Cisplatin

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Introduction

The platinum co-ordination complex cis-diaminedichloroplatinum(II) (cis-DDP or cisplatin; Figure 1) was first synthesized in 1845. The useful biological effects of the compound, however, were not discovered for more than a century. In 1965, biophysicist Barnett Rosenberg was examining the effects of electrical fields on the bacterium Escherichia coli and observed that cells held between charged platinum electrodes grew in size but did not divide. A number of compounds had been produced during electrolysis and one, later identified as cisplatin, showed the ability to hinder cell division. The inhibitory effect on cell division suggested that cisplatin might have potential as an anticancer agent.

Cisplatin was approved by the United States Food and Drug Administration in 1979 and is now one of the most widely used chemotherapeutic agents for the treatment of human cancer. Cisplatin demonstrates significant activity against tumours of the ovary, bladder, lung, head and neck, but it is most strikingly effective against testicular cancer. According to recent estimates, over 90% of testicular tumours are curable, largely through the use of cisplatin-based chemotherapy. Whereas cisplatin slows the clinical course of other solid tumours, it rarely affords complete remission. In these cancers, treatment failure is often a result of drug resistance. Given the limitations of

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cisplatin therapy, achieving a complete understanding of the mechanism of action of cisplatin may lead to the design of novel compounds that are effective against a wider range of tumour types, to agents that ablate drug-resistant tumours, as well as to agents with fewer toxic side effects. The side effects associated with cisplatin treatment include gastrointestinal distress, kidney damage, nerve damage, hearing loss and bone-marrow suppression; some of these side effects can be alleviated partially in the clinic.

Although cisplatin’s mechanism of action is not entirely known, it is believed that cisplatin exerts its cytotoxic effects through the formation of covalent adducts in which the chloride ligands of the drug are replaced by specific DNA bases. The DNA adducts of cisplatin are proposed to mediate cytotoxicity by inhibiting DNA replication and transcription and, ultimately, by activating a pathway termed programmed cell death, or apoptosis. The geometric isomer of cisplatin, \textit{trans}-diamminedichloroplatinum(II) (\textit{trans}-DDP; Figure 1), also binds to DNA and inhibits DNA replication, but it is at least 20-fold less toxic to cells than cisplatin and is inactive as an anti-tumour agent. This observation suggests that the cytotoxicity of cisplatin cannot be explained simply by its ability to cause DNA damage. Rather, more complex cellular and biochemical mechanisms may underlie the observed differences in toxicity.

\textbf{Figure 1. Structures of the anti-cancer drug cisplatin (cis-DDP) and other platinum compounds discussed in this Chapter}
between cis- and trans-DDP. Studies comparing adduct formation, repair and toxicity of the cis and trans platinum isomers have provided important insights into the mechanisms by which cisplatin, alone, displays clinical efficacy. This Chapter will summarize work on how cells differentially process platinum adducts. The review will focus on the following five areas: DNA adducts formed by cis- and trans-DDP; effects on DNA replication and transcription; repair of platinum adducts; recognition of adducts by cellular proteins; and the mechanisms of cisplatin resistance.

**DNA adducts formed by cis- and trans-DDP**

Cisplatin is administered intravenously to patients. In the bloodstream where the concentration of chloride ions is high (~100 mM), cisplatin, a neutral molecule, is relatively unreactive (Figure 2). Inside the cell, however, the low ambient chloride concentration (~4 mM) facilitates hydrolysis of the chloride ligands of the drug. Water molecules displace the two chlorides in a stepwise manner to form an aquated positively charged complex. Since water is a good leaving group, the aquated electrophilic species can react readily by ligand substitution with nucleophiles in the cell, including DNA, RNA, proteins and cellular thiols, such as glutathione and metallothioneins. The critical cellular target for cisplatin is widely believed to be DNA. The most compelling evidence in support of this view is the high sensitivity of cells with defects in DNA repair to the lethal effects of this drug. Cisplatin binds primarily at the

![Figure 2. Schematic representation of cisplatin entering the cell and its interaction with cellular nucleophiles, including DNA](image)

The major DNA adducts formed by cisplatin are monofunctional adducts, intrastrand crosslinks and interstrand crosslinks.
N-7 positions of purine (guanine and adenine) bases, which are exposed in the major groove of the DNA double helix and are not involved in base-pair hydrogen-bonding interactions (Figure 3). Cisplatin binds to DNA in two successive steps. First, monofunctional adducts are formed by co-ordination to a single guanine or adenine. Subsequently, the remaining electrophilic centre on these monofunctional adducts will either react with a nearby purine on the same strand of the DNA to form a bifunctional intrastrand crosslink, or it will react with a purine on the complementary DNA strand to form an interstrand crosslink (Figure 2). The major DNA adducts formed by reaction of cisplatin with DNA in vitro include 1,2-intrastrand d(GpG) adducts between adjacent guanines (65% of detected adducts), 1,2-intrastrand d(ApG) adducts between an adjacent adenine and guanine (25%) and 1,3-intrastrand d(GpNpG) adducts between guanines separated by an intervening nucleotide (6%). Interstrand crosslinks, which form between guanines at d(G°pC)/d(G°pC)
sites (where the asterisks denote sites of platinum co-ordination), and monofunctional adducts occur at a lower frequency (1–2%; Table 1). Interestingly, 1,2-intrastrand crosslinks at d(GpA) sites are very rare. A similar DNA-adduct profile is observed in DNA isolated from the white blood cells of cancer patients following cisplatin treatment, so the pattern observed in vitro is also seen in vivo. Unfortunately, the levels of cisplatin adducts are similar in tumours and other tissues of cancer patients following cisplatin therapy, indicating that cisplatin does not localize specifically in tumour tissue.

