

## 48 ALKYLATING AGENTS AND PLATINUM ANTITUMOR COMPOUNDS

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The alkylating agents and the platinum antitumor compounds form strong chemical bonds with electron-rich atoms (nucleophiles), such as sulfur in proteins and nitrogen in DNA. Although these compounds react with many biologic molecules, the primary cytotoxic actions of both classes of agents appear to be the inhibition of DNA replication and cell division produced by their reactions with DNA. However, the chemical differences between these two classes of agents produce significant differences in their antitumor and toxic effects.

### ALKYLATING AGENTS

The alkylating agents were the first nonhormonal drugs to be used effectively in the treatment of cancer, and the story behind the recognition of the antitumor effects of these compounds is a remarkable one. During World War I, toxic gases were used as military weapons. The most devastating of these gases was sulfur mustard (Fig. 48.1). The compound was used as a weapon because of its vesicant effects, which produce skin irritation, blindness, and pulmonary damage. However, it was observed that troops and civilians who were exposed to sulfur mustard also developed bone marrow suppression and lymphoid aplasia. Because of these findings, sulfur mustard was evaluated as an antitumor agent.<sup>1</sup> The closely related, but less toxic, nitrogen mustards of World War II vintage were selected for further study. Trials in patients with lymphoma demonstrated regression of tumors, with relief of symptoms.<sup>2-4</sup> These results encouraged the search for nitrogen mustards that were more effective and less toxic and stimulated efforts to find other chemicals with antitumor activity.

**CHEMISTRY OF THE ALKYLATING AGENTS** The alkylating agents are compounds that react with electron-rich atoms in biologic molecules to form covalent bonds. Traditionally, these agents have been divided into two types: those that react directly with biologic molecules and those that form a reactive intermediate, which then reacts with the biologic molecules. These types are termed SN1 and SN2, respectively, and are illustrated in Figure 48.2. The terms refer to the kinetics of the reactions; the rate of reaction of an SN1 agent is dependent only on the concentration of the reactive intermediate, whereas the rate of reaction of an SN2 agent is dependent on the concentration of the alkylating agent and of the molecule with which it is reacting. This distinction has important implications in understanding the cellular and molecular pharmacology of specific alkylating agents. The nitrogen mustards and nitrosoureas are examples of SN1 agents, whereas busulfan is an SN2 agent.

A large number of chemical compounds are alkylating agents under physiologic conditions, and a variety of such compounds have been found to have antitumor activity. Although it is not possible to describe all of the compounds that have been used clinically, those compounds that are currently used look promising in clinical trials or represent a type of alkylating agent will be discussed.

**TYPES OF ALKYLATING AGENTS Nitrogen Mustards.** The most frequently used alkylating agents are the nitrogen mustards. Although thousands of nitrogen mustards have been synthesized and tested, only five are commonly used in cancer therapy today. These are mechlorethamine (the original "nitrogen mustard"), cyclophosphamide, ifosfamide, melphalan, and chlorambucil, and they are illustrated in Figure 48.3. The characteristic chemical constituent of the nitrogen mustards is the bischloroethyl group, and all of the nitrogen mustards react

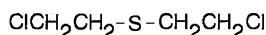


Figure 48.1. Structure of sulfur mustard (bischloroethylsulfide).

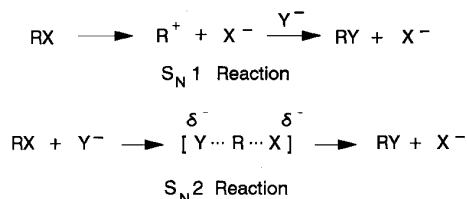


Figure 48.2. SN1 and SN2 reactions of alkylating agents.

through an aziridinium intermediate as shown in Figure 48.4. The remainder of the molecule is important in determining the physical properties of the molecule and affects the transport, distribution, and reactivity of the specific agents. The importance of the total molecule is demonstrated by cyclophosphamide.

Cyclophosphamide is not a reactive compound, but it undergoes activation in the body. The complex activation scheme<sup>5</sup> is shown in Figure 48.5. The initial activation reaction is carried out by cytochrome P450 mediated microsomal oxidation in the liver to produce 4-hydroxycyclophosphamide, which is in spontaneous equilibrium with the tautomer, aldophosphamide.<sup>6</sup> At physiologic pH, this equilibrium is predominantly in the form of 4-hydroxycyclophosphamide.<sup>7</sup> This equilibrium mixture diffuses from the hepatocyte into the plasma and is distributed throughout the body. Since 4-hydroxycyclophosphamide is relatively nonpolar, it enters target cells readily by diffusion. Aldophosphamide spontaneously decomposes to produce phosphoramidate mustard, which is the first reactive alkylating agent produced in the metabolism of cyclophosphamide. Although phosphoramidate mustard is also produced extracellularly, this compound is very polar, and enters cells poorly, and phosphoramidate mustard in the plasma probably plays a minor role in the therapeutic and toxic effects of cyclophosphamide. Thus, 4-hydroxycyclophosphamide/aldophosphamide serves as an efficient mechanism to deliver the alkylating phosphoramidate mustard into cells. Recent evidence suggests that after one of the chloroethyl groups of phosphoramidate mustard cyclizes to form a chloroethyl aziridinium moiety, the molecule cleaves to produce chloroethylaziridine.<sup>8</sup> Accordingly, free chloroethylaziridine may contribute significantly to the alkylation and cross-linking of DNA by cyclophosphamide.

The toxic compound acrolein was demonstrated to be produced by the metabolism of cyclophosphamide by Alarcon,<sup>9</sup> but administration of didechlorocyclophosphamide, a compound that could produce acrolein but not the chloroethyl alkylating species, did not demonstrate antitumor activity in an animal model.<sup>10</sup> In 1992, Lee and colleagues<sup>11</sup> reported that a decrease in the enzyme O6-alkylguanine-alkyltransferase in circulating lymphocytes was produced by the administration of high doses of cyclophosphamide for bone marrow

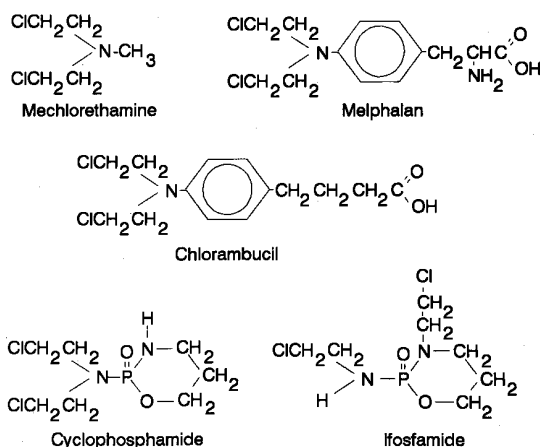


Figure 48.3. Structures of nitrogen mustards currently used in therapy.



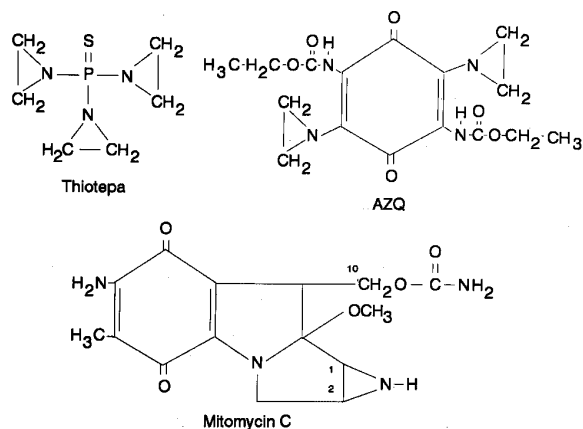


Figure 48.6. Structures of aziridine alkylating agents.

exposure to TEPA may exceed those of thiotepa.<sup>45</sup> The AUC exposure to thiotepa has been shown to correlate with the degree of myelosuppression in patients, whereas the AUC exposure to TEPA did not.<sup>45</sup> Some studies have suggested that a metabolite is produced that is more reactive than the parent compound.<sup>46,47</sup> However, such a metabolite has not been characterized, and the activity of thiotepa may be enhanced by low pH within tumor cells. At the lower pH, the aziridine ring will be protonated and more reactive.

Mitomycin C is a natural product that has been used in the treatment of breast cancer and cancers of the gastrointestinal tract.<sup>48-50</sup> This compound contains an aziridine ring and appears to exert its cytotoxic effect through the cross-linking of DNA.<sup>51,52</sup> Mitomycin C undergoes reduction in the cell, with enhancement of the affinity of the carbon-1 atom of the aziridine ring for nucleophiles, such as the extracyclic nitrogen atom on guanylic acid in DNA. Following this alkylation, there is displacement of the activated carbamate group on the 10 carbon atom of mitomycin C by an extracyclic amino nitrogen of a guanylic acid molecule on the complementary DNA strand to produce an interstrand DNA cross-link.<sup>53-55</sup>

AZQ was designed to be sufficiently lipophilic to readily cross the blood-brain barrier for the treatment of CNS tumors.<sup>56</sup> It has demonstrated clinical activity against brain tumors,<sup>57</sup> other solid tumors, and leukemia.<sup>58</sup> AZQ has been shown to undergo reduction of the quinone ring in cells. This reduction results in protonation of the aziridine rings and enhancement of reactivity of the compound.<sup>59,60</sup>

The epoxides, such as dianhydrogalactitol<sup>61,62</sup> (Fig. 48.7), are chemically related to the aziridines and alkylate through a similar mechanism of attack of a nucleophile, such as an amino nitrogen, on a carbon of a strained three-member ring. Dibromodulcitol<sup>63</sup> is hydrolyzed to dianhydrogalactitol and thus is a pro-drug to an epoxide.<sup>64</sup>

**Alkyl Sulfonates.** The alkyl alkane sulfonate busulfan (Fig. 48.8) was one of the earliest alkylating agents.<sup>65</sup> This compound is one of the few currently used agents that clearly alkylate through an SN<sub>2</sub> reaction, as shown in Figure 48.9. Hepsulfam, an alkyl sulfamate analogue of busulfan with a wider range of antitumor activity in preclinical

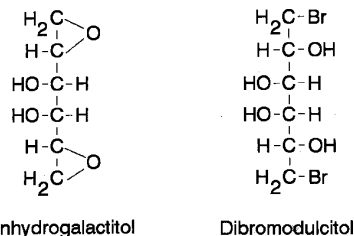


Figure 48.7. Structures of an epoxide alkylating agent (dianhydrogalactitol) and an epoxide prodrug (dibromodulcitol).

