantly operative. Because of this complexity, the development of rational strategies for preventing or overcoming resistance to an agent is seriously hampered by the unavailability of methods to establish which type of resistance is operative in the malignant cells of individual cancer patients.

Only recently has the spectrum of mechanisms by which cells in culture may become resistant to cisplatin and its analogues begun to unfold. No all of these may be relevant in the clinical setting, and some mechanisms may occur more frequently than others. This paper gives an overview of the pertinent findings and discusses some potential clinical implications.

Decreased uptake of CDDP

Little is known about the transport of CDDP across the cell membrane. The agent passes the cell membrane by passive diffusion [34], or it may require an active amino acid transport system [14, 16]. The latter concept is supported by the finding that the uptake of CDDP can be completely inhibited by concomitant incubation with certain amino acids; conversely, CDDP inhibits the membrane transport of neutral amino acids, which make use of a common transport system [80]. In addition, proliferating cells are known to be more sensitive to water-soluble, carrier-dependent alkylating agents (including CDDP) than resting cells, presumably because of increased activity of the transport system [15].

The decreased uptake of CDDP has been described in CDDP-resistant Chinese hamster cells [92], murine L1210 cells [11, 44, 45, 51, 75, 93], human head and neck squamous cell carcinoma [88], and human prostate tumor [57] and ovarian cancer [2, 3, 52] cell lines; in other resistant cell lines, however, CDDP uptake is comparable to that in sensitive cells. The decreased uptake of CDDP appears to be a phenomenon occurring at low levels of resistance that does not become progressively pronounced as cells attain higher levels of resistance [75]. Sensitivity to CDDP has been shown to correlate with intracellular platinum content in HeLa, Chinese hamster, and HaK cells [24]. Interestingly, hyperthermia potentiates CDDP cytotoxicity in Chinese hamster ovary cells, particularly in a transport-defective CDDP-resistant line, by enhancing the cellular accumulation of CDDP [92].

In human chronic myelogenous leukemia K562 cells, changes in plasma mebrane transport systems for neutral amino acids closely parallel the development of resistance to CDDP [82]; CDDP-resistant murine L1210 cells also show distinct changes in amino acid transport [36]. These findings suggest that resistance to CDDP may be caused in part by its decreased uptake, which may result from a decrease in the number and/or affinity of membrane receptors involved in amino acid transport. The corresponding structural and biochemical changes in the cell membrane remain to be characterized [4].

Increased efflux, as implicated in multiple drug resistance, has not been shown to play a role in resistance to CDDP [66].

Increased metallothioneins

Metallothioneins (MT) are intracellular proteins rich in sulfhydryl groups, which are thought to play a role in the metabolism and storage of zinc and other heavy metals [94]. The inducement of MT synthesis probably constitutes a protective mechanism against toxic metals such as zinc, copper, or cadmium. Exposure of cell lines to cadmium (or zinc) chloride may result in cadmium resistance, mediated by increased intracellular MT levels; amplification of the metallothionein-I gene has been reported in a cadmium-resistant Friend leukemia cell line [8].

Mouse fibroblast and human epithelial cell lines made resistant to cadmium and containing high levels of MT are cross-resistant to CDDP [7]. Of the CDDP in the cytosol, 70% is bound to the MT fraction in these cells. In Ehrlich ascites tumor cells, which naturally contain a high level of MT, CDDP displaces about 40% of the normal amount of zinc from MT [50], indicating the high specificity of CDDP binding to MT. Cross-resistance to CDDP has also been reported in human ovarian carcinoma [5, 61] and head and neck squamous cell carcinoma [47] cell lines, all of which were selected for resistance to cadmium. Increased metallothionein levels were also found in CDDP-resistant human head and neck squamous cell carcinoma [88] and prostate tumor [57] cell lines that had not been exposed to cadmium. Subcutaneous tumors in nude mice, induced by the injection of cells from an MT-rich variant of a mouse fibroblast line, are resistant to CDDP, as opposed to tumors induced by the injection of cells from the parent cell line, which contains only trace amounts of MT [25]. These findings provide evidence of the role for the MT content of tumors in vivo in CDDP resistance. On the other hand, exposure to CDDP in vitro does not induce MT synthesis [5, 22, 61, 99], suggesting that a role for MT in acquired resistance may be unlikely.