trans-DDP (Figure 1), the clinically inactive isomer of cisplatin, binds primarily, but not exclusively, at the N-7 positions of purine bases in DNA and forms intrastrand and interstrand crosslinks, as well as monofunctional adducts. In general, the DNA-adduct spectrum of trans-DDP is less well characterized than that of cisplatin. The trans-DDP–DNA adducts include 1,3- and 1,4-intrastrand crosslinks between purine bases separated by one or two intervening nucleotides (≈40%) and interstrand d(G*pC)/d(G*pC) and d(G*pC)/d(GpC*) crosslinks between complementary guanine and cytosine bases (20%; Table 1). However, due to steric constraints, trans-DDP cannot form the 1,2-intrastrand adducts, and this observation has led to the suggestion that intrastrand crosslinks, which comprise over 90% of the adduct spectrum of cisplatin, are responsible for the anti-tumour activity singularly seen with cisplatin.

The structures of platinum–DNA adducts have been investigated by several methods. Gel electrophoretic-mobility studies of oligonucleotides containing site-specific platinum adducts reveal that the 1,2-d(GpG), 1,2-d(ApG) and 1,3-d(GpTpG) intrastrand crosslinks of cisplatin bend the DNA helix 34° in the direction of the major groove. The degree of unwinding induced by the 1,2-intrastrand crosslinks is 13°, whereas the 1,3-intrastrand adduct unwinds the helix to a greater extent (23°; summarized in Table 2). Recently, the structure of the 1,2-d(GpG) cisplatin intrastrand crosslink within a 12-base-pair DNA duplex was determined by X-ray crystallography [1] (Figure 4). The

| Table 1. Comparison of DNA adducts formed by cis-DDP and trans-DDP |
|------------------------|------------------------|
|                        | cis-DDP                | trans-DDP              |
| **Monofunctional adducts** |                        |                        |
| dG                     | Yes                    | Yes                    |
| **Intrastrand crosslinks** |                        |                        |
| 1,2-d(GpG)             | 65%                    | No                     |
| 1,2-d(ApG)             | 25%                    | No                     |
| 1,3-d(GpNpG)           | 6%                     | ≈40%                   |
| **Interstrand crosslinks** |                        |                        |
| d(G*pC)/d(G*pC)        | 2%                     | ?                      |
| d(G*pC)/d(GpC*)        | ?                      | 20%                    |

Asterisks denote sites of platinum co-ordination.
Table 2. Structural alterations induced by platinum–DNA adducts

<table>
<thead>
<tr>
<th>Adducts</th>
<th>Degree of bending</th>
<th>Degree of unwinding</th>
<th>HMG binding</th>
</tr>
</thead>
<tbody>
<tr>
<td>cis-DDP</td>
<td>Monofunctional</td>
<td>None</td>
<td>6°</td>
</tr>
<tr>
<td>1,3-d(GpNpG)</td>
<td>34°</td>
<td>23°</td>
<td>Yes</td>
</tr>
<tr>
<td>1,2-d(GpG)</td>
<td>34°</td>
<td>13°</td>
<td>Yes</td>
</tr>
<tr>
<td>1,2-d(ApG)</td>
<td>34°</td>
<td>13°</td>
<td>Yes</td>
</tr>
<tr>
<td>d(G<em>pC)/d(G</em>pC)</td>
<td>20–40°</td>
<td>80°</td>
<td>Yes</td>
</tr>
<tr>
<td>trans-DDP</td>
<td>1,3-d(GpNpG)</td>
<td>Flexible</td>
<td>9°</td>
</tr>
<tr>
<td>d(G<em>pC)/d(GpC</em>)</td>
<td>Flexible, 26°</td>
<td>12°</td>
<td>No</td>
</tr>
</tbody>
</table>

Asterisks denote sites of platinum co-ordination.

Figure 4. The structure of the 1,2-d(GpG) cisplatin intrastrand crosslink in a 12-base-pair DNA duplex

Co-ordination of platinum is to the N-7 atoms of the guanine bases. Co-ordinate data taken from [1].
platinum atom lies in the major groove of the DNA, and the DNA duplex is significantly bent towards the major groove at the site of platination. Coordination of the platinum to its guanine ligands destacks the bases, while base-pair hydrogen bonding is maintained. By contrast, the structure of a cisplatin interstrand $d(G^\circ pC)/d(G^\circ pC)$ crosslink in duplex DNA reveals that the platinum atom lies in the minor groove of the DNA. The DNA helix is bent $\approx 20\text{–}40^\circ$ towards the minor groove and is significantly unwound ($\approx 80^\circ$) [2].

