

## MINIREVIEW

# Is Cisplatin-Induced Cell Death Always Produced by Apoptosis?

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The platinum drugs represent a unique and important class of antitumor agents. The clinical development of the neutral, square planar, coordination complex *cis*-diamminedichloroplatinum(II) (cisplatin), marked in the 1970s a watershed in the treatment of cancer. Cisplatin is widely used for the treatment of many malignancies, including testicular, ovarian, bladder, cervical, head and neck, and small-cell and non-small-cell lung cancers (Rosenberg, 1999). The cisplatin analog 1,1-cyclobutanedicarboxylate platinum(II) (carboplatin), has also been used increasingly in the last 2 decades. Despite the great efficacy at treating certain kinds of cancers, cisplatin, carboplatin, and other cisplatin analogs introduced into clinics have major problems, such as several side effects and the acquisition or presence of resistance to these drugs that undermines their curative potential (Kelland, 1993). Thus, considerable efforts are being directed toward the development of novel platinum compounds with clinical profiles complementary to that of cisplatin and its analogs, such as *trans*-Pt(II) compounds, Pt(IV) compounds, and polynuclear platinum compounds (Wong and Giandomenico, 1999; Pérez et al., 2000).

Cisplatin is a well known DNA-damaging agent and the current thinking is that DNA platination is an essential first step in the cytotoxic activity of the drug. However, the mechanism(s) whereby these DNA adducts kill cells is not fully understood. One potentially important way by which cisplatin-DNA adducts may kill cells is by induction of pro-

grammed cell death or apoptosis (Eastman, 1999). Is cisplatin-induced cell death always the result of an apoptotic pathway? This review tries to shed light on this important issue.

## Molecular and Cellular Pharmacology of Cisplatin

**Intracellular Accumulation.** Cisplatin cellular uptake is barely understood. The current data indicate that cisplatin enters cells through transmembrane channels but these data are also consistent with high-capacity facilitated transport (Gately and Howell, 1993). So far, the search for a specific cisplatin membrane transport system has been unsuccessful. Once cisplatin enters the cell, the chloride concentration drops to  $\approx 20$  mM and the drug undergoes strong hydration to form positively charged active species for subsequent interaction with cellular nucleophiles (Andrews and Howell, 1990).

**Binding to DNA and Other Cellular Targets.** Many cellular components that have nucleophilic sites such as DNA, RNA, proteins, membrane phospholipids, cytoskeletal microfilaments, and thiol-containing molecules react with cisplatin, although only approximately 1% of the intracellular cisplatin reacts with nuclear DNA to yield a variety of adducts that include interstrand and intrastrand DNA cross-links and DNA-protein cross-links (Fig. 1). The most common adduct is an intrastrand cross-link between adjacent guanines (Pérez, 1998). Although genomic DNA is generally accepted as the critical pharmacological target of cisplatin-induced cytotoxicity, there is evidence that other cellular targets may also be involved in the cytotoxicity of the drug. Thus, cisplatin binds to mitochondrial DNA, interacts with phospholipids and phosphatidylserine in membranes,

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**ABBREVIATIONS:** cisplatin, *cis*-diamminedichloroplatinum(II); NER, nucleotide excision repair; MMR, mismatch repair; DNA-PK, DNA-dependent protein kinase; HMG, high-mobility group; caspase, cysteine aspartate-specific proteinase; transplatin, *trans*-diamminedichloroplatinum(II); DISC, death-inducing signaling complex; FasL, Fas ligand; bFGF, basic fibroblast growth factor.