Increased glutathione and/or glutathione-S-transferase

Glutathione (GSH) is a predominantly intracellular nonprotein sulfhydryl compound that plays a part in detoxification and in the repair of cellular injury [6]. Glutathione reductase (GR) is an NADPH-dependent enzyme that helps to maintain cellular levels of GSH; glutathione-S-transferase (GST) catalyzes the conjugation of electrophilic groups with GSH, leading to the formation of thio ethers. Intracellular GSH levels can be reduced by treatment with buthionine-S-R-sulfoximine (BSO), an inhibitor of GSH synthesis, and can be increased by exposure to 2-oxothiazolidine-4-carboxylate (OTZ).

The detailed mechanism by which GSH decreases CDDP cytotoxicity has not been established. The follow-

ing possibilities have been proposed [12]: (a) alteration of CDDP membrane transport, (b) drug inactivation by the formation of an inactive GSH-CDDP complex in the cytosol, (c) decreased binding of CDDP to DNA, and (d) increased repair of DNA lesions.

Increased levels of GSH have been demonstrated in CDDP-resistant murine L1210 leukemia [44, 75], human ovarian carcinoma [3, 12, 38], human bladder carcinoma [9, 10], and human small-cell lung cancer [43] cell lines. In some cell lines, GR and GST were elevated [9, 43, 88]. After the depletion of GSH with BSO, sensitivity to CDDP was increased in some normally sensitive [38] and some resistant [3, 38, 44] cell lines. In other cell lines, however, resistance was not reversible after treatment with BSO [1, 42, 75, 88]. A decrease in cross-resistance to CBDCA after treatment with BSO has also been described in a human ovarian carcinoma cell line [39]. It is conceivable that BSO only potentiates CDDP cytotoxicity after the sustained and severe depletion of GSH; differences in the attained GSH level may be responsible for the observed differences in the response to treatment with BSO.

The oral administration of BSO in nude mice with a transplanted intraabdominal human ovarian cancer resulted in decreased levels of GSH in the tumor and increased CDDP toxicity [39]. Pretreatment with OTZ induced an elevation in GSH levels and protection against CDDP cytotoxicity in a human lung fibroblast cell line, whereas no changes were observed in a human lung adenocarcinoma cell line (A549) that naturally contains a high level of GSH [78]. Potential clinical significance is suggested by the findings in two ovarian adenocarcinoma cell lines that were derived from ascites of the same patient, one prior to the clinical response to a CDDP-containing regimen and the other after relapse [96]. The second (clinically resistant) cell line demonstrated CDDP resistance in the clonogenic assay and contained higher levels of GSH and GST than the first.

Most investigators now agree on the importance of the glutathione system in CDDP resistance. However, there is no correlation between the GSH level and the degree of resistance to CDDP. Moreover, it appears that the severe depletion of GSH by BSO is required in many model systems to reverse CDDP resistance. To assume a direct correlation between glutathione levels and CDDP resistance may therefore be an oversimplification, and it may well be that the intracellular activity of glutathione-S-transferase, a glutathione-dependent enzyme, is the more important determinant.