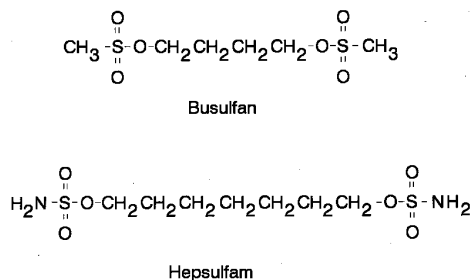


Figure 48.8. Structure of alkyl sulfonate (busulfan) and alkyl sulfamate (hepsulfam) agents.

studies,<sup>66</sup> has been evaluated in clinical trials but thus far has demonstrated no superiority to busulfan. Busulfan has a most interesting, but poorly understood, selective toxicity for early myeloid precursors.<sup>67,68</sup> This selective effect is probably responsible for its activity against chronic myelocytic leukemia (CML).<sup>69,70</sup>

The use of busulfan as first-line therapy for the treatment of CML has been succeeded by the use of the less toxic hydroxyurea. The current major use of busulfan is as a component of bone marrow ablative regimens for bone marrow and stem cell transplantation of patients with acute myeloid leukemia and other malignancies.<sup>71,72</sup>

**Nitrosoureas** The nitrosoureas are a class of alkylating agents that have received considerable attention during the past 3 decades.<sup>73-75</sup> Several nitrosoureas currently in clinical use or clinical trials are shown in Figure 48.10. These compounds decompose to produce alkylating compounds under physiologic conditions. Although there are several mechanisms by which this may occur, the predominant mechanism is that shown in Figure 48.11, a base catalyzed decomposition to a chloroethyl diazonium moiety,<sup>76</sup> which has been shown to react with DNA,<sup>77,78</sup> as discussed below.

Carmustine (BCNU) was the first agent to demonstrate significant activity against a preclinical model of intracerebral tumor<sup>74</sup> and is currently used for the treatment of primary brain tumors<sup>79</sup> and in the treatment of multiple myeloma.<sup>80</sup> Lomustine (CCNU) and Semustine (methyl CCNU) demonstrated greater activity against solid tumors in preclinical studies.<sup>81</sup> CCNU is used in the treatment of CNS tumors<sup>82,83</sup> and lymphomas,<sup>84,85</sup> and methyl CCNU has been used particularly in the treatment of gastrointestinal tumors.<sup>86,87</sup> ACNU, which is more water soluble than most of the other nitrosoureas, has been employed for the intra-arterial and intrathecal treatment of CNS tumors<sup>88,89</sup> and the treatment of solid tumors.<sup>90</sup> The clinical use of the nitrosoureas has been limited by marked and prolonged hematopoietic toxicity and by renal toxicity. The development of nitrosoureas with a higher therapeutic index, such as fotemustine<sup>91,92</sup> and others,<sup>93,94</sup> remains a very active area of endeavor.

**TRIAZENES, HYDRAZINES, AND RELATED COMPOUNDS** These are nitrogen-containing compounds that spontaneously decompose or can

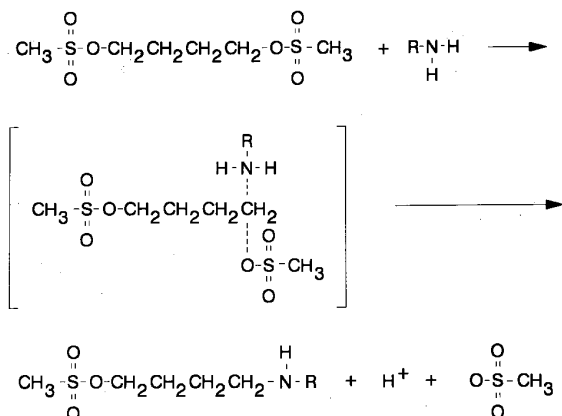


Figure 48.9. Mechanism of alkylation by busulfan.

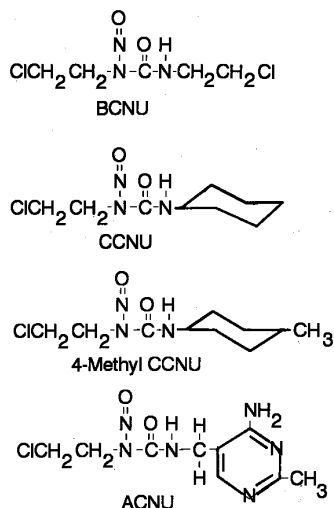


Figure 48.10. Structures of nitrosoureas.

be metabolized to produce alkyl diazonium intermediates that alkylate biologic molecules. Procarbazine and dacarbazine, which are illustrated in Figure 48.12, are metabolized to reactive intermediates that decompose to produce methyl diazonium, which methylates DNA.<sup>95</sup> The metabolism of procarbazine is complex, and there are different pathways through which a reactive methyl group can be produced.<sup>95</sup> It is most likely that the pathway responsible for the DNA methylation and cytotoxicity is the generation of methylazoxypcarbazine.<sup>96,97</sup> The activation of dacarbazine via N-methyl oxidation by a microsomal P450 enzyme is illustrated in Figure 48.13.<sup>98,99</sup> Both procarbazine and dacarbazine are used in the treatment of Hodgkins disease<sup>100,101</sup>; procarbazine is a component of combination regimens used for the treatment of primary brain tumors,<sup>102</sup> and dacarbazine is used in the treatment of melanoma.<sup>103,104</sup> Procarbazine was originally developed as a monoamine oxidase inhibitor, and it can produce CNS depression and acute hypertensive reactions after the ingestion of tyramine-rich foods.<sup>105</sup>

Temozolomide (see Fig. 48.13) spontaneously decomposes under physiologic conditions to produce the same active metabolite produced by DTIC.<sup>106</sup> Temozolomide, which is administered orally, has demonstrated antitumor activity against gliomas and melanomas in phase I and II trials<sup>107–109</sup> and is now approved for glioma treatment in the United States and Europe.

**Hexamethylmelamine.** Hexamethylmelamine (Fig.48.14) is an active antitumor agent that has been considered to be acting as an alkylating agent because the methyl groups are required for antitumor activity. The methyl groups are hydroxylated with subsequent demethylation

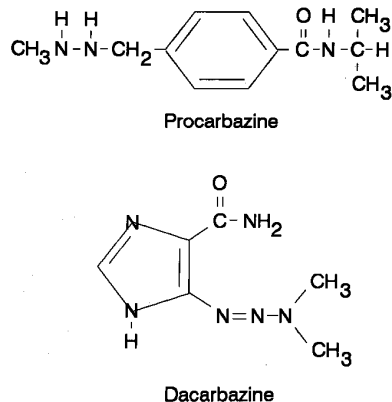


Figure 48.12. Structures of monofunctional alkylating agents.

in vivo,<sup>110,111</sup> a reaction that can generate a reactive methyl group. Analogues in which the methyl groups are hydroxylated are also active.<sup>112,113</sup> Few studies of the cross-resistance of this agent have been carried out, but one study<sup>114</sup> found that O-6-alkylguanine-alkyltransferase was not inactivated in vivo by hexamethylmelamine, as would be expected from an O-6 guanyl methylating agent. Therefore, the mechanism of cytotoxic activity of hexamethylmelamine remains in question. The agent does have significant antitumor activity against ovarian cancer<sup>115</sup> and is used primarily in the third-line treatment of that tumor.

**DECOMPOSITION AND METABOLISM** The alkylating agents react with water and are inactivated by this hydrolysis. The alkylating agents also are inactivated by reaction with thiols, such as glutathione. The reaction of alkylating agents with glutathione can be increased by the glutathione S-transferase enzymes, as will be discussed below in mechanisms of cellular resistance. The alkylating agents also undergo microsomal and other types of xenobiotic metabolism. Such metabolism may activate agents, as described above, inactivate them, or change their physical properties without inactivating them. Nitrosoureas have been reported to be denitrosated and inactivated by microsomal metabolism.<sup>116,117</sup>

Chlorambucil is metabolized to bischloroethylphenylacetic acid, which is an active alkylating agent, and probably contributes to the therapeutic and toxic effects of chlorambucil.<sup>118,119</sup> Mitomycin C must be reductively activated intracellularly to alkylate DNA bases and cross-link the DNA, and glutathione appears to play a role in this process.<sup>120</sup> **MECHANISM OF CYTOXICITY** Although the alkylating agents react with a number of biologic molecules, including amino acids, thiols, RNA, and DNA, a number of lines of evidence have led to the generally accepted conclusion that the cytotoxic effects of the agents are due to reactions with DNA. Bifunctional agents are much more effective antitumor agents than monofunctional agents, but addition of more than two alkylating groups does not further increase the cytotoxic activity. These observations<sup>121</sup> and the early studies of Brookes and Lawley<sup>122,123</sup> led to the suggestion that interstrand cross-linking of DNA was responsible for the cytotoxic activity of the bifunctional alkylating agents. A good correlation has been shown between cytotoxicity and the formation of interstrand cross-links by bifunctional

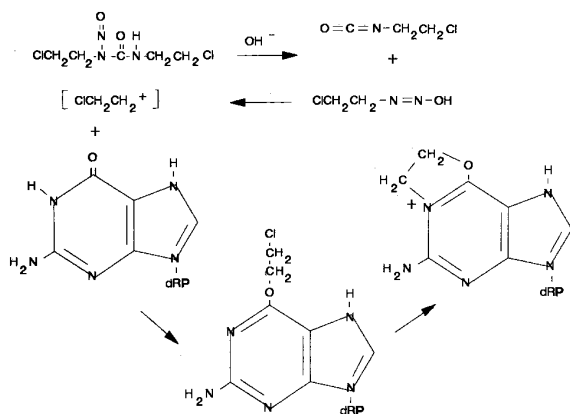


Figure 48.11. Mechanism of nitrosourea activation and alkylation of deoxyguanylic acid.