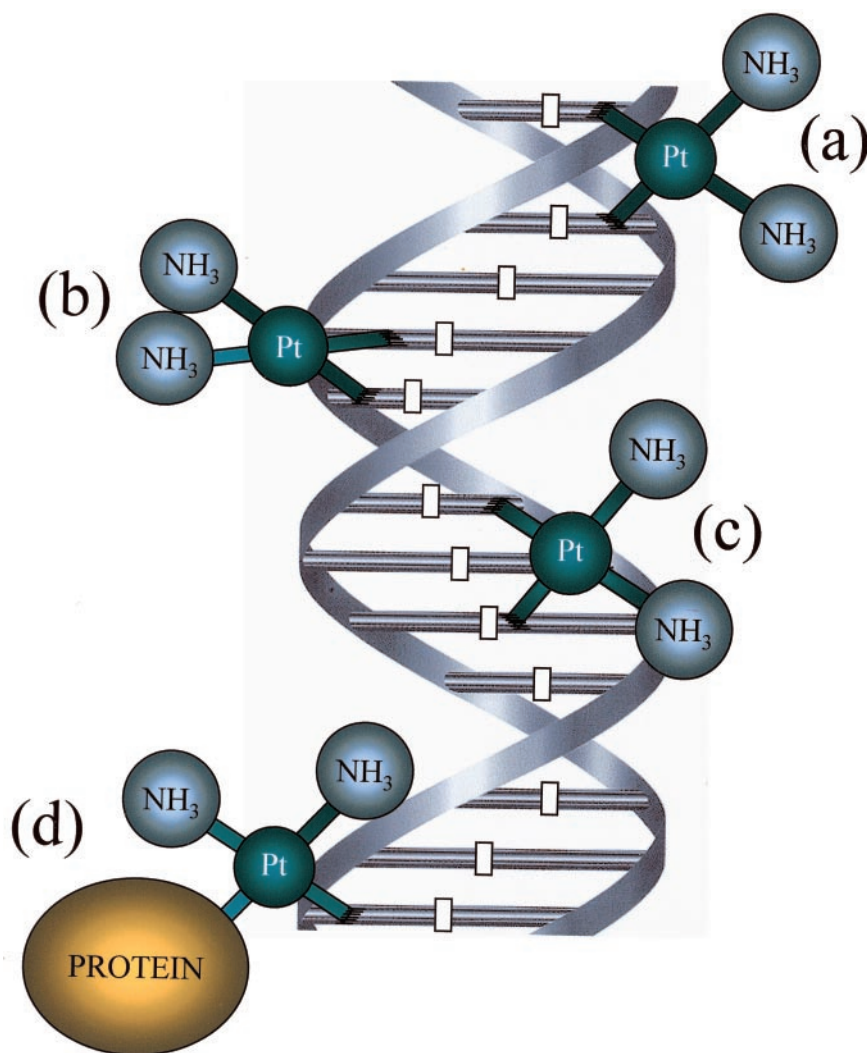
disrupts the cytoskeleton, and affects the polymerization of actin (Jamieson and Lippard, 1999).

**Proteins That Recognize Cisplatin-Induced DNA Damage.** The mechanism of cisplatin-induced DNA damage toward cell killing is beginning to be disentangled. In the past, it was thought that cisplatin cytotoxicity was the result of inhibition of DNA synthesis. However, DNA repair-deficient cells die at concentrations of cisplatin that do not inhibit DNA synthesis. Moreover, DNA repair-proficient cells survive at concentrations of cisplatin high enough to inhibit DNA synthesis and arrest the cells in S phase (Sorenson and Eastman, 1988). Thus, cisplatin-induced cell death does not always correlate with inhibition of DNA synthesis. To help understand the initial events that link cisplatin-induced DNA damage to the cell death pathway, considerable attention has recently focused on identification and characterization of proteins that recognize cisplatin-induced DNA damage. At present, several families of proteins are implicated as important: 1) nucleotide excision repair (NER) proteins, 2) mismatch repair (MMR) proteins, 3) DNA-dependent protein kinase (DNA-PK), and 4) high-mobility group (HMG) proteins.