Decreased DNA binding and increased DNA repair

Most evidence suggests that the cytotoxicity of platinum compounds is a result of their interactions with nucleic acids, leading to the inhibition of DNA replication [76]. After crossing the cell membrane and undergoing aquation reactions, cisplatin is monofunctionally bound to a guanine base on a DNA strand at the N7 position [74]; since CDDP contains two reactive sites, a second reaction takes place, resulting in the formation of bifunctional Pt-DNA adducts. The major adducts are intrastrand (within one DNA strand) cross-links formed by the binding of CDDP to two neighboring guanines or, less frequently, to adenine and guanine or to two guanines separated by one or more nucleobases. Other types of adducts are interstrand cross-links of two guanines on complementary DNA strands, and DNA protein cross-links. Interstrand cross-links can be demonstrated by the alkaline elution assay [49] or the ethidium bromide assay [19], intrastrand cross-links by ELISA methods, using antibodies against CDDP-DNA adducts [68, 70].

There is no agreement as to which lesion is predominantly responsible for the antitumor effets of CDDP. Some investigators favor the interstrand cross-link [69, 100], others consider the intrastrand cross-link to be the more likely candidate [31, 67, 86]. There is a correlation between the extent of interstrand cross-link formation and CDDP cytotoxicity [26, 53, 100, 101]. A decreased formation of interstrand cross-links has been described in CDDP-resistant fibroblast [26], murine L1210 [101], human small-cell lung cancer [43], human head and neck squamous cell carcinoma [88], and human bladder carcinoma [9] cell lines. The possibility has been suggested that in resistant cells monofunctional platinum-DNA adducts are inactivated (e.g., by GSH [12, 26, 59]) and/or removed before the formation of interstrand cross-links takes place [53, 58, 59]. Obviously, these correlations do not necessarily imply a causal relationship. If the relative proportion of the different Pt-DNA adducts remains the same in sensitive and resistant cell lines, a decreased formation of interstrand cross-links automatically implies an equally decreased formation of other types of adducts. Since interstrand cross-links comprise less than 1% of all cross-links, the intrastrand variety may be even more important.

The measurement of intrastrand cross-links has only recently become possible and has therefore been less extensively studied. A correlation between the formation of intrastrand cross-links and CDDP cytotoxicity has been shown in a human ovarian carcinoma cell line [72]: the formation of intrastrand cross-links in the CDDP-resistant subline was clearly decreased. There are large interindividual variations in the formation of intrastrand cross-links in the leukocytes of patients treated with CDDP [28, 29, 73]. In a series of 33 patients, correlations were found between the formation of intrastrand cross-links in nucleated white blood cells and the response to chemotherapy [73].

Decreased adduct formation in CDDP-resistant cells may in turn be a result of several factors such as (1) a decreased concentration of CDDP in the nucleus caused by a decreased in its transport across the cell membrane or by its inactivation in the cytosol; (2) changes in DNA or chromatin, which decrease the binding of Pt to DNA; and (3) the increased repair of DNA lesions. Changes in DNA or chromatin in CDDP-resistant cells have never been demonstrated but cannot be ruled out as potential mechanisms of resistance. The possibility of increased repair has been studied extensively. Xeroderma pigmentosum cell lines, which are defective in DNA repair, are extremely sensitive to CDDP [69]. An increase in unscheduled DNA synthesis (possibly reflecting increased repair) has been demonstrated in CDDP-resistant human ovarian carcinoma cell lines [12, 55]. The inhibition of DNA repair with aphidicolin (an inhibitor of DNA polymerase alpha) partially restores CDDP sensitivity in a CDDP-resistant human ovarian carcinoma cell line [40]. An enhanced DNA repair capacity has been suggested in CDDP-resistant L1210 cells in an elegant host-reactivation assay of DNA repair using a CDDP-treated plasmid vector [81].

The hypothesis that the increased repair of monofunctional adducts may be important [53, 58, 59] was men-

tioned above. However, the ability of some cell lines to repair DNA containing O6-alkylated guanine (Mer phenotype) does not protect against the cytotoxicity of CDDP [53]. This mechanism may be of importance in the repair of monofunctional adducts [32].