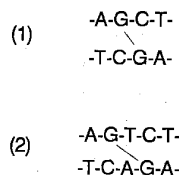
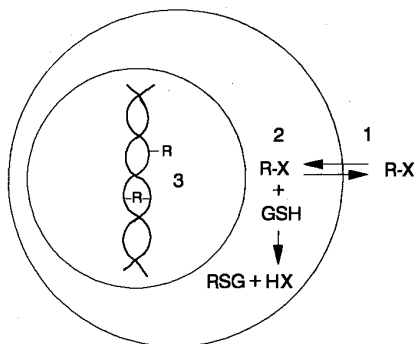


Figure 48.13. Interstrand cross-linking of DNA by nitrogen mustards. (1) Site of crosslinking proposed by Brookes.<sup>65</sup> (2) Site of crosslinking found by Loechler<sup>330</sup> and Hopkins.<sup>307</sup>

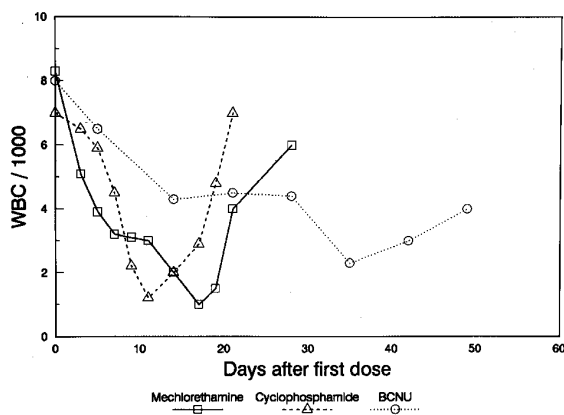


1. Decreased entry into or increased exit of agent from cell
2. Inactivation of agent in cell
3. Enhanced repair of DNA lesions produced by alkylation

**Figure 48.14.** Mechanisms of resistance to alkylating agents.

alkylating agents. The alkaline elution technique developed by Ewis and Kohn<sup>124</sup> has been especially important in these studies. More recently, nitrogen mustard interstrand cross-links in oligonucleotides have been chemically characterized.<sup>125-127</sup>

Although the alkylating agents can react with virtually all of the nitrogens in the DNA bases, there is selectivity, based on the electron density of the nitrogens and the local structure of the DNA. The nitrogen mustards react most readily with the N-7 position of guanylic acid.<sup>128</sup> This nitrogen atom has a high electron density, which has been proposed to be enhanced by base stacking in the DNA helical structure.<sup>122</sup> Brookes and Lawley suggested that the nitrogen mustard cross-link in DNA was between the N-7 guanine atoms in base-paired G-C sequences in DNA.<sup>122</sup> However, more recent studies have found the nitrogen mustard cross-link to occur between the N-7 atoms of guanylic acids in a G-X-C sequence, as illustrated in Figure 48.15.<sup>125-127</sup> The cross-linking of mitomycin C between two extracyclic guanylic acid amino groups is described above.<sup>53</sup> This site of cross-linking may be determined by the orientation of mitomycin C in the minor groove of DNA.<sup>54</sup> The reactive species of the nitrosoureas is more reactive than the aziridiniums of the nitrogen mustards and initially alkylates the O-6 position of guanylic acid.<sup>129,130</sup> According to a mechanism proposed by Ludlum, after a series of rearrangements involving a reactive cyclic five-membered intermediate of the N-1, C-6, and O-6 atoms of guanylic acid and two carbons from the chloroethyl group of the nitrosourea (see Fig. 48.11), a cross-link is formed between N-1 of guanylic acid and N-3 of a cytidylic acid on the complementary DNA strand.<sup>131,132</sup> Such a cross-link has been demonstrated to occur in an oligonucleotide treated with BCNU.<sup>133</sup>



**Figure 48.15.** Hematopoietic toxicity of alkylating agents.

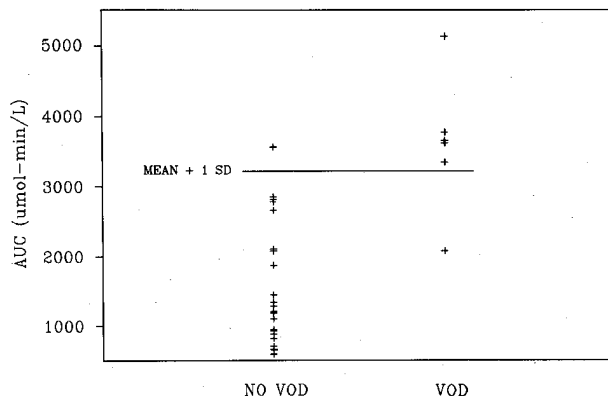
Alkylating agents such as methylnitrosourea, procarbazine, and dacarbazine are not bifunctional and produce methylation of DNA, predominantly on the O-6 and N-7 positions of guanylic acid. These lesions can produce both spontaneous and enzyme mediated single-strand breaks,<sup>134,135</sup> which are cytotoxic. However, it has now been demonstrated, initially by Modrich and colleagues,<sup>136,137</sup> that active mismatch DNA repair is a major mediator of the cytotoxicity of mono-functional alkylating agents.

**CELLULAR RESISTANCE TO ALKYLATING AGENTS** Cellular resistance to antitumor agents is a critical determinant of the effectiveness of therapy. Resistance mechanisms in normal tissues provide selectivity and an improved therapeutic index. Resistance of tumor cells allows these cells to escape the effects of therapy. Consideration of the pharmacology and chemistry of the alkylating agents predicts four general types of cellular resistance to alkylating agents (Fig. 48.16). These are (a) decreased uptake of agents into or increased export out of the cell, (b) increased inactivation of agents in the cell, (c) enhanced repair of the DNA damage produced by the alkylating agents, and (d) the absence of cellular mechanisms that produce cytotoxicity in response to DNA damage. All four of these mechanisms have been described. Resistance of tumor cells to mechlorethamine can occur on the basis of decreased transport into the cell,<sup>138,139</sup> and it has also been demonstrated that certain cells resistant to melphalan have decreased active transport of the agent and of amino acid.<sup>140,141</sup> Most alkylating agents enter cells by diffusion, however, and the alkylating agents, with the exception of mitomycin C, are not substrates for the multiple drug resistance export systems.

The second mechanism of cellular resistance to alkylating agents is intracellular inactivation of the agent. As discussed above, the enzyme aldehyde dehydrogenase detoxifies the primary metabolites of cyclophosphamide and ifosfamide, and the presence of this enzyme in bone marrow precursor cells and gastrointestinal epithelial cells protects these organs from toxicity of the agents. Aldehyde dehydrogenase has also been demonstrated to be a mechanism of cyclophosphamide resistance of murine,<sup>142</sup> rat,<sup>143</sup> and human leukemia cells<sup>144</sup> and human ovarian,<sup>145</sup> colon,<sup>146</sup> and breast<sup>147</sup> cancer cells.

An association between cellular resistance to alkylating agents and increased cellular levels of glutathione<sup>148-150</sup> and the enzyme glutathione transferase has been described by a number of investigators.<sup>151-154</sup> Glutathione (GSH) is a thiol-containing tripeptide that is present at millimolar concentrations in many cells, reacts with electrophilic (electron-deficient) molecules, and protects cells from such electrophiles.<sup>155-157</sup> Mulcahy and colleagues<sup>158</sup> have demonstrated that increased GSH in cells resistant to melphalan can be related to increased transcription of gamma-glutamylcysteine synthetase, the enzyme that catalyzes the rate-limiting step in de novo synthesis of GSH.

Although most electrophiles of biologic significance react spontaneously with glutathione, glutathione S-transferases catalyze the reaction between glutathione and electrophiles. The glutathione conjugates of several alkylating agents have been characterized<sup>159-161</sup> and their formation shown to be enhanced by a glutathione S-transferase.



**Figure 48.16.** Relationship between plasma AUC of busulfan and occurrence of veno-occlusive disease of the liver. (From Grochow et al.<sup>183</sup>)

There are three principal isozymes of glutathione S-transferase, and recent studies indicate that specific isozymes may catalyze the conjugation of different alkylating agents. The alpha isozyme of GST has been found to catalyze the glutathione conjugation of the aziridinium forms of melphalan,<sup>162</sup> chlorambucil,<sup>163</sup> and phosphoramide mustard.<sup>164</sup> The GSH conjugation of 4-hydroxycyclophosphamide<sup>165</sup> was found to be enhanced by all three classes of GST. The mu isozyme has been implicated in the inactivation of BCNU.<sup>166</sup> At this time it, is evident that glutathione alone or glutathione plus an appropriate glutathione S-transferase can render cells resistant to alkylating agents and that this mechanism is probably an important mechanism of resistance to electrophilic antitumor drugs, such as the alkylating agents and platinum compounds.