It is becoming clear that the NER pathway is responsible for the repair of cisplatin-DNA adducts. It seems that only 16 genes are essential for the DNA damage recognition and

excision function of the intrastrand adduct between two adjacent guanines (Mu et al., 1996). MMR is a postreplication repair system that corrects unpaired or mispaired nucleotides. The relationship between DNA damage recognition by MMR proteins and cytotoxicity remains incompletely defined. Human mismatch repair complex hMutS- $\alpha$  detects but does not remove cisplatin-DNA adducts. This protein has been shown to recognize specifically a single cisplatin intrastrand adduct between two adjacent guanines within a double-strand oligonucleotide (Yamada et al., 1997). As for the molecular pharmacology of cisplatin DNA adduct repair, it is currently a matter of debate as to whether NER is more important than MMR in the repair of DNA damage by cisplatin. However, in ovarian cancer and colon cancer, at least, MMR is a comparatively small contributor to the cisplatin resistance phenotype because an intact MMR system seems to be essential for the linkage of DNA damage/repair with the initiation of apoptosis (Reed, 1999). The current thinking is that MMR proteins would try to insert the "correct" nucleotide on the nondamaged strand opposite the intrastrand adduct between two adjacent guanines and that this "futile" repair cycle might then induce apoptosis.

DNA-PK is another repair system that is required for the elimination of DNA double-strand breaks that are induced by ionizing radiation. DNA-PK also reportedly interacts with



**Fig. 1.** Main adducts formed in the interaction of cisplatin with DNA. (a), interstrand cross-link. (b), 1,2-intrastrand cross-link. (c), 1,3-intrastrand cross-link. (d), protein-DNA cross-link.

cisplatin-DNA lesions (Turchi and Henkels, 1996). Binding to DNA of Ku subunits of DNA-PK is essential *in vitro* to activate the kinase activity of DNA-PK to phosphorylate itself or other transcription factors. It has been shown in apoptotic ovarian cancer cells that the presence of cisplatin-DNA adducts serves to inhibit the ability of the Ku subunits of DNA-PK to translocate on a duplex DNA substrate so that kinase activity is abrogated and that the ability of Ku subunits to bind DNA is decreased. The decrease in kinase activity could be caused by proteolytic degradation of the catalytic subunit of DNA-PK by caspases (Henkels and Turchi, 1997).

The HMG proteins are a family of small, nonhistone chromatin-associated proteins involved in gene regulation and maintenance of chromatin structure. The HMG box proteins do have the common feature of binding to DNA involved in structural deformation and some of them also bind to cisplatin-DNA adducts (Zamble and Lippard, 1999). Thus, a HMG protein called structure specific recognition protein-1 (SSRP-1) binds to cisplatin but not transplatin adducts (Bruhn et al., 1992). HMG1 and HMG2 proteins recognize the intrastrand adduct between adjacent guanines and it is hypothesized that the affinity of HMG box proteins for cisplatin-DNA adducts seems to be a case of "mistaken identity" (Hughes et al., 1992).

### Translation of Cisplatin-DNA Adducts into Cytotoxicity: Apoptosis as a Model of Cell Death

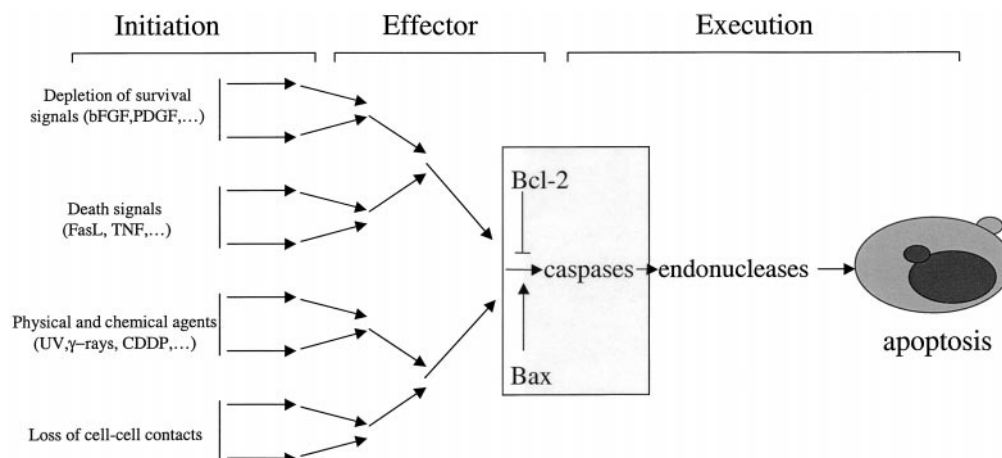
One potentially important mechanism of translation of cisplatin-DNA damage into cell death is apoptosis. Considerable evidence indicates that cisplatin can kill cells through the induction of apoptosis (Eastman, 1999).