The rate of removal of cross-links may reflect the efficacy of the repair systems. In two studies, no differences could be found in the removal of total DNA adducts, DNA protein cross-links, or DNA interstrand cross-links between CDDP-hypersensitive and normally sensitive Walker rat carcinoma cells [71] and CDDP-sensitive and -resistant L1210 cells [86]. However, the methods used in these experiments measured only the first step in DNA repair and therefore gave no indication as to whether the remaining steps in repair were carried out with equal efficiency [69, 76]. Few data have been published on the removal of intrastrand cross-links. Generally, more intrastrand cross-links remain in sensitive than in resistant cell lines 16–24 h after treatment with CDDP [30].

In view of these data, it is likely that increased DNA repair is a mechanism of CDDP resistance. It is reasonable to assume that persistent Pt-DNA adducts have major effects on DNA replication. Studies of DNA repair in CDDP-treated cells are hampered (a) by the fact that it is still unclear as to which adduct is responsible for the antitumor effect of CDDP, (b) by the lack of knowledge about repair mechanisms in human tumors, and (c) by the lack of sensitive methods for measuring the different steps of repair.

The inducement of sister chromatid exchanges (SCE) is generally thought to reflect some type of DNA damage [98]. Since DNA is the primary target of CDDP, the induction of SCE in CDDP-treated cells may reflect cellular drug sensitivity. A correlation between the formation of SCE and CDDP cytotoxicity has been found in human primary tumor cell cultures [90]. The SCE assay has the potential to identify heterogeneity in drug response within a tumor.

Increased tolerance to unrepaired lesions in DNA

This is largely a theoretical possibility. Support of this hypothesis may lie in the fact that at equitoxic doses of CDDP, resistant L1210 cells have up to 15-fold higher levels of interstrand cross-links than sensitive cells [86]. This mechanism may also be present in a CDDP-resistant bladder carcinoma cell line [10]. Mechanisms by which cells could become tolerant include alterations in DNA replication and postreplication repair [23].

Cross-resistance to platinum-analogues (see Fig. 1)

In almost all cell lines investigated, there is partial or complete cross-resistance to the most frequently used platinum analogues carboplatin (CBDCA) and *cis*-dichloro-*bis*-isopropylaminetranshydroxyplatinum(IV) (CHIP) [10, 12, 42, 46, 51, 52, 76, 87, 88]. Interestingly, there is a nearly complete lack of cross-resistance between CDDP and 1,2-diamminecyclohexaneplatinum(II) (DACH) derivatives, e.g., tetraplatin [12, 23, 46, 51, 77, 93, 95].

CBDCA has been the most extensively investigated analogue in both the experimental and clinical settings. Because CDDP and CBDCA differ only in the leaving ligand, the bifunctional adducts formed with DNA are assumed to be chemically identical with respect to the platinum moiety. Thus, differences between the two drugs are probably not due to their effect at the DNA level but may reflect differences in pharmacokinetics, particularly in the rate of hydrolysis reaction [48, 60]. The overall antitumor activities of CDDP and CBDCA are similar [91]. There have been reports of patients resistant to CDDP who responded to CBDCA [17, 27, 35], but this situation appears to be the exception rather than the rule. The major clinical advantage of CBDCA over CDDP is its different toxicity pattern, not its potential to overcome CDDP resistance. The use of other platinum compounds (notably the DACH-Pt derivatives) for this purpose remains to be investigated.

Potential clinical implications

Although the detailed biochemical mechanisms of cisplatin resistance are still elusive, general patterns are beginning to emerge that may help in the design of rational strategies for overcoming resistance in the clinic. The decreased transport of CDDP across the cell membrane, enhanced inactivation by intracellular detoxification systems, and increased DNA repair appear to be the most prominent mechanisms. It is very likely that there is variation in sensitivity to CDDP due to tumor cell heterogeneity, as has been observed in clonal human glioma cell lines isolated from a single nontreated tumor [97]. The selection of a CDDP-resistant subclone has been suggested in a cytogenetic study of two ovarian carcinoma cell lines derived from the same patient, one prior to therapy and the other after the development of clinical resistance to CDDP [96]. Of particular importance is the difficulty, as with other alkylating agents, involved in reaching high levels of CDDP resistance in vitro [32]. This resistance "ceiling" at relatively low drug levels contrasts with that of other agents, such as methotrexate or doxorubicin, where very high degrees of resistance can be induced with relative ease. This may explain the steep dose-response relationship in ovarian carcinoma cell lines [62] and provides the rationale for the use of high-dose CDDP [63] or CBDCA [64].