Structural distortions induced by the DNA adducts of trans-DDP are distinct from those of the cisplatin adducts (Table 2). Gel electrophoresis indicates that the 1,3-$d(GpTpG)$ intrastrand crosslink of trans-DDP introduces a point of flexibility into the DNA helix rather than a directed bend, and produces a smaller degree of helix unwinding ($9^\circ$) than the cisplatin-intrastrand adducts. The trans-DDP $d(G^\circ pC)/d(GpC^\circ)$ interstrand crosslink produces a bend of $26^\circ$ towards the major groove and unwinds the DNA helix by $12^\circ$. In addition, the adduct introduces a degree of flexibility to the helix [3]. Finally, monofunctional platinum adducts do not bend the DNA, but unwind the DNA helix by $6^\circ$ [4].

**Effects on DNA replication and transcription**

It has been proposed that, like many anti-tumour agents, cisplatin exerts its cytotoxic effects through the inhibition of DNA replication. By inhibiting the synthesis of DNA in cells, cisplatin–DNA adducts would slow cell division, which in turn could provide a trigger for cell death. In this way, cisplatin would have a selective effect on rapidly dividing cells, such as tumour cells. The ability of DNA polymerases to perform synthesis on platinated DNA templates *in vitro* has been investigated in several studies. Cisplatin–DNA adducts block the progression of bacterial as well as eukaryotic DNA polymerases *in vitro*. However, DNA adducts of the inactive cisplatin isomer, trans-DDP are equally effective as cisplatin adducts at inhibiting the DNA polymerases. In other work, the effects of cis- and trans-DDP on replication *in vivo* was investigated using African green monkey CV-1 cells infected with the DNA tumour virus, simian virus 40 (SV40). SV40 is a model minichromosome that is replicated by the host-cell enzyme machinery and then can be recovered. SV40-infected CV-1 cells were treated with various amounts of cis- or trans-DDP and then SV40 DNA replication was monitored. It was found that the two platinum compounds were equally effective at blocking DNA replication when equal numbers of platinum adducts were bound to the SV40 DNA. These results indicate that the anti-tumour activity of cisplatin cannot be based solely on its ability to inhibit DNA replication and that additional mechanisms must be invoked in order to explain the different toxicities of cis- and trans-DDP.

Inhibition of transcription is another way that cisplatin may exert its anti-tumour effects. RNA synthesis, like DNA replication, would be more critical
for a rapidly dividing tumour cell than for a stationary cell. Recent evidence suggests that, whereas the DNA adducts of cis- and trans-DDP block DNA replication equally, the adducts of these platinum isomers may inhibit RNA synthesis differentially. In vitro studies demonstrate that, when present on the transcribed strand, the 1,2- and 1,3-intrastrand crosslinks of cisplatin completely block the processivity of E. coli and wheat germ RNA polymerases. By contrast, monofunctional platinum adducts and the 1,3-d(GpTpG) intrastrand adduct of trans-DDP are bypassed by the polymerases [5,6]. In recent experiments in vivo, plasmids containing a reporter gene were modified with cis- or trans-DDP, introduced into mammalian cells, and the levels of reporter-gene transcription measured [7]. A 2–3-fold higher level of transcription was observed from plasmids modified with trans-DDP as compared with plasmids modified with cis-DDP. In particular, cis- and trans-DDP–DNA adducts are bypassed by RNA polymerase with relative efficiencies of 0–16% and 60–76%, respectively. These results suggest that the anti-tumour activity of cisplatin may be derived, at least in part, by efficient inhibition of transcription. Moreover, by inducing changes in the delicate balance of gene transcription within tumour cells, cisplatin adducts may provide a signal for cell death.

**Repair of platinum adducts**

Repair of cisplatin-induced DNA damage is one mechanism by which cells treated with cisplatin may increase their likelihood of survival. Nucleotide-excision repair is believed to be the main process by which bulky DNA adducts, including those formed by UV light or by cisplatin, are removed from DNA. The general repair mechanism involves recognition of the damage, incision of the DNA strand on both sides of the lesion, excision of the damaged oligonucleotide and resynthesis to fill the gap. In mammalian cells, nucleotide-excision repair requires the action of at least 30 proteins. The disease xeroderma pigmentosum (XP) results from defects in nucleotide-excision repair and is characterized by extreme sensitivity to UV light. The genes required for mammalian excision repair in rodents include those defined by the seven (A–G) complementation groups of XP as well as the excision-repair cross-complementing (ERCC) genes isolated from UV-sensitive rodent cell lines.

The repair of individual cisplatin–DNA adducts has been investigated in in vitro DNA repair assay systems employing human cell extracts. These in vitro repair assays either monitor DNA resynthesis after removal of the platinum damage or detect excision of the damage-containing fragment directly. Early results demonstrated that human cell extracts could carry out repair synthesis on plasmids modified globally with cisplatin. A subsequent study concluded that repair of cisplatin-modified templates results from the removal of minor adducts rather than the major 1,2-intrastrand cisplatin crosslinks. When examined directly in vitro, the 1,2-d(GpG) intrastrand adduct located at a specific
site is less efficiently repaired (3–20-fold) by human cell extracts than the 1,3-d(GpTpG) intrastrand crosslink. Incubation of either the 1,2- or 1,3-intrastrand crosslinked substrate with human cell extracts results in the release of a 26–29-nucleotide fragment that contains the adduct. Repair of the 1,3-intrastrand adduct is also more efficient in a reconstituted repair system containing purified repair factors, suggesting that differences in the rates of repair are due to structural differences in the platinum adducts rather than to unidentified factors in the cell extracts. Taken together, these results suggest that poor repair of the major 1,2-d(GpG) intrastrand adduct may contribute to the anti-tumour activity of cisplatin.