**The Apoptotic Pathway and Its Phases.** Apoptosis or "programmed cell death" is a genetically regulated mechanism of cell turnover that occurs during embryonic development, normal cellular homeostasis, and spontaneous and drug-induced tumor cell death (Hickman, 1992). Apoptosis is characterized by unique morphological and biochemical features. These features include cell shrinkage, blebbing of the cell surface, loss of cell-cell contact, chromatin condensation with activation of endogenous endonucleases, recognition by phagocytic cells, and dependence on the energy supplied by

ATP as well as on active protein synthesis (Dive and Wyllie, 1993).

Given that numerous stimuli, including antitumor drugs, induce apoptosis, one can see the potential complexity in the regulatory networks needed to integrate this information and to decide the fate of a cell. To help understand apoptosis, it is necessary to consider three different stages (Fig. 2). The first one is an initiation phase, in which a stimulus is received followed by engagement of any one of several possible pathways that respond to the stimulus. The second one is an effector phase, in which all the possible initiating signals are integrated and a decision to live or die is made. The last one is a common irreversible execution phase, in which some proteins autodigest and DNA is cleaved (Eastman, 1999). *Bcl-2* is an oncogene that seems to be at the convergence of many apoptotic pathways and the ratio of *Bcl-2* to *Bax* protein (Fisher, 1994) might be the final determinant of whether a cell enters the execution phase (see Fig. 2). *Bax* is a gene that encodes a dominant inhibitor of *Bcl-2* (Eliopoulos et al., 1995). A conserved feature of the execution phase of apoptosis is the specific degradation of a series of proteins by the cysteine-aspartate-specific proteases, or caspases. Caspases are activated when an apoptotic stimulus induces the release of cytochrome *c* from mitochondria (Alnemri, 1997). However, little is known about what initiates activation of the first caspase and what constitute the critical substrates for caspase cleavage.

**Models for Translation of Cisplatin-Induced DNA Damage into Apoptosis.** The specific mechanism(s) that trigger apoptosis in response to cisplatin insult have not yet been defined. In principle, such mechanism(s) must include ways to detect DNA damage and determine it is strong enough to be lethal. Among the mechanisms that have been proposed for how proteins that bind to cisplatin-DNA adducts might modulate the sensitivity of cells to the drug, two seem to be the most feasible (Jamieson and Lippard, 1999). In the "hijacking model", HMG proteins binding to cisplatin-DNA adducts could modulate cell cycle events after DNA damage and trigger apoptosis. In the "repair shielding model", HMG proteins could protect cisplatin-DNA adducts from recognition by DNA repair enzymes. These mechanisms of cisplatin-induced cytotoxicity are not necessarily exclusive and could work in concert. Figure 3 illustrates the "repair shielding model", in which some HMG box proteins bind with high



**Fig. 2.** Scheme of the converging pathways leading to apoptosis in mammalian cells. A great variety of stimuli such as depletion of survival signals, death signals, physical and chemical agents, and loss of cell-cell contacts can initiate apoptosis through numerous different means. The effector phase integrates these signals leading to the decision of life or death. PDGF, platelet-derived growth factor; TNF, tumor necrosis factor; CDDP, cisplatin.