Several investigators have demonstrated that buthionine sulfoxime (BSO), an inhibitor of glutathione synthesis, can reduce cellular glutathione levels and sensitize tumors to alkylating agents *in vitro* and *in vivo*.<sup>167-169</sup> However, normal cells can also be sensitized by BSO administration<sup>170,171</sup> to produce significant toxicity. BSO in combination with alkylating agents is now undergoing clinical trials. Phase I trials of the combination of BSO and melphalan have demonstrated increased myelotoxicity and depletion of tumor GSH, compared with the same dose of melphalan alone.<sup>172-174</sup> Phase II trials are in progress to determine if the tumor response rate is greater with the addition of BSO to melphalan.

Inhibitors of glutathione S-transferases have been shown to enhance the cytotoxicity of melphalan on cells resistant to alkylating agents,<sup>175</sup> and such inhibitors are being examined in clinical trials. A phase I trial of the GST inhibitor sulfasalazine<sup>176</sup> with melphalan doses of 20 mg/m<sup>2</sup> and greater demonstrated reductions of glutathione and GST levels in the peripheral mononuclear cells of some patients, and the main toxicity of the combination was nausea and vomiting. Increased myelosuppression was not seen.

An association between increased cellular concentrations of metallothionein and resistance to platinum agents has been established<sup>177,178</sup> and is probably due to binding of the platinum agents to the multiple thiol groups of this cellular protein. Lazo and colleagues<sup>179</sup> found that transfection-induced increased cellular metallothionein also produced resistance to alkylating agents. Yu and colleagues and Wei and colleagues have demonstrated binding of melphalan<sup>180</sup> and phosphoramidate mustard<sup>181</sup> to thiol groups in metallothionein. Thus, increased metallothionein content of cells is another mechanism of inactivation of alkylating agents.

Since the cytotoxicity of the alkylating agents appears to be mediated through the alkylation of DNA, the repair of alkylation lesions is an obvious mechanism of resistance to these agents and has been the subject of intense investigation. The best-defined DNA repair resistance to alkylating agents is resistance to the nitrosoureas and other compounds that alkylate the 0-6 position of guanylic acid in DNA. The protein 0-6-alkylguanine-DNA-alkyltransferase (O6-AT) has been shown to remove alkyl groups from the 0-6 position of guanine and thus prevent the formation of an interstrand cross-link.<sup>129,131,182</sup> The removed alkyl group is covalently and irreversibly bound to the alkyltransferase so that the protein can catalyze the removal of only one alkyl molecule and is then rapidly catabolized. It is now obvious that elevated O6-AT is a major mechanism of resistance to nitrosoureas in human gliomas<sup>183-185</sup> and other human tumors.<sup>186-188</sup>

The fact that O6-AT is irreversibly inactivated by the transfer to it of an alkyl group from the 0-6 position of guanine provides an approach to counteracting this mechanism of resistance. If cells are treated with a monofunctional 0-6 alkylating agent, such as streptozotocin, there follows a period when the 0-6 alkyltransferase activity is decreased. This decrease in activity is due to the removal of alkyl groups from the 0-6 guanine sites on the DNA and a subsequent reduction of the level of active enzyme before enzyme synthesis can restore functional levels of the enzyme. If the cells are treated with a nitrosourea (or other 0-6 guanine alkylating agent) during this period of decreased 0-6 alkyltransferase, the cells are more sensitive to nitrosourea.<sup>189</sup> The enzyme will also remove 0-6 benzyl groups from acid-soluble guanine analogs, and compounds such as 0-6 benzylguanine, administered prior to nitrosoureas, will reverse alkyltransferase

resistance in cells and animal models,<sup>190-192</sup> and clinical trials with 0-6 benzylguanine are now in progress.<sup>193-195</sup>

Although O6-BG and related compounds can reverse tumor resistance to nitrosoureas and methylating agents such as temozolamide, the bone marrow toxicity of these agents is increased by O6-BG. However, human O6-AT enzymes that are resistant to the combination of an alkylating agent and O6-BG have been isolated and transfected into cell lines.<sup>196,197</sup> It has been shown in a mouse model that transfection of such an enzyme into hematopoietic cells in mice produces protection of the bone marrow from the cytotoxicity of the combination of BCNU and O6-BG,<sup>196</sup> and currently similar studies are planned in patients.

Removal of interstrand cross-links from DNA in cells can be shown to occur in studies using alkaline elution and other techniques.<sup>198</sup> Friedman and colleagues have described a human medulloblastoma cell that is resistant to cyclophosphamide on the basis of repair of the interstrand cross-links.<sup>199</sup> This cell does not appear to repair the BCNU or busulfan cross-link, suggesting that the recognition and repair of interstrand cross-links are quite structure specific.

Recently, evidence has been presented that poly(ADP ribose) polymerase is involved in the repair of nitrogen mustard lesions.<sup>200</sup> Also, there is good evidence that cells that react to alkylation damage by arresting in the G2 phase of the cell cycle can repair DNA during this period and are more resistant to alkylating agents than cells that proceed through mitosis despite alkylation damage. A human tumor cell line has been described that exhibits G2 arrest in response to alkylating damage and demonstrates increased resistance to nitrogen mustard.<sup>201</sup> This cell line was found to have increased accumulation of phosphorylated (and inactivated) cdc2 kinase associated with G2 arrest after nitrogen mustard treatment. This alteration should allow repair of DNA damage before the cell enters mitosis. This mechanism of resistance to alkylating agents is probably important for tumor cells but also may provide a degree of drug specificity for many other tumors, because normal cells may be more likely to exhibit this protective mechanism. Inhibitors of DNA repair have been shown to enhance the cytotoxicity of alkylating agents,<sup>202-204</sup> and some of these inhibitors are being examined in clinical trials. It seems likely that increased understanding of the DNA repair process will allow more effective use of alkylating agents.

**In Vivo Resistance.** Murine tumors that are resistant to alkylating agents *in vivo*, but not *in vitro*, have been reported.<sup>205,206</sup> Further studies of these tumors that are resistant to cyclophosphamide, cisplatin, and thiotepa *in vivo* have demonstrated that the tumors are also resistant to these agents in three-dimensional *in vitro* culture but not in two-dimensional *in vitro* culture.<sup>207</sup> Such resistance may be acquired rapidly after drug exposure<sup>208</sup> and may be associated with enhanced metastatic properties.<sup>209</sup> The mechanisms responsible for this type of resistance have not yet been established. There may be differences between known cellular resistance factors or between membrane properties in the three-dimensional milieu, compared with the two-dimensional configuration, and adhesion molecules may alter drug sensitivity. Other potential mechanisms for drug resistance *in vivo* are poor perfusion of the tumor and changes in the intracellular pH.<sup>210</sup>

**CLINICAL PHARMACOLOGY Cyclophosphamide.** After the administration of a systemic dose of 50 mg/kg, plasma levels of the parent compound of up to 400 micromolar may be achieved and decay with a half-life of 3 to 10 hours.<sup>211-213</sup> The rate of metabolism of the parent compound varies considerably among individuals and can be modulated by the administration of compounds that affect the rate of microsomal metabolism, such as phenobarbital<sup>214</sup> or a previous dose of cyclophosphamide.<sup>215,216</sup> However, at conventional doses, the clearance rate of the parent compound does not appear to significantly affect the toxicity or therapeutic effect of the agent.<sup>217</sup> This independence of effect from the rate of metabolism is probably because the parent compound is not rapidly excreted and continues to be activated, so that the AUC for systemic exposure to the active metabolites is similar after a given dose.

At the higher doses currently used in bone marrow transplantation regimens, however, the plasma concentrations of cyclophosphamide

should be close to the capacity of the microsomal activating enzymes. Grochow and colleagues<sup>218</sup> demonstrated that in patients receiving 4 g/m<sup>2</sup> of cyclophosphamide over 90 minutes and achieving initial plasma concentrations of greater than 500 mM, saturable pharmacokinetics are seen. These investigators concluded that when the dosing rate equals or exceeds 4 g/m<sup>2</sup> in 90 minutes or the plasma concentration of cyclophosphamide exceeds 150 mM (the lowest Km seen in the patients), nonlinear disposition may occur, with variable exposure to the active metabolites. This study also confirmed previous reports that cyclophosphamide can induce its own metabolism.

Studies of pharmacokinetics of the critical metabolite 4-hydroxycyclophosphamide have been limited in the past by the difficulty of accurately measuring this labile molecule. However, more specific methods are now available, and the pharmacokinetics of this important metabolite are now being elucidated. Anderson and colleagues measured 4-hydroxycyclophosphamide in patient blood after cyclophosphamide administration, using a very specific gas chromatographic-mass spectrometric technique.<sup>219</sup> After a dose of cyclophosphamide of 110 mg/kg over 90 minutes, peak concentrations of 9 to 12 micromolar and AUCs of 105 to 110 micromolar hours were measured; a cyclophosphamide dose of 170 mg/kg given as a continuous infusion over 4 days produced plasma concentrations of 1 to 5 micromolar, with a total AUC of about 98 to 110 micromolar hours. Subsequent studies have found similar results.<sup>220,221</sup> All studies have found a considerable patient variation in the exposure to 4-hydroxycyclophosphamide after the same dose of cyclophosphamide and differences in the exposure and ratios of cyclophosphamide/4-hydroxycyclophosphamide each day when short-duration infusions are given on subsequent days. These findings are most likely to be due to differences in the cytochrome p450 complements in patients and the differing exposures to drugs that modulate the activities of these enzymes. These findings indicate that pharmacokinetically guided dose adjustment will be the best method to produce consistent patient exposures to the active metabolites of cyclophosphamide.