Evidence suggests that DNA adducts of the inactive trans-DDP isomer may be preferentially repaired in mammalian cells compared with adducts of cis-DDP. This observation is in line with the lower toxicity of the trans isomer to cells. DNA repair synthesis assays in vitro indicate that human cell extracts carry out repair synthesis twice as efficiently on trans-DDP- than on cis-DDP-modified plasmids. In addition, extracts prepared from excision-repair-deficient XP complementation group A cells are unable to perform repair synthesis on cis-DDP- and trans-DDP-modified plasmids, suggesting that both types of adduct are repaired by the nucleotide excision-repair pathway. In an SV40-based in vitro replication assay, preincubation of trans-DDP-modified plasmids, but not cis-DDP-modified plasmids, with human cell extracts restores DNA synthesis by 30%, suggesting that an activity present in the extracts repairs the trans-DDP-damaged template preferentially. In support of this view, a repair assay in vitro demonstrated that the extracts contained a specific repair activity for trans-DDP adducts. Studies in vivo, however, have yielded conflicting results. One study reported that in SV40-infected CV-1 cells, cis-DDP adducts accumulate continuously over a period of 48 h, whereas trans-DDP adducts reach a maximum at 6 h and then levels drop dramatically. By contrast, another investigation found that DNA adducts of cis- and trans-DDP are removed from DNA at similar rates. Differences in experimental conditions, however, may not make the two studies directly comparable. It remains to be determined whether preferential repair of trans-DDP adducts can account for the differential toxicity between the two isomers.

Recognition of platinum adducts by cellular proteins

The structural differences among the DNA adducts formed by cis- and trans-DDP suggest that the adducts of these isomers may be processed differently by cellular proteins. To test this hypothesis, a technique called the gel-mobility-shift assay was used to search for proteins in mammalian cell extracts that bind to platinated DNA. In this assay, proteins that bind non-covalently to a radioactive DNA probe are detected by their ability to retard the migration of the probe through an electrophoresis gel. By this tool, several proteins are observed in mammalian cell extracts that bind selectively to DNA
modified with cisplatin, but not to DNA adducts of the trans-DDP isomer. Moreover, the 1,2-d(GpG) and 1,2-d(ApG) intrastrand crosslinks of cisplatin, but not the 1,3-d(GpTpG) intrastrand adducts, are recognized by these proteins. Significantly, cisplatin-DNA binding activity is also observed in more clinically relevant extracts prepared from human testicular, ovarian and cervical tumours [8].

In parallel work, DNA modified with cisplatin was used as a probe to clone a gene that encodes a cisplatin–DNA-binding protein. In this method, a human B-cell cDNA library is constructed in the expression vector, bacteriophage λgtll. After growth of the recombinant bacteriophage on plates, isopropyl β-D-thiogalactopyranoside is added to induce expression of fusion proteins consisting of β-galactosidase linked to polypeptide sequences encoded by the cloned DNA. The screening of these fusion proteins with DNA probes modified with cisplatin led to the isolation of a positive cDNA clone encoding an 81-kDa cisplatin–DNA-binding protein. Amino acid sequence analysis revealed that the protein, termed structure-specific recognition protein 1 (SSRP1), contained a 75-amino acid conserved DNA-binding motif called the high-mobility group (HMG) domain. A family of proteins having in common the HMG DNA-binding domain has since been identified. The prototype of this family is HMG1, named for its fast mobility on SDS/PAGE. HMG1 is an abundant chromosomal protein containing two tandem HMG domains that bind to DNA cruciform structures; its function in the cell, however, is unknown. The similarity between the HMG domains of HMG1 and SSRP1 suggested that HMG1 may also recognize cisplatin-modified DNA. This notion was confirmed in gel-mobility-shift assays with purified HMG1 protein. In particular, HMG1 had the same profile of cisplatin-adduct recognition as that observed with mammalian cell extracts. Further experiments demonstrated that a single HMG domain mediates binding to cisplatin-modified DNA. To date, cisplatin–DNA-binding activity has been demonstrated for several HMG-domain proteins (summarized in Table 3). It should be emphasized that besides binding to platinated DNA, many of these proteins function in important roles in the cell, such as in the regulation of transcription.

What is the structural basis for cisplatin-adduct recognition by HMG-domain proteins? Proteins with HMG domains interact with bent DNA structures such as cruciforms and four-way junctions and induce bends in linear DNA. The interaction of the HMG domain with bent DNAs is probably due to its L-shaped tertiary structure. These observations suggest that structural distortions induced in DNA by cisplatin adducts may serve as structure-specific recognition signals for HMG-domain proteins. Moreover, the proteins appear able to distinguish the different structures induced by individual cis- and trans-DDP–DNA adducts (summarized in Table 2). For example, the major 1,2-intrastrand adducts of cisplatin, which bend the DNA 34° and unwind it 13°, are recognized by HMG-domain proteins, whereas the 1,3-intrastrand adducts, which unwind the DNA to a greater degree (23°), fail to
Table 3. HMG-domain proteins shown to bind to cisplatin-modified DNA
For details of proteins, see text.