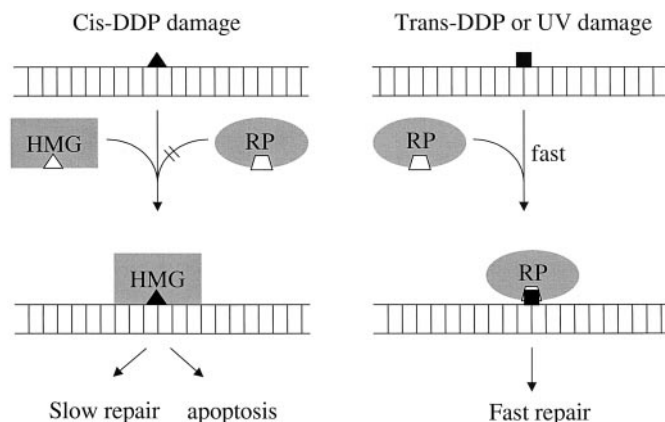
affinity to cisplatin-DNA adducts but not to transplatin, UV-induced or other DNA lesions. In contrast, a repair protein (RP) possessing DNA binding affinity flexible enough to recognize different lesions would have a much lower affinity for cisplatin-DNA adducts than for either transplatin-DNA adducts or UV-induced DNA lesions. Consequently, DNA repair would proceed slowly because the repair protein would displace HMG box proteins from the cisplatin-DNA adduct very slowly. Alternatively, the binding of HMG box or other proteins to the cisplatin-DNA adduct might lead to cell cycle arrest and the induction of apoptosis. Therefore, in a particular cell line, cisplatin cytotoxicity might be determined by a "dynamic contest" between proteins that repair DNA and proteins that interfere with DNA repair and trigger apoptosis.

### Factors That May Be Involved in Cisplatin-Induced Apoptosis

Multiple factors that may influence the ability of cisplatin to induce apoptosis have been identified at the cellular and molecular levels.

**Defective Apoptotic Program.** It is generally accepted that the main mechanisms affecting the occurrence of cisplatin resistance include increased drug efflux, decreased drug influx, increased cellular glutathione and metallothionein levels, increased DNA repair, and oncogene expression (Pérez, 1998). However, emerging evidence suggests that an important number of cases of cisplatin resistance might be the result of a defective apoptotic program. In this case, increased levels of DNA damage would be required to induce the signal initiating apoptosis (Henkels and Turchi, 1997). The current thinking is that most, if not all, tumor cell types have the potential to undergo apoptosis in response to anti-cancer drugs and that there is a "threshold level" of cellular damage that triggers apoptosis. This threshold level would be dependent on the type of tumor cell exposed to the insult (Fisher, 1994).

**Pro-Apoptotic and Anti-Apoptotic Proteins.** Several members of the so-called proapoptotic (i.e., Bax, Bak, Bad, BclXs) and antiapoptotic (i.e., Bcl-2, Bcl-X<sub>L</sub>, Mcl-1, Bcl-w and A1) families of proteins reportedly regulate the effector phase



**Fig. 3.** Model for the repair of DNA damage induced by cisplatin. HMG box or other DNA conformation-binding proteins interact specifically with cisplatin-DNA adducts. Binding limits access by repair proteins (RP), inhibiting DNA repair or triggering apoptosis. The HMG proteins do not recognize *trans*-DDP-DNA adducts or damage induced by UV or other agents, so that DNA repair proceeds quickly for cisplatin-DNA adducts. *cis*-DDP, cisplatin; *trans*-DDP, transplatin.

of apoptosis (Reed, 1997). Accordingly, it has been observed that cisplatin-induced apoptosis in both sensitive and resistant ovarian cancer cells is associated with an increased level of Bax and Bak proteins (Jones et al., 1998). In addition, a decrease in *Bcl-2* expression has been reported in cisplatin-resistant ovarian cancer cells after cisplatin treatment (Henkels and Turchi, 1999).