The majority of a dose of cyclophosphamide (< 70%) is excreted in the urine as the inactive metabolite carboxyphosphamide.<sup>222,223</sup> Renal function does not significantly affect the toxicity of cyclophosphamide,<sup>224</sup> most likely because spontaneous decomposition, and not renal excretion, determines the clearance of the principal active metabolites.

The clinical pharmacology of ifosfamide has been much less studied but is similar to that of cyclophosphamide, except that microsomal activation is somewhat slower, and chloroethyl side chain oxidation plays a greater role in its metabolism.<sup>225-227</sup> Thus, for a dose of ifosfamide, lower systemic concentrations of the 4-hydroxy metabolite are achieved than for the same dose of cyclophosphamide.<sup>228</sup> Both cyclophosphamide and ifosfamide are well absorbed after oral administration.<sup>229</sup> Boddy and colleagues<sup>17</sup> have demonstrated that ifosfamide, like cyclophosphamide, can autoinduce its own metabolism. Because of the greater and more variable side chain oxidation of ifosfamide, differences in the P450 drug metabolizing enzymes between individuals, and the modulation of these enzymes by concomitantly administered agents, may play a greater role in altering the clinical effects of ifosfamide than cyclophosphamide.<sup>230-232</sup>

**Melphalan.** Alberts and colleagues found that peak plasma levels of 4 to 13 micromolar were present after intravenous administration of a 0.6-mg/kg dose of melphalan, and the half-life ( $t_{1/2}$ ) was 1.8 hours.<sup>233,234</sup> At this dose, the mean AUC for melphalan was 8 micromolar hours. Similar AUC per dose and pharmacokinetics have been demonstrated by other investigators after high intravenous doses of melphalan.<sup>235</sup> After conventional oral doses of 0.25 mg/kg, peak plasma levels of up to 0.625 micromolar were found.<sup>236</sup> There is variable systemic availability after oral dosing,<sup>237,238</sup> and it has been shown that oral administration of food with melphalan will inhibit absorption of the agent.<sup>239</sup> It has been reported that myelosuppression from melphalan is increased in patients with decreased renal function.<sup>240</sup> The half-life of melphalan is prolonged in anephric dogs,<sup>241</sup> and significant renal clearance of the parent compound in patients has been shown by Reece and colleagues.<sup>242</sup>

**Chlorambucil.** After the oral administration of 0.6 mg/kg of chlorambucil, peak levels of 2 to 6 micromolar parent compound were found at 1 hour by Alberts and colleagues.<sup>118,234</sup> Peak plasma levels of phenylacetic acid mustard of 2 to 4 micromolar occurred at 2 to 4 hours after chlorambucil administration. The plasma half-life ( $t_{1/2}$ ) of chlorambucil was 92 minutes and that of phenylacetic acid mustard was 145 minutes. At a dose of 0.6 mg/kg of chlorambucil, the plasma AUC of chlorambucil was 3 to 9 micromolar hours.<sup>118</sup> Similar values were found by Hartvig and colleagues,<sup>243</sup> who also found a two- to four-fold variation in systemic availability of chlorambucil and phenylacetic acid mustard after oral administration of chlorambucil.

**Thiotepa.** The pharmacokinetics of thiotepa have been studied by Cohen and colleagues,<sup>244</sup> after an intravenous injection of 12 mg/m<sup>2</sup>. Peak plasma levels of about 5 micromolar were achieved and were found to decay with a  $t_{1/2}$  of 7.7 minutes and a  $t_{1/2}$  of 125 minutes. The mean AUC was 9 micromolar hours. Plasma concentrations of TEPA of up to 1 micromolar were found and remained in plasma longer than thiotepa. Henner and colleagues<sup>245</sup> examined the plasma levels of thiotepa after 4-day continuous intravenous infusions of up to 900 mg/m<sup>2</sup>. Peak plasma levels of thiotepa of 7 micromolar were initially attained on the first day, and then the levels gradually decreased. Plasma AUC values of up to 600 micromolar hours were achieved. When given intraperitoneally, there is rapid loss of thiotepa from the intraperitoneal cavity and a concomitant increase in plasma levels to those associated with the same dose if given intravenously.<sup>246</sup> After intravenous injection, cerebrospinal fluid levels comparable with plasma levels are found.<sup>247</sup> Recent studies have indicated that the simultaneous administration of thiotepa and cyclophosphamide will result in lower exposure to the active metabolite of cyclophosphamide, 4-hydroxycyclophosphamide.<sup>218</sup>

**Nitrosoureas.** The pharmacokinetics of BCNU have been studied by Levin and colleagues<sup>248</sup> after intravenous infusion of 60 to 170 mg/m<sup>2</sup>, peak plasma concentrations of 5 micromolar were reached and then decayed with an initial half-life of 6 minutes and a second half-life of 68 minutes. Henner and colleagues<sup>249</sup> measured the pharmacokinetics of BCNU after intravenous doses of 600 mg/m<sup>2</sup>. The peak plasma level of ultrafilterable BCNU was found to be 4.7 micromolar and the mean AUC was 5.4 micromolar hours. The ultrafilterable BCNU was 23% of the total plasma BCNU. The pharmacokinetics of CCNU after administration of 130 mg/m<sup>2</sup> to patients have also been described.<sup>250</sup> The parent compound could not be detected in plasma, but the mono-hydroxylated metabolites, trans-4-hydroxy CCNU and cis-4-hydroxy CCNU, were found in a ratio of 6:4 and at total peak concentrations of about 3 micromolar. The plasma clearance half-lives of the hydroxy-CCNU metabolites varied from 1 to 3 hours between patients.

**Busulfan.** Because of its insolubility in aqueous solutions, busulfan has previously been available only as an oral preparation. However, an intravenous preparation has been recently made available<sup>251</sup> but has not been extensively used, so that most of the published pharmacokinetic information is after oral administration. For myeloablative therapy prior to bone marrow transplantation, busulfan is widely used at a dose of 1 mg/kg every 6 hours for 4 days. After a 1 mg/kg dose in adults and older children, there is a considerable variation in bioavailability, with peak plasma levels of 1 to 10 micromolar and elimination half-times between 1 and 7 hours.<sup>252-254</sup> The AUC after a single dose in adults and older children varies between 10 and 80 micromolar hours. However, in young children (age 1-3), the peak plasma concentrations are less (1-5 micromolar), the mean elimination time about 40% faster, and the AUC consistently less at 6 to 17 micromolar hours.<sup>255</sup> Grochow and colleagues<sup>252</sup> have demonstrated that AUCs of busulfan greater than one standard deviation from the mean values for all patients are associated with a very high risk of veno-occlusive disease of the liver (Fig. 48.17). It has now been demonstrated that pharmacokinetic guided adjustment of the busulfan dose can reduce the incidence and severity of this toxicity.<sup>256,257</sup>

**TOXICITIES** The characteristic toxicities of the alkylating agents are hematopoietic, gastrointestinal, gonadal, and CNS toxicity. However, each of the agents has a characteristic set of toxicities, determined by the reactivity, metabolism, and distribution of the agent, and the clinician should be aware of these idiosyncrasies of the agents.

**Hematopoietic Toxicity.** In general, the clinical dose-limiting toxicity for alkylating agents is hematopoietic toxicity, particularly suppression of granulocytes and platelets. The nadir of granulocyte depression after alkylating agents is usually 8 to 16 days, and the granulocytes usually return to normal within 20 days after a single dose of the agent.<sup>258</sup> Cyclophosphamide and ifosfamide are less hematopoietically toxic than other alkylating agents<sup>258,259</sup>; granulocyte levels return to normal more rapidly, platelets are affected less, and repeated doses of cyclophosphamide and ifosfamide do not produce cumulative damage and progressive deterioration of the hematopoietic elements. The reduced hematopoietic toxicity of cyclophosphamide and ifosfamide is due to the presence of aldehyde dehydrogenase in the hematopoietic stem cells and the early megakaryocytes, as discussed earlier. In contrast, the nitrosoureas produce severe hematopoietic toxicity, with a delayed onset and nadirs of granulocytes and platelets occurring as late as 45 days.<sup>75,260</sup> Busulfan also produces severe hematopoietic depression, with a selectivity for early myeloid precursors.<sup>67,68</sup> The variations in the cellular patterns and time courses of hematopoietic suppression after the administration of different alkylating agents indicate that the individual agents have selectivity for different hematopoietic precursor cells (Fig. 48.18).