<table>
<thead>
<tr>
<th>Full-length protein</th>
<th>Molecular mass (kDa)</th>
<th>Species</th>
<th>Number of HMG domains</th>
<th>Function</th>
</tr>
</thead>
<tbody>
<tr>
<td>SSRP1</td>
<td>81</td>
<td>Human</td>
<td>1</td>
<td>Unknown, human homologue of V(D)J recombination sequence-binding protein</td>
</tr>
<tr>
<td>lkr1</td>
<td>80</td>
<td>Yeast</td>
<td>2</td>
<td>Transcriptional receptor</td>
</tr>
<tr>
<td>HMG1</td>
<td>28</td>
<td>Rat</td>
<td>2</td>
<td>Unknown, binds to DNA cruciforms</td>
</tr>
<tr>
<td>HMG2</td>
<td>26.5</td>
<td>Calf</td>
<td>2</td>
<td>Unknown</td>
</tr>
<tr>
<td>hUBF</td>
<td>97/94</td>
<td>Human</td>
<td>6</td>
<td>Ribosomal RNA transcription factor</td>
</tr>
<tr>
<td>tsHMG</td>
<td>23</td>
<td>Mouse</td>
<td>2</td>
<td>Testis-specific HMG protein, spermatogenesis</td>
</tr>
<tr>
<td>hSRY</td>
<td>24</td>
<td>Human</td>
<td>1</td>
<td>Testis-determining factor</td>
</tr>
<tr>
<td>MtTFA</td>
<td>24</td>
<td>Human</td>
<td>1</td>
<td>Mitochondrial transcription factor</td>
</tr>
<tr>
<td>LEF-1</td>
<td>44</td>
<td>Mouse</td>
<td>1</td>
<td>Lymphoid-enhancer-binding factor</td>
</tr>
</tbody>
</table>
attract these proteins. It is noteworthy that the binding of HMG-domain proteins to the 1,2-intrastrand adducts increases the DNA bend angle from 34° to 70–90°, in striking agreement with the 80° angle between the two arms of the L-shaped HMG domain.

Recognition of cisplatin adducts by HMG-domain proteins may be fortuitous; that is, the adducts may mimic naturally bent DNA substrates for these proteins. Cisplatin-adduct binding by this protein family could, however, potentially play a role in the mechanism of action of the drug. In particular, the selective affinity for the major DNA adducts of cisplatin, but not for DNA adducts of the clinically ineffective trans-DDP isomer, indicates that these proteins may act to enhance cisplatin cytotoxicity. One proposed model (Figure 5B) suggests that the binding of HMG-domain proteins to cisplatin adducts blocks removal of the lesions by the repair machinery of the cell. Slow repair would allow the adducts to persist on the DNA, enhancing cisplatin cytotoxicity. By contrast, DNA adducts not recognized by HMG-domain proteins would be repaired more readily, improving the chances for cell survival. Supporting this hypothesis are results from in vivo experiments in yeast. A yeast strain with a deletion for the HMG-domain protein Ixr1 (intrastrand crosslink recognition protein) is 2–6-fold less sensitive to cisplatin than the parental strain containing the Ixr1 protein; by contrast, sensitivity to trans-DDP or UV light is not altered by the absence of Ixr1. Further experiments demonstrate that the effect of Ixr1 on cisplatin sensitivity is significantly decreased in a series of yeast strains deficient in nucleotide-excision repair, establishing a direct link to repair. Additional evidence for the repair-shielding model is obtained from excision-repair assays carried out in vitro. The addition of HMG-domain proteins to the assay specifically inhibited excision repair of the major 1,2-d(GpG) intrastrand cisplatin crosslink, but not the 1,3-d(GpTpG) intrastrand adduct. Finally, the XP group A-complementing (XPAC) protein, which is responsible for damage recognition in nucleotide-excision repair, exhibits a lower binding affinity in vitro for cisplatin-modified DNA compared with the HMG proteins HMG1, Ixr1 and human upstream-binding factor (hUBF). This result suggests that XPAC may not easily displace HMG-domain proteins from cisplatin–DNA adducts. Taken together, these results indicate that HMG-domain proteins may potentiate cisplatin cytotoxicity by shielding lesions from excision repair.