**Caspases.** As mentioned already, the caspase cascade is activated in response to cisplatin insult; this activation leads to an irreversible commitment to apoptotic cell death. Caspases are usually classified into two groups (Eastman, 1999): initiators (i.e., caspase-2, caspase-8, and caspase-9) and effectors (i.e., caspase-3, caspase-6, caspase-7, and caspase-14). Initiator caspases interact with signaling adaptor molecules through motifs in the prodomains called caspase recruitment domains (Hofmann et al., 1997). Two regulatory pathways of the caspases cascade have been reported. The first pathway begins with the assembly of a death-inducing signaling complex (DISC) at the Fas receptor (Enary et al., 1996). Activation of Fas receptor by its natural Fas ligand (FasL) induces the formation of a DISC consisting of the adaptor molecule Fas-associated death domain and caspase-8. Activated caspase-8 in the Fas/FasL initiated DISC activates effector caspase-3, and the activated caspase-3 can directly initiate certain caspase-activated deoxynucleases (Muzio et al., 1998). This first pathway may be activated in tumor cells in response to cisplatin (Fulda et al., 1998). The second pathway begins with the release of cytochrome C from the mitochondria, which subsequently causes apoptosis by activation of caspase-9 and caspase-3 (Li et al., 1997). In the presence of ATP and cytochrome *c*, the apoptotic protease-activating factor-1 (Apaf-1) binds through its caspase recruitment domain region to the corresponding motif in caspase-9, causing the activation of this caspase that in turn activates caspase-3 (Srinivasula et al., 1998). Cisplatin may cause mitochondrial release of cytochrome *c* and caspase-3 activation (Kojima et al., 1998). In addition, in human osteosarcoma cells, cisplatin induces apoptosis through a sequential activation of caspase-8, caspase-3, and caspase-6 (Seki et al., 2000). However, it has been also reported that cisplatin-induced apoptosis in A2780 ovarian tumor cells may proceed via a caspase-3 independent pathway (Henkels and Turchi, 1999). This lack of caspase-3 activation after cisplatin treatment in A2780 tumor cells is consistent with inefficient formation of DNA ladders and altered apoptotic morphology. In fact, caspase-3 has been specifically implicated as the effector caspase responsible for cleavage of the human DNA fragmentation factor that subsequently activates the DNA endonuclease (DFF40) required for formation of apoptotic DNA ladders (Liu et al., 1998).

**Apoptotic Endonucleases and Divalent Cations.** Several endonucleases that catalyze the internucleosomal fragmentation of DNA have been implicated in apoptosis and some of them require specific levels of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  (Peitsch et al., 1993; Eastman 1999). Both in vitro and in vivo, the maximal activity of these  $\text{Ca}^{2+}/\text{Mg}^{2+}$ -dependent endonucleases is reached at concentrations of  $\text{Ca}^{2+}$  and  $\text{Mg}^{2+}$  of 2.5 to 5 mM and 5 mM, respectively (Yakovlev et al., 2000). However,  $\text{Ca}^{2+}$  is not always required for apoptotic DNA digestion. In fact, the DNA endonuclease called caspase-activated DNase is a  $\text{Ca}^{2+}$ -independent endonuclease.

ase that has also been involved in apoptosis (Reynolds and Eastman, 1996).

**Cell Cycle Check Points.** Cell cycle arrest and a specific cell cycle check point at the boundary of the G<sub>2</sub> and M phases have been suggested to be involved in apoptosis induction in a variety of proliferating cells treated with cisplatin (Eastman, 1999). If cisplatin-induced cellular damage were irreparable during the G<sub>2</sub> phase, controlled elimination of platinated cells via apoptosis would prevent the passage of these cells into mitosis. Thus, it has been hypothesized that in the cell cycle checkpoint, the DNA lesions would be "sensed" (Evans et al., 1994).