Peptide growth factors, such as granulocyte-macrophage colony-stimulating factor (sargramostim, GM-CSF) and granulocyte colony-stimulating factor (filgrastim, G-CSF), which stimulate the differentiation and proliferation of hematopoietic precursors,<sup>261</sup> are now used clinically. The degree and duration of granulocyte depression after antitumor drug administration can be reduced by the concomitant use of these growth factors.<sup>262-264</sup> Currently, growth factors that may stimulate the proliferation and restoration of megakaryocytes and platelets are under investigation.<sup>265,266</sup> The use of these factors with the alkylating agents has been particularly attractive because of the steep dose-response curve of the alkylating agents and because, with several alkylating agents, a considerable increase in dose may be administered before another dose-limiting toxicity is reached. For these same reasons, combinations of alkylating agents have been used extensively in association with allogeneic and autologous bone marrow transplantation.<sup>71,267</sup>

**Gastrointestinal Toxicity.** Damage to the gastrointestinal tract is a toxicity that frequently occurs with high-dose regimens. Mucositis, stomatitis, esophagitis, and diarrhea occur with high doses of alkylating agents and in particular after high doses of melphalan and thiotepa or combinations of alkylating agents including melphalan or thiotepa.<sup>268-270</sup> Significant mucositis is unusual even after very high doses of cyclophosphamide or ifosfamide. This lack of gastrointestinal toxicity is probably due to the presence of the enzyme aldehyde dehydrogenase in the epithelial cells of the gastrointestinal tract.<sup>13</sup>

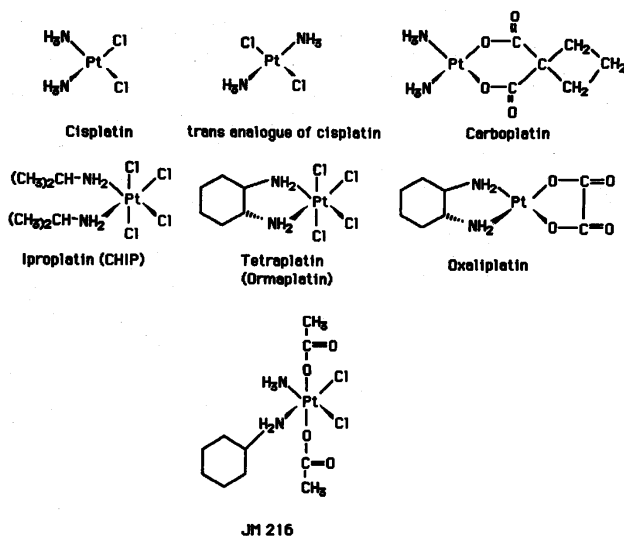


Figure 48.17. Structures of platinum antitumor agents.

Nausea and vomiting are frequent side effects of alkylating agents. Although these side effects are not usually life threatening, they are major discomforts to patients and may result in the delay or discontinuation of therapy. The nausea and vomiting are, at least in part, mediated through the CNS and are not due to direct gastrointestinal toxicity.<sup>271,272</sup> These effects are variable between patients in that some people tolerate high doses of these drugs without nausea and vomiting, whereas other patients are incapacitated by even low doses of alkylating agents. The frequency of nausea and vomiting does increase as the dose of alkylating agents is increased. Therefore, it is important, especially with the use of increasing doses of alkylating agents, to provide the patient with adequate antiemetic medication. Such medications include phenothiazines, other antiemetics, acute doses of corticosteroids, and, more recently, antiserotonin agents.<sup>273-275</sup>

**Veno-Occlusive Disease of the Liver.** This syndrome is characterized clinically by hepatomegaly, right upper quadrant pain, jaundice, ascites, and a high mortality rate from hepatic failure. Pathologically, the syndrome is associated with subendothelial thickening and narrowing of the hepatic venule lumen.<sup>276</sup> This complication has been seen in about 25% of patients receiving high-dose cyclophosphamide and busulfan (see Fig. 48.17) or cyclophosphamide and total body irradiation prior to allogeneic or autologous bone marrow transplantation for leukemia or lymphoma,<sup>276</sup> and has also been seen after other high-dose alkylating agent therapy.<sup>277,278</sup> Liver transplantation has been used for the treatment of veno-occlusive disease in patients after bone marrow transplantation.<sup>279,280</sup>

**Gonadal Damage.** A serious toxicity of the alkylating agents is gonadal damage. The characteristic lesion in men, depletion of testicular germ cells with preservation of Sertoli cells, was first described in 1948 in patients treated with mechlorethamine.<sup>281</sup> This lesion has subsequently been observed with other alkylating agents<sup>282</sup> and frequently results in aspermia or oligospermia in men treated with drug combinations including alkylating agents.<sup>283</sup> However, spermatogenesis and fertility may return after several years.<sup>284,285</sup>

Amenorrhea, associated with disappearance of mature and primordial ovarian follicles, is seen in women treated with alkylating agents.<sup>70,286,287</sup> The frequency of amenorrhea increases with the age of the woman and is more likely to be irreversible in older women.<sup>288</sup>

**Pulmonary Damage.** Pulmonary damage in the form of interstitial pneumonitis and fibrosis has been associated with almost all of the alkylating antitumor drugs. Although the exact mechanism of the pulmonary toxicity is not known, it is presumably due to direct toxicity of the alkylating agents to pulmonary epithelial cells. The typical presentation of this toxicity is the onset of a nonproductive cough and dyspnea, which may progress to tachypnea and cyanosis and even to severe pulmonary insufficiency and death. This complication was

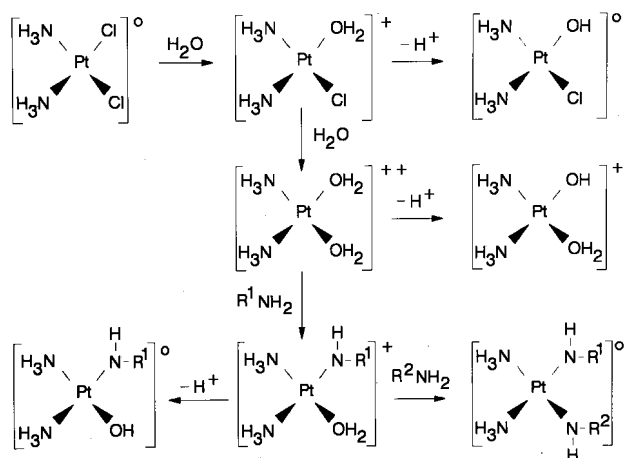


Figure 48.18. Aquation of platinum compounds and reaction with nucleophiles.

first described in association with busulfan therapy,<sup>289</sup> but subsequently it has been described after cyclophosphamide,<sup>290,291</sup> nitrosoureas,<sup>292,293</sup> melphalan,<sup>294</sup> chlorambucil,<sup>295</sup> and mitomycin C.<sup>296</sup> A significant incidence of pulmonary toxicity has been reported in patients receiving high doses of cyclophosphamide, cisplatin, and BCNU.<sup>297</sup>

**Hemorrhagic Cystitis.** The oxazophosphorines, cyclophosphamide and ifosfamide, produce bladder toxicity, which is not seen with other alkylating agents. This toxicity is a hemorrhagic cystitis, which may progress to massive hemorrhage.<sup>298,299</sup> The toxicity has been demonstrated to be due to metabolites of these drugs, which are excreted into the urine. The metabolite principally responsible for this toxicity is acrolein,<sup>300</sup> although phosphoramidate mustard and chloracetaldehyde may contribute to the effect. Hemorrhagic cystitis is seen more commonly after ifosfamide therapy than cyclophosphamide, partly because higher doses of this agent are used (see above). Renal tubular damage has also been seen after ifosfamide, including a Fanconi-type syndrome with azotemia, elevated serum creatinine, and enzymuria.<sup>301</sup>

The systemic administration of thiols can prevent or ameliorate the bladder damage from cyclophosphamide and ifosfamide because the thiols conjugate the aldehyde functions of acrolein and chloracetaldehyde. The most widely used compound to prevent oxazophosphorine bladder toxicity is the sodium salt of 2-mercaptoethane sulfonate (MESNA).<sup>302</sup> MESNA is usually administered to all patients receiving ifosfamide and to patients who are receiving high-dose cyclophosphamide. Subclinical renal toxicity has been observed in children receiving ifosfamide,<sup>16,303</sup> despite MESNA administration, so that administration of MESNA does not eliminate the need for adequate hydration and careful observation of the patient.

**Antidiuresis.** An antidiuretic effect is commonly seen in patients receiving doses of cyclophosphamide of 50 mg/kg or greater.<sup>304,305</sup> This syndrome is characterized by a decrease in urine output 6 to 8 hours after drug administration, weight gain, a marked increase in urine osmolality, and a decrease in serum osmolality and sodium concentration. Pericardial and pleural effusions may be seen, and seizures due to hyponatremia have occurred after cyclophosphamide therapy,<sup>306</sup> especially if low-sodium replacement fluids have been administered. This antidiuretic syndrome appears to be due to an effect of cyclophosphamide metabolites on the distal renal tubule and is self-limited, with the excess fluid excreted over a period of about 12 hours. Administration of furosemide will promote free water clearance and ameliorate the syndrome.<sup>307</sup>

**Renal Toxicity.** Renal toxicity has proven to be a serious toxicity of the nitrosoureas.<sup>308,309</sup> This effect is dose-related and may produce severe renal failure and death after administration of more than 1,200 mg of BCNU. Elevation of serum creatinine and other clinical evidence of renal toxicity may not be seen until after the completion of therapy. The histology of the kidneys in patients with renal nitrosourea damage is similar to that in radiation nephritis. A case of acute renal failure after melphalan therapy has been reported.<sup>310</sup>

**Alopecia.** Although the association between an alkylating agent and alopecia was first described with busulfan therapy,<sup>311</sup> this toxicity has been predominantly associated with cyclophosphamide and ifosfamide therapy. The alopecia produced by these agents may be quite severe, especially if the agent is given in combination with vincristine or doxorubicin. Regrowth of the hair occurs after cessation of therapy and may be associated with a change in the texture and color of the hair.<sup>312</sup> The structure-function studies of Feil and Lamoureaux<sup>313</sup> suggest that this toxicity is due to the entry of lipophilic metabolites into the hair follicles. This suggestion is consistent with the fact that busulfan, vincristine, and adriamycin are all lipophilic molecules.