A second, as yet equally plausible, model (Figure 5C) proposed to explain how HMG-domain proteins may effect cisplatin toxicity is based on the fact that several HMG-domain proteins have important functions as regulators of transcription. The titration (or ‘hijacking’) of HMG-domain proteins away from their natural binding sites by cisplatin adducts might impair the expression of genes critical to a growing tumour cell and lead to cell death. Supporting this model are results from DNA-binding experiments in vitro with the HMG-domain protein hUBF, an important regulator of ribosomal RNA synthesis. The interaction of hUBF with the 1,2-d(GpG) intrastrand
Figure 5. Models to explain how HMG domain and other nuclear proteins may enhance cisplatin cytotoxicity

(A) A normal cell with a nuclear protein interacting with the DNA. (B) Repair-shielding model. Cisplatin–DNA adducts (lollipop symbol) attract the nuclear protein and are shielded from DNA-repair enzymes, promoting the persistence of the adducts and sensitizing the cell to cisplatin. (C) Hijacking model. The adducts titrate the nuclear protein away from its normal site of binding, resulting in reduced expression of a critical gene. (D) Abortive repair model. Misdirected repair attempts at sites of cisplatin damage by mismatch DNA-repair proteins (e.g. hMSH2) may generate DNA-strand breaks that signal for cell death.
adduct of cisplatin is very favourable and rivals that of the protein for its natural promoter sequence. Moreover, the binding of hUBF to its promoter can be competed in vitro with a cisplatin–DNA adduct concentration (5 nM) significantly lower than that found in the DNA of platinum-drug-treated cancer patients (0.1–1 μM). These results suggest that cisplatin adducts may act as ‘molecular decoys’ in cells for HMG-domain-containing transcription factors. Thus cisplatin could have profound effects on the expression of specific genes.

As already mentioned, an important aspect of the anti-tumour activity of cisplatin is its increased effectiveness in the treatment of testicular cancer as compared with other tumour cell types. Most HMG-domain proteins are expressed in all tissue cell types and thus cannot account for the tissue-specific cytotoxicity of cisplatin. Recent evidence, however, indicates that some HMG-domain proteins are specifically expressed in the testis and thus could potentially contribute to the selective efficacy of cisplatin against testicular tumours by one or both of the mechanisms described above. In support of this view, cisplatin-adduct-binding activity has been demonstrated for a mouse testis-specific HMG-domain protein (tsHMG) [9] and the human testis-determining factor (hSRY) [10]. In the case of hSRY, the affinity of the protein for the 1,2-d(GpG) cisplatin adduct is very similar to that for its putative DNA target sequence. Furthermore, the testis-specific proteins hSRY and tsHMG inhibit excision repair of the 1,2-d(GpG) intrastrand crosslink in vitro.

It has also been demonstrated that the human mismatch repair protein hMSH2, which does not contain an HMG domain, binds to DNA modified with cisplatin [11,12]; importantly, this protein is overexpressed in testicular tissue [12]. As part of the mismatch DNA-repair system, hMSH2 normally functions in the recognition and repair of mismatched base pairs or small loops in DNA. The importance of mismatch repair is emphasized by the fact that mutations in mismatch repair genes are associated with over 90% of hereditary non-polyposis colorectal cancer cases. Recently, it has been proposed that mismatch repair may also be involved in the mechanism of action of cisplatin. hMSH2, in concert with other elements of the mismatch repair system, may attempt to repair cisplatin adducts and thereby generate DNA-strand breaks (Figure 5D). The latter could signal programmed cell death, or apoptosis. Accordingly, this protein, which is expressed at high levels in testis, may potentiate toxicity by this abortive repair mechanism. In addition, however, hMSH2 could participate in cell killing by binding to adducts and blocking repair, as described above for the HMG-domain proteins.

**Mechanisms of cisplatin resistance**

The development of drug resistance is a major factor in the failure of cisplatin-based chemotherapy to cure cancer patients. In the clinic, levels of resistance are of the order of 2–4-fold. A corresponding increase in cisplatin dose, however, would lead to severe toxicity to the patient. There are two types of
cisplatin resistance, intrinsic and acquired. Intrinsic resistance is encountered with patients whose tumours are inherently resistant and do not respond to cisplatin at the time of first treatment. Acquired resistance can emerge in tumour cell populations after an initial drug response. Model systems have been valuable in the elucidation of the mechanisms of cisplatin resistance. For example, cell lines that have the ability to grow continuously in cultured media in vitro have been established from patient tumours. Intrinsic cisplatin resistance/sensitivity has been studied in testicular-tumour cell lines and bladder- or colon-tumour cell lines as representatives of inherently cisplatin-sensitive and -resistant tumour cell types, respectively. Cell lines that have acquired resistance by repeated or continuous exposure of the cells to cisplatin in vitro have been used extensively to identify mechanisms of acquired resistance. These cell lines, however, often acquire a much higher level of resistance (10–1000-fold) than tumours observed in vivo (2–4-fold), suggesting that these lines may not be clinically relevant models for the disease. Cell lines have also been established from tumour cells made resistant in vivo by cisplatin treatment, and these lines may more faithfully reflect the clinical situation. Another caveat to studying resistance in established cell lines is that some resistance mechanisms may operate only at the level of the organism, and not at the cellular level. For example, murine mammary tumours made resistant to cisplatin in vivo by treatment of tumour-bearing animals are surprisingly sensitive to cisplatin when the tumour cells are grown in vitro as continuous cultures.

Despite the caveats above, several mechanisms of cisplatin resistance have been identified by the various cellular model systems. These include: (i) reduced drug accumulation; (ii) increased drug inactivation by sulphur-containing molecules, such as glutathione and metallothionein; (ii) enhanced repair of cisplatin–DNA adducts; (iv) increased tolerance of cisplatin damage; and (v) altered expression of regulatory proteins. It is emphasized that development of resistance is a multifactorial process and, therefore, a given tumour may become resistant by one or more mechanisms.