**Oncogenes and Tumor Suppressor Genes.** Oncogene expression may also affect apoptosis induction by cisplatin and other antitumor drugs, but the effect of a given oncogene on apoptosis depends on the cellular genetic background in which it is immersed. Consequently, there is a pool of seemingly disparate findings and no clear patterns have yet begun to emerge. For instance, although cisplatin resistance is directly related to the levels of *c-myc* transcript in Friend murine erythroleukemia (Sklar and Prochownik, 1991), enforced *c-myc* expression induces apoptosis in fibroblasts (Zhan et al., 1997). In addition, although in a panel of 16 human ovarian carcinoma cell lines, *H-ras* overexpression does not either sensitize or confer resistance to cisplatin-induced apoptosis (Holford et al., 1998), cisplatin is unable to induce apoptosis in murine keratinocytes overexpressing *H-ras* oncogene (Pérez et al., 1999). Moreover, although the *Bcl-2* oncogene is a well-known member of the antiapoptotic family and high Bcl-2 protein levels inhibit cisplatin-induced apoptosis in cells of hematopoietic origin and in HeLa cells (Reed, 1997), high levels of Bcl-2 protein confer a trend toward sensitivity to cisplatin in human ovarian cancer cells (Beale et al., 2000).

Tumor suppressor genes also influence cisplatin-induced apoptosis. *p53* is considered a "guardian of the genome" and facilitates DNA repair before DNA replication. Cisplatin DNA damage may lead to expression of p53 protein that subsequently induces both expression of downstream *p21WAF* protein and G<sub>1</sub> phase cell cycle arrest (Reed, 1999). In the event of irreparable DNA damage, p53 protein induces apoptosis (Fisher, 1994). The *p53* gene also directly affects expression of other downstream genes that regulate sensitivity to apoptosis, activating transcription of proapoptotic Bax and repressing transcription of antiapoptotic Bcl-2 proteins (Eastman, 1999). However, it recently has been reported that in some ovarian tumor cell lines, cisplatin treatment decreases or even abolishes p53 protein levels. Interestingly, most of these cisplatin-treated cells have features characteristic of necrotic cell death (González et al., 2000). On the other hand, although cisplatin may initiate apoptosis through pathways modulated by *p53*, this tumor suppressor gene is not always required for apoptosis. Thus, cisplatin induces apoptosis in cells expressing either wild type or mutant p53 protein or even in cells lacking p53 (Zamble et al., 1998). Examination of Bcl-2, Bax, and p53 protein levels in pairs of cisplatin-sensitive and -resistant ovarian cancer cell lines demonstrated that high levels of Bcl-2 protein protected the cells from cisplatin-induced apoptosis by decreasing the rate and levels of p53 protein accumulation. Moreover, Bcl-2 indirectly suppressed Bax induction, thereby prolonging cell survival (Eliopoulos et al., 1995). On the other hand, it has

been found recently that p38 mitogen-activated protein kinase is involved in the activation of p53 by cisplatin insult and that p38 associates physically with p53. Interestingly, inhibition of p38 mitogen-activated protein kinase diminished the apoptotic fraction of cells exposed to cisplatin and increased cell survival (Sánchez-Prieto et al., 2000).

**Growth Factors and Cytokines.** Signals from the extracellular environment, such as certain growth factors and cytokines, may also modulate cisplatin-induced apoptosis because they can regulate the apoptotic response to chemotherapeutic drugs. In fact, it has been reported that basic fibroblast growth factor (bFGF) sensitizes NIH 3T3 cells to cisplatin-induced apoptosis. Interestingly, the concentrations of bFGF required to sensitize 3T3 cells to cisplatin-induced apoptosis ( $\geq 10$  ng/ml) are significantly higher than those needed to stimulate cell growth (0.5 ng/ml). These data suggest that the signal by which bFGF confers sensitivity to cisplatin-induced apoptosis might be propagated through a specific low-affinity receptor and a signal transduction pathway different from those that stimulate mitogenesis (Coleman et al., 2000). Perhaps some growth factors, such as bFGF, have two membrane receptors: a high-affinity receptor involved in mitogenesis and a low-affinity receptor involved in cell arrest and sensitization to apoptosis.