**Allergic and Hypersensitivity Reactions.** Since the alkylating agents react with many biologic molecules, it is not surprising that they would serve as haptens and produce allergic reactions.<sup>314-316</sup> The most frequent reactions that have been reported have been cutaneous hypersensitivities. Anaphylactic reactions are rare, but they have occurred.<sup>317</sup> Patterns of cross-reactivity have not been carefully

defined, but cross-reactivity between agents of similar structure, such as the nitrogen mustards, have been described.<sup>316,318</sup>

**Cardiotoxicity.** The nonhematologic dose-limiting toxicity of cyclophosphamide is cardiac toxicity.<sup>319-321</sup> The fulminant syndrome has been seen most frequently in patients receiving a total dose of cyclophosphamide greater than 200 mg/kg preparatory to bone marrow transplantation. The clinical course of the syndrome consists of the rapid onset of severe heart failure, which is fatal within 10 to 14 days. The hearts of such patients are dilated, with patchy transmural hemorrhage and pericardial effusion. The microscopic findings consist of interstitial hemorrhage and edema, myocardial necrosis and vacuolar changes, and specific changes in the intramural small coronary vessels.<sup>320</sup> Decreased electrocardiographic voltage and a transient increase in heart size is seen in high-dose cyclophosphamide patients without clinical symptoms, and the characteristic pathologic findings are present in such patients who die of other causes. Cardiotoxicity and cardiomegaly have been seen in patients receiving lower doses of cyclophosphamide in combination with other alkylating agents.<sup>322</sup> Age greater than 50 and previous adriamycin exposure appear to increase the risk of cyclophosphamide cardiotoxicity.<sup>321</sup>

**Neurotoxicity.** In preclinical studies of alkylating agents, convulsions have often been seen.<sup>323</sup> At the usual clinical doses of these agents, frank neurotoxicity is not usually seen but drowsiness and alterations of consciousness can be seen.<sup>324</sup> With the increasing use of higher doses of alkylating agents and combinations of alkylating agents, more clinical neurotoxicity is being seen.<sup>325</sup> At BCNU doses of 1,200 mg/m<sup>2</sup>, severe CNS toxicity has been seen,<sup>326</sup> and the intracarotid administration of BCNU has produced severe eye pain and blindness.<sup>327</sup> High-dose busulfan therapy produces seizures, and anti-convulsants are often used prophylactically in these patients.<sup>328</sup>

**Teratogenicity.** Studies carried out in vivo and in embryo cultures have demonstrated that virtually all of the alkylating agents are teratogenic.<sup>329,330</sup> The teratogenic effect is probably due to cytotoxic effects on the embryo by the same mechanisms by which the compounds are toxic to tumor cells.<sup>331-334</sup> The available clinical information indicates that there is a definite risk of a malformed infant if the mother is treated with an alkylating agent during the first trimester of pregnancy.<sup>335-337</sup> In a review of the literature, Nicholson<sup>338</sup> found that of 25 women who had received alkylating agents during the first trimester of pregnancy there were four fetal malformations. However, the administration of alkylation agents during the second and third trimesters is not associated with an increased risk of fetal malformation.<sup>338-340</sup>

**Carcinogenesis.** Since the initial reports of acute leukemia occurring in patients treated with alkylating agents,<sup>341-344</sup> it has become increasingly obvious that this type of oncogenesis is a significant complication of alkylating agent therapy. Several studies have indicated that the rate of acute leukemia after alkylating agent therapy may be 10% or higher in certain groups of patients.<sup>345-347</sup> Procarbazine and other methylating agents appear to be the most potent oncogenic agents,<sup>348</sup> and melphalan appears to produce a higher rate of acute leukemia than cyclophosphamide.<sup>349</sup> The lesser leukemogenic potential of cyclophosphamide may well be related to the hematopoietic stem cell sparing effect of this agent.<sup>13</sup> An increased rate of solid tumors is also seen in patients treated with alkylating agents.<sup>350,351</sup> Although sufficient data are not yet available to be certain, it appears that high-dose alkylating agent therapy administered in intermittent pulses over a relatively short period of time is less oncogenic than prolonged alkylating agent therapy.

**Immunosuppression.** The immunosuppressive effect of alkylating agents was first described by Hektoen and Corper<sup>352</sup> for sulfur mustard. Cyclophosphamide is particularly immunosuppressive<sup>353</sup> and is used for the treatment of autoimmune diseases,<sup>354-356</sup> Cyclophosphamide is also used in preparative regimens for allogeneic transplantation because of its immunoablative activity.<sup>357</sup> Low doses of cyclophosphamide and melphalan can enhance the immune response by selectively inhibiting the immune suppressor cells.<sup>358-360</sup> Because of this effect, moderate doses of cyclophosphamide have been used in conjunction with immunotherapy and biologic response modifiers, such as interleukin-2.<sup>361,362</sup>

The clinical significance of the immunosuppression produced by alkylating agents in their role as antitumor agents is not certain. The two major concerns are susceptibility to infection in the immunosuppressed host and the potential interference with a host immune response to the tumor. The available evidence indicates that most intermittent antitumor regimens do not produce a profound or prolonged immunosuppression.<sup>363</sup>

**Platinum Antitumor Compounds.** The platinum antitumor agents are complexes of platinum with ligands that can be displaced by nucleophilic (electron-rich) atoms to form strong bonds with covalent characteristics. Thus, like the alkylating agents, the platinum agents form strong chemical bonds with thiol sulfurs and amino nitrogens in proteins and nucleic acids.

The first platinum antitumor compound was discovered by Rosenberg and colleagues<sup>364,365</sup> while studying the effects of electric current on bacterial growth. The growth inhibition observed was found to be caused by a platinum complex of ammonia and chloride, which was produced in the medium from the platinum electrode. These investigators found several such compounds to have antitumor activity against murine tumors *in vivo*.<sup>365</sup> The most active of these compounds was the one now known as cisplatin (Fig. 48.19).

Cisplatin went into clinical trials in the early 1970s<sup>366-368</sup> and was found to have significant antitumor activity against testicular cancer, lymphoma, squamous cell carcinoma of the head and neck, ovarian cancer, and bladder cancer. Because of its significant therapeutic effect in these tumors and activity against a number of other solid tumors, it became the most frequently used antitumor agent. Because of the renal and neurotoxicities of cisplatin, there were intensive efforts to devise analogues with fewer of these toxicities. This work led to the development of carboplatin, which produces primarily hematopoietic toxicity and appears to have an antitumor effect similar to cisplatin<sup>369-373</sup> against the tumors against which it has been used. A number of other platinum compounds are currently under investigation and are discussed below.

**Chemistry.** The platinum compounds that are active antitumor agents can have either four or six ligands (see Fig. 48.19), with a square planar or hexahedral configuration, respectively. Those with four ligands have an oxidation state of +2, and those with six ligands an oxidation state of +4. The chloride ligands of cisplatin and the other complexes with the 12 oxidation state can be exchanged for nucleophilic atoms in the biologic milieu, including the nitrogens of the DNA bases. The chloride ligands of the +4 compounds are much less reactive than those of the +2 compounds,<sup>374</sup> and it is likely that the +4 compounds are reduced *in vivo* to produce the reactive +2 complexes.<sup>375-377</sup> The ligand substitution reactions of the square planar complexes occur with retention of the configuration of the platinum complex.<sup>378</sup> Since the trans-platinum compounds are essentially inactive as antitumor compounds, the ability of the cis compounds to form certain stereo-specific cross-links probably accounts for their antitumor activity.

In some cis-platinum compounds in clinical use, the chloride leaving ligands are replaced with carboxyl ester groups, as in carboplatin and oxaliplatin (see Fig. 48.19). These ligands are less readily displaced; thus, these compounds require higher concentrations for cytotoxicity. The decreased renal and neurologic toxicity of these compounds is also probably due to the fact that they are less chemically reactive than cisplatin. Substitutions on the amino groups alter the lipophilicity and distribution of the agent.

**CELLULAR AND MOLECULAR PHARMACOLOGY** Although the chloride and carboxyester ligands can probably be directly displaced by bio-

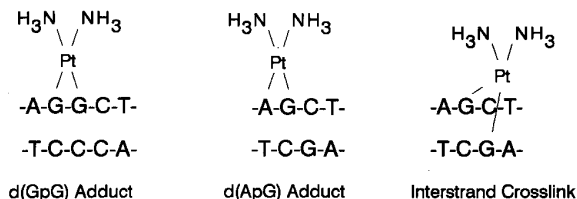


Figure 48.19. Platinum-DNA adducts.

logic atoms, it is likely that, in the biologic milieu, the chloride or carboxy ligands are displaced by water molecules to form the aquo ligand, which is a better leaving group than the chloride or carboxy groups.<sup>378</sup> The high chloride content of the extracellular fluid maintains the platinum compounds in the chloride and less reactive form. However, in the lower chloride content of the cell, the more reactive aquo species is formed. The loss of a proton produces the hydroxy ligand, which is unreactive.<sup>379</sup> The proposed aquation pathway for cisplatin is shown in Figure 48.20. The platinum compounds react with many biologic molecules, but there is considerable evidence that these compounds, like the bifunctional alkylating agents, exert their cytotoxic effect by reacting with DNA and interfering with DNA replication and cell division. Roberts and Pera<sup>380</sup> demonstrated that the amount of platinum bound to DNA was directly related to the degree of toxicity of platinum compounds. Zwelling and colleagues<sup>381</sup> demonstrated that the degree of DNA interstrand cross-linking *in vitro* and *in vivo* was directly related to the degree of cytotoxicity in rodent tumor cells.