Reduced drug accumulation
Cells can limit the formation of toxic DNA adducts by reducing the intracellular accumulation of cisplatin. Decreased drug accumulation is a consistent feature of many cisplatin-resistant cell lines, but the effects are small (2–5-fold) even when levels of resistance are high. The mechanisms underlying changes in cisplatin accumulation have not been elucidated. In fact, the pathway by which cisplatin enters cells is unclear; evidence exists both for passive diffusion and a carrier-mediated transport system. Recent studies suggest that an export pump may act to efflux cisplatin from cells. This pump is distinct, however, from the membrane drug-efflux pump encoded by the mdr (multidrug resistance) gene, which removes a variety of functionally different drugs from resistant cells, but is not involved in cisplatin resistance.
Drug inactivation by sulphur-containing molecules

A second mechanism by which cells may limit the number of cisplatin adducts formed is by increasing the concentration of sulphur-containing molecules that can react with the drug before it reaches the DNA (see Figure 2). Such molecules include glutathione, the most abundant thiol in the cell, and metallothioneins, small cysteine-rich proteins involved in detoxification of heavy metals. Following cisplatin treatment, a significant proportion of the total platinum content in cells is involved in a complex with either glutathione (60%) or metallothionein (25%). Elevated glutathione levels exist in cisplatin-resistant cells made resistant in vitro and in vivo and, in some cases, increased glutathione correlates with a reduction in interstrand-crosslink formation. This result supports a mechanism in which glutathione can react with monofunctional platinum adducts, preventing their closure to the more toxic bifunctional crosslinks. Glutathione is not increased, however, in other cisplatin-resistant cell lines. Similarly, metallothioneins are overexpressed in some, but not all, cell lines selected for resistance to cisplatin. Taken together, these results suggest that drug inactivation by glutathione or metallothioneins may contribute to cisplatin resistance, but additional resistance mechanisms are probably involved.

Enhanced repair of cisplatin–DNA adducts

In tumour cell lines that have acquired cisplatin resistance by incubation with the drug in vitro, enhanced repair of cisplatin adducts is often a major mechanism of resistance. Similarly, increased DNA repair has been observed in cell lines and primary tumour cells from patients with in vivo-acquired resistance. For example, an ovarian-cancer cell line established from a cisplatin-treated patient after the onset of resistance exhibits 3-fold-higher levels of DNA repair compared with a sensitive cell line derived from the same patient prior to the development of resistance.

Intrinsic resistance to cisplatin is associated with an elevated capacity for DNA repair, as evidenced in cell lines and tumours from untreated patients. Cells from non-small-cell lung tumours, which are generally resistant to cisplatin therapy, had 2–4-fold higher levels of DNA repair than cells derived from small-cell lung tumours, which are usually responsive to cisplatin treatment. Cisplatin sensitivity also correlates with DNA-repair capacity in testicular and bladder-tumour cell lines, which represent inherently cisplatin-sensitive and -resistant tumour cell types, respectively. In particular, no significant removal of the major 1,2-d(GpG) intrastrand cisplatin crosslink is detected in five testicular-tumour cell lines, whereas a bladder-tumour cell line is proficient in the repair of this adduct. These results suggest that the extreme sensitivity of testicular tumours to cisplatin may be attributed to a deficient ability to repair cisplatin–DNA adducts. Moreover, deficient DNA-repair capacity appears to be a good indicator of response to cisplatin chemotherapy.
Taken together, the results detailed above implicate enhanced repair of cisplatin–DNA adducts as a very viable mechanism of cisplatin resistance. Increases in DNA repair are usually only 2–3-fold, however, even in cells that have acquired 20–500-fold levels of resistance, suggesting that cells may activate mechanisms other than repair in order to achieve very high degrees of resistance. Enhanced repair is not always associated with cisplatin resistance. In fact, cisplatin-resistant cells have been described that are deficient in cisplatin-adduct removal [13].

**Increased tolerance of cisplatin damage**

An alternative strategy that cells may use to protect themselves against cisplatin is to develop an increased tolerance of cisplatin–DNA damage. Indeed, several cisplatin-resistant cell lines appear able to tolerate high levels of cisplatin–DNA adducts [14]. For resistant cells that have little or no enhanced capacity for DNA repair, this mechanism may be particularly significant. One way that resistant cells may tolerate damage is through enhanced replicative bypass of cisplatin lesions. Recently, it has been shown that calf thymus DNA polymerase β efficiently bypasses the major 1,2-d(GpG) cisplatin adduct in vitro. Thus, increased expression of this enzyme in cisplatin-resistant cells may enhance replicative bypass of cisplatin adducts. In support of this view, increased levels of DNA polymerase β exist in some cisplatin-resistant tumour cells. Further studies are needed in order to elucidate the mechanisms by which cisplatin-resistant cells are able to tolerate high levels of cisplatin-induced damage.