**Viral Proteins.** It recently has been found that in adult T-cell leukemia, in which the human T-cell leukemia virus type 1 is the causative agent, expression of viral Tax protein favors cisplatin-induced apoptosis. Tax protein might indirectly sensitize cells to apoptosis through inhibition of nucleotide excision repair of cisplatin-induced DNA damage, because it is well known that the presence of unrepaired genomic damage typically induces apoptotic cell death (Kao et al., 2000).

## Summary

It is generally accepted that DNA damage and subsequent induction of apoptosis may be the primary cytotoxic mechanism of cisplatin and other DNA-binding antitumor drugs (Fisher, 1994). Because the final step of apoptosis is characterized by morphological changes in the nucleus, the death signals of the execution phase must be transmitted from the cytoplasm to the nucleus. Thus, the recognition and processing of cisplatin-induced DNA damage through "classic" apoptosis, requires that a nuclear signal, generated at the initiation phase, be transmitted to the cytoplasm to be processed through the effector and execution phases. At the end of the execution phase, the apoptotic signal must come back to the nucleus to produce internucleosomal DNA degradation. Therefore, the induction of apoptosis from detection and subsequent processing of cisplatin-induced DNA damage seems to be a long and complex process of cell death. However, because cisplatin is a nonspecific drug and reacts not only with DNA but also with proteins, we cannot rule out the possibility that in some cases of cisplatin-induced apoptosis, an easier process of initiation, such as damage to cytoplasmic proteins, may take place (Pérez, 1998). Thus, damage to proteins is worth considering as a factor contributing to cisplatin-induced apoptosis. Moreover, it is possible that cisplatin damage to proteins could induce apoptosis at the execution phase level. In fact, initiation of apoptosis at the execution phase (activation of caspases) has been previously

reported for the cell killing produced by cytotoxic T lymphocytes (Golstein et al., 1991).

Although apoptosis and necrosis are conceptually distinct forms of cell death with very different morphological and biochemical characteristics, these two types of demise may occur simultaneously in tissues or cell cultures exposed to the same insult (Eguchi et al., 1997, Zhan et al., 1999). In fact, both types of cell death have been found in the same population of cisplatin-treated cells (Pestell et al., 2000). Moreover, it has been hypothesized that in a tissue or cell population, apoptosis and necrosis might be two extremes of a continuum of possible types of cell demise. Individual cell death would be decided by factors such as the availability of energy and the metabolic condition of the cell (Leist et al., 1997). Thus, some cells might die as a result of an unfinished apoptotic program. In fact, in L1210 leukemic cells, cisplatin-induced cell death seems to be the result of a defective apoptotic program that lacks some morphological and biochemical characteristics attributed to apoptosis (Segal-Bendirdjian and Jacquemin-Sablon, 1995). In addition, at high doses, cisplatin could damage molecules involved in cellular energy supply (i.e., ATP) and also proteins directly or indirectly involved in the apoptotic process (i.e., p53, Bax, Bcl-2, and caspases), leading to necrotic cell death. In fact, in cisplatin-resistant keratinocytes transformed by H-ras oncogene, a high dose of cisplatin (312  $\mu$ M) induces characteristic features of necrotic cell death (Pérez et al., 1999). Thus, depending on the level of cellular damage induced by cisplatin, necrosis could take place either directly or as a consequence of an unfinished apoptotic program.

In summary, a growing body of evidence suggests that cisplatin-induced cell death does not always come from "classic" apoptosis. Depending on both cisplatin dose and cellular status, cisplatin may also induced cell death by a defective apoptotic program or even by necrosis. Elucidation of the conditions under which the apoptotic program induced by cisplatin as well as other antitumor drugs is totally or partially executed may have important implications for the outcome of cancer chemotherapy.

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