Obviously, nearly all data on CDDP resistance are derived from in vitro models or experimental animal systems, and it is far from clear as to which of the mechanisms of resistance in cell lines (if any) are prevalent in human tumors. Unfortunately, experimental resistance may be quite different from clinical resistance. Much higher degrees of resistance can be induced in vitro than in vivo. Pharmacokinetic factors have not been taken into account in most experimental work but may compound CDDP resistance in vivo. Most of the work on clinical CDDP resistance has been done in patients with ovarian and testicular cancer. Response prediction has been attempted using clonogenic assays [37], tritium-thymidine incorporation assays [79, 83], or the extent of intrastrand cross-link formation [73]. No studies focusing on specific mechanisms of CDDP resistance in patient samples have been published.

Insight into some of the mechanisms of resistance mentioned above can possibly be exploited in the clinic. The use of high-dose CDDP or CBDCA has been shown to be effective in some cases of CDDP resistance. It is tempting to speculate that this strategy may overcome the defective transport of CDDP or CBDCA across the cell membrane, but the simple overwhelming of detoxification or repair systems may provide an alternative explanation. Very high local concentrations of CDDP, with similar effects, can al-

so be reached by i.p. administration in ovarian cancer patients with minimal residual disease limited to the peritoneal cavity. A third approach to overcome transport defects could be the use of liposome-trapped lipophilic platinum derivatives [21]. Hyperthermia might increase CDDP cytotoxicity by augmenting its uptake in both sensitive and resistant tumors, particularly in localized disease (e.g., head and neck cancer).

Attempts to modulate clinically CDDP cytotoxicity focus on the intracellular detoxifying systems. If BSO proves to increase CDDP or CBDCA cytotoxicity to tumors without increasing adverse effects in other tissues (perhaps by local administration), it may be a worthwhile strategy for overcoming CDDP resistance. Preclinical toxicologic and pharmacokinetic studies are now in progress [65, 84].

To our knowledge, strategies for overcoming resistance associated with the increased expression of metallothioneins or enhanced DNA repair have not been proposed. The latter mechanism is anticipated to prove much more important than the former, but the tools required for the detailed study of complex DNA repair processes are still highly inadequate. As stated above, the nature of the "lethal" DNA lesion has not been defined, and it is therefore unclear as to which of the many types of repair processes should be the focus of drug resistance research.

The use of CBDCA and some other cisplatin analogues to overcome CDDP resistance has proven to be effective in only a minority of cases. The potential of other platinum analogues, particularly those with unconventional cellular uptake kinetics or different reactivity patterns, remains to be investigated.

Conclusions

It is clear that some patients clinically benefit from strategies for overcoming cisplatin resistance while others do not. It is reasonable to assume that the chances of responding to, for example, high-dose carboplatin or the regional administration of cisplatin depend on both the level of resistance in the individual tumor and the specific mechanism(s) of resistance involved. The potential mechanisms of resistance available to malignant cells may depend on their tissue of origin and on their stage and degree of differentiation, i.e., on the specific repertoire of genes expressed. Thus, favored mechanisms of resistance activated in response to drug exposure are likely to depend on many factors, the combination of which may be unique for each individual tumor. If this is true, the clinical application of the knowledge gained in model systems will require the examination of individual human tumors. The development of a methodology for the routine detection and classification of CDDP resistance in human tumor samples is now both feasible and urgently required. The clinical validation and rational use of such determinations may provide a major challenge for medical oncology in the coming decade.

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