**Altered expression of regulatory proteins**

The altered expression of regulatory proteins by tumour cells may also contribute to cisplatin resistance. For example, cisplatin-resistant cells have demonstrated increased expression of the proto-oncogenes c-fos, c-myc and H-ras. In addition, the tumour-suppressor gene p53 may play a significant role in cellular drug sensitivity. The protein encoded by p53 is a transcriptional regulator that, in response to DNA damage, can regulate the cell cycle and, in some cell types, activate apoptosis. Significantly, functional p53 is required for the induction of apoptosis by various anti-cancer agents [15]. Thus inactivation of p53 could lead to resistance to cisplatin and other DNA-damaging agents. It is well established that cells treated by cisplatin can die by apoptosis and several studies have examined the role of p53 in cisplatin resistance/sensitivity. Lymphoma cell lines expressing mutant p53 exhibited a decreased sensitivity to cisplatin and a reduced ability to undergo apoptosis compared with lines expressing wild-type p53 [16]. Furthermore, inherently cisplatin-sensitive testicular-tumour cells, which rarely exhibit p53 mutations [17], readily undergo drug-induced apoptosis [18]. These results suggest that the extreme sensitivity of testicular tumours to cisplatin may be derived from their predominantly wild-type p53 genotype. Taken together, the observations
described above indicate that \( p53 \) gene status is an important determinant of cisplatin resistance/sensitivity in tumour cells.

**Conclusions and outlook**

Testicular cancer, in contrast to most other types of solid tumour, is curable by cisplatin-based chemotherapy. Extending the success of cisplatin in the treatment of testicular cancer to other cancers will require a full understanding of the mechanism of action of the drug and its specificity for testicular tumours. Fortunately, progress has been made in recent years. DNA appears to be the critical cellular target for cisplatin, and the drug–DNA adducts, which severely distort the DNA structure, are believed to be responsible for the anti-tumour effects of the drug. It is interesting that \textit{trans}-DDP, the geometric isomer of cisplatin, also binds to DNA, but is ineffective as a chemotherapeutic agent. This differential toxicity may stem from the formation of a different spectrum of adducts by the two compounds as well as differential processing by DNA-repair factors and other cellular proteins, such as the HMG-domain proteins. The mechanisms responsible for the development of cisplatin resistance are beginning to be elucidated. Finally, studies of testicular-tumour cells suggest that the exquisite sensitivity of this tumour cell type to cisplatin may be related to a reduced capacity to repair cisplatin-induced DNA damage. As a second factor, lethality may also be enhanced by a predominantly wild-type \( p53 \) genotype that poises the cells to undergo programmed cell death, apoptosis, when confronted with DNA damage.

In the 20 years since the first clinical use of cisplatin, several new platinum compounds have been identified. Carboplatin (see Figure 1), a cisplatin analogue that is in use clinically, has reduced systemic toxicity compared with cisplatin, owing to its slower rate of hydrolysis. A promising new set of drugs, the platinum (IV) dicarboxylate compounds, such as JM216 (see Figure 1), has been developed recently. These compounds, which are currently undergoing clinical trials, are reduced \textit{in vivo} to the corresponding Pt(II) species and, unlike cisplatin, can be administered orally to patients [19]. Because of their similarity in structure to cisplatin, both carboplatin and the platinum (IV) dicarboxylate drugs form a spectrum of DNA adducts that is nearly identical to that of cisplatin and, thus, they have activity against the same subset of tumours as the parent drug. At present, there is a search for platinum anti-cancer drugs that are effective against a broader range of tumour types than cisplatin. Towards this end, it has been discovered that platinum compounds of the formula \( \textit{cis} \cdot \text{Pt(NH}_3)_2\text{(Am)Cl}^+ \), where Am is a derivative of pyrimidine (see Figure 1), form only monofunctional DNA adducts, yet demonstrate anti-tumour activity [20]. Since these compounds form lesions that are distinct from those of cisplatin, it is possible that these new agents may have activity
against tumours that are inherently resistant to cisplatin. We eagerly await the elucidation of the mechanism of action of this class of platinum compounds.

Finally, it is evident from the foregoing discussion that much has been learned about the unusual ways that cells respond to cisplatin. For example, the binding of proteins to adducts with the outcome of enhanced lethality is a novel notion that has stimulated thinking towards the design of a new generation of anti-cancer drugs. Recently, progress has been made with the synthesis of novel toxins that selectively kill cancer cells, based upon the lessons learned from cisplatin [21].

Summary

- Cisplatin is a widely used anti-cancer drug that is exceptionally effective against testicular cancer. trans-DDP, the geometric isomer of cisplatin, is ineffective as a chemotherapeutic agent.
- The anti-tumour activity of cisplatin is generally attributed to its formation of DNA adducts, both intrastrand and interstrand crosslinks, which induce structural distortions in DNA.
- The DNA adducts of cisplatin are thought to mediate its cytotoxic effects by inhibiting DNA replication and transcription and, ultimately, by inducing programmed cell death, or apoptosis.
- The adducts of both cis- and trans-DDP are removed from DNA by the nucleotide-excision-repair pathway.
- Cellular proteins possessing certain DNA-binding motifs, including the HMG domain, bind selectively to DNA modified by cisplatin, but not to DNA adducts of trans-DDP; evidence suggests a possible role for these proteins in modulating cisplatin cytotoxicity.
- Both intrinsic and drug-induced resistance often limit the success of cisplatin; several specific mechanisms of cisplatin resistance have been identified.

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Further reading

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