The cis-platinum compounds, like the alkylating agents,<sup>382-384</sup> react with nitrogen atoms of DNA and preferentially react with the N-7 atom of deoxyguanylic acid. Specific adducts of Pt compounds with DNA have now been characterized and studied.<sup>380</sup> The consensus of the studies is that the most frequent adducts are dGpG and dApdG, which result from the cisplatinum complex binding to adjacent deoxyguanylates or an adjacent deoxyadenylate and deoxyguanylate in a strand of DNA to produce an intrastrand cross-link in both situations. A less common lesion is one that results from binding of the platinum atom to the N-7 of a deoxyguanylate in one strand of DNA and to the N-7 atom of a deoxyguanylate in the complementary strand of DNA, thereby producing an interstrand cross-link. Repair of these lesions does occur, and the cytotoxicity to the cell is probably determined by the resultant formation and repair of the lesions.<sup>385</sup> As mentioned above, a close correlation between interstrand DNA cross-linking has been demonstrated, but equally precise methods for quantifying intrastrand cross-links in whole cells after drug exposure are not available. Thus, intrastrand DNA cross-links might correlate equally well or better with cytotoxicity. The DNA adducts formed by Pt compounds other than cisplatin have been less well studied but appear to be similar to those formed by cisplatin.<sup>386-388</sup>

Although there is considerable evidence that the formation of DNA adducts is responsible for the cytotoxicity of the platinum antitumor agents, the mechanisms through which the cytotoxic effects are mediated are not well understood. Evidence has been presented that the platinum adducts inhibit replication.<sup>389,390</sup> Heiger-Bernays and colleagues<sup>391</sup> have demonstrated that as few as two platinum adducts per genome were sufficient for inhibition of DNA replication by cisplatin. Sorenson and Eastman<sup>392</sup> found that cytotoxicity with cisplatin

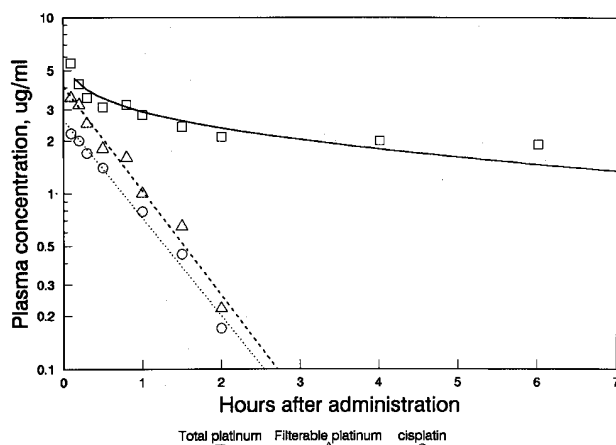


Figure 48.20. Clinical pharmacokinetics of cisplatin after single injection of 100 mg/m<sup>2</sup>. (Adapted from Patton et al.<sup>344</sup>)

was correlated with the duration of arrest in the G<sub>2</sub> phase of the cell cycle and postulated that the G<sub>2</sub> arrest was due to the inability of the cells to transcribe the Pt-damaged DNA and produce the mRNA essential for mitosis.

In 1996, Drummond and colleagues<sup>393</sup> demonstrated that ovarian tumor cells resistant to cisplatin were deficient in the MutL alpha MLHI subunit and suggested that the mismatch repair system recognized the cisplatin cross-link and played a role in the cytotoxicity of cisplatin. Similar findings were reported by others,<sup>394</sup> and Mello and colleagues<sup>395</sup> postulated that the mismatch repair protein hMSH2 played an active role in mediating cisplatin cytotoxicity. Vaisman and colleagues have suggested that mismatch repair defects result in increased replicative bypass of cisplatin adducts.<sup>396</sup> It has also been established that transplatin lesions, and those produced by oxaliplatin, tetraplatin, and JM-216, are not recognized by the mismatch repair system,<sup>397</sup> and that these agents produce cytotoxicity in mismatch repair deficient cells. The latter three agents produce a cross-link containing the bulky cyclohexylamino group (see Fig. 48.19). Takahara and colleagues And Gelesco and Lippard have now reported both the crystal structure<sup>398</sup> and the NMR solution structure<sup>399</sup> of the cisplatin d(GpG) cross-link. This type of structural understanding should increase the interpretation of the functional effects of the platinum cross-links.

**MECHANISMS OF CELLULAR RESISTANCE TO PLATINUM AGENTS** A number of mechanisms of cellular resistance to platinum compounds have been described. These mechanisms include decreased uptake of the platinum compound into resistant cells, inactivation of the drug by cellular thiol compounds, enhanced repair of the platinum-related DNA damage, and the absence of mismatch repair, as described above.

Decreased cellular uptake of cisplatin by cells resistant to the compound has been described by a number of investigators.<sup>400–404</sup> The uptake of cisplatin into cells is linear for over an hour and does not appear to be an active transport process, although it is partially inhibited by metabolic inhibitors.<sup>400</sup> There has also been a report of increased efflux of cisplatin in a resistant cell line.<sup>405</sup> Mann and colleagues<sup>406</sup> could not demonstrate changes in the physical properties of the cell membrane of cells resistant to cisplatin. Thus, although decreased cellular accumulation of the platinum compounds appears to be one type of cellular resistance, the mechanism of this type of resistance remains undefined and may be related to altered binding of the agents to cellular proteins, rather than alteration of passage through the cell membrane.<sup>407</sup>

A number of investigators have demonstrated that both rodent and human tumor cells that are selected *in vitro* or *in vivo* by exposure to the platinum antitumor compounds frequently demonstrate elevated glutathione levels in association with resistance to these drugs.<sup>408–413</sup> Tumor cell lines derived from patients resistant to therapy with cisplatin have also been found to have elevated glutathione levels.<sup>414,415</sup>

Further evidence that glutathione is involved in resistance to platinum compounds can be inferred from the fact that several investigators have shown that tumor cells can be sensitized to the platinum agents by depletion of cellular glutathione by treatment with buthionine sulfoximine, an inhibitor of glutathione synthesis.<sup>413–418</sup>

The mechanism(s) through which glutathione-associated resistance is mediated have not been definitively elucidated. Andrews and colleagues<sup>416</sup> demonstrated that cisplatin binds to glutathione, and Dedan and Borch<sup>419</sup> have studied the reaction rates of cisplatin with various thiols, including glutathione, and characterized a reaction product in which two glutathiones appeared to be bound to each platinum through the cysteine residues of the glutathiones. The thiol platinum ligand is very stable and thus will not react further. Eastman<sup>410</sup> has presented evidence that glutathione may react with monofunctional adducts on DNA to quench the second reactive ligand and prevent cross-link formation. Resistance to cisplatin has also been associated with elevation of glutathione transferase enzyme activity, increased levels of the pi (acidic) isozyme of the protein, and increased levels of the mRNA for the pi isozyme.<sup>420–422</sup> However, the catalysis of the conjugation of glutathione with platinum agents by this enzyme has not been characterized.

Cellular resistance to platinum agents has also been associated with another sulfhydryl-containing protein, metallothionein. Several investigators have found that tumor cells exposed to heavy metals, such as cadmium, develop resistance to cisplatin, which is associated with increased cellular levels of metallothionein.<sup>423–425</sup> In one report, transfection of cells with the metallothionein gene resulted in increased metallothionein levels and resistance of the cells to cisplatin, melphalan, and chlorambucil.<sup>425</sup> Naganuma and colleagues<sup>426</sup> have reported that administration of bismuth subnitrate to mice produced increased levels of metallothionein in the kidneys and resulted in protection of the mice from the renal and gastrointestinal toxicity of cisplatin but did not affect the response of transplanted tumors to cisplatin in the mice. Cisplatin binds to metallothionein in Ehrlich ascites tumor cells<sup>427</sup> and in the liver and kidney of rats,<sup>407,428</sup> and the systemic administration of cisplatin or its hydrolyzed product can induce metallothionein in the liver and kidney.<sup>429</sup> These findings indicate that metallothionein can protect both tumor and normal cells from cisplatin, although the binding of the drug to this protein has not been characterized.

As with the alkylating agents, there is extensive evidence that enhanced DNA repair can be responsible for resistance to the platinum compounds. Van Den Berg and Roberts<sup>430</sup> first reported that caffeine, a known inhibitor of DNA repair, potentiated cytotoxicity and chromosomal damage in mammalian cells, and shortly thereafter Fravel and Roberts demonstrated that excision repair of cisplatin-damaged DNA does occur in treated cells.<sup>431</sup> Many subsequent studies have demonstrated that cells deficient in DNA repair, such as those from patients with xeroderma pigmentosum or Fanconi's anemia, are very sensitive to cisplatin.<sup>432–436</sup>

Agents that are known to inhibit the activity of enzymes involved in the repair of DNA, such as aphidocolin and novobiocin, have been shown to sensitize cells to cisplatin and to reverse the resistance of repair-resistant cell lines.<sup>437–440</sup> The antitumor agents hydroxyurea and cytosine arabinoside, which inhibit DNA repair synthesis, both produce a synergistic cytotoxic effect with cisplatin.<sup>441,442</sup>

Studies by Becket and colleagues And Husain and colleagues have supported the above indications that platinum adducts in DNA are repaired by an excision repair mechanism,<sup>443,444</sup> and these investigators<sup>445</sup> have now reported that the nucleotide excision repair system can remove DNA adducts produced from cisplatin, oxaliplatin, and JM 216 (Bis-aceto-ammine-dichloro-cyclohexylamine-platinum IV). These findings are consistent with studies by Dabholkar and colleagues and Li and colleagues demonstrating that ERCC-1 is involved in platinum repair.<sup>446,447</sup>

It has also been shown that platinum interstrand DNA cross-links are removed more rapidly in cisplatin-resistant cells<sup>448</sup> and that very sensitive tumor cells may have a decreased ability to remove DNA interstrand cross-links.<sup>449</sup> A protein, XPE-BF (xeroderma pigmentosum complementation group E binding factor), which binds to Pt-damaged DNA<sup>450–452</sup> and may mark it for repair, has been identified. A series of proteins, the HMG domain proteins, which bind to Pt intrastrand cross-links, produce bending of the DNA, and may inhibit the repair of these lesions, has also been described.<sup>453,454</sup> It has also been found that cisplatin-resistant cells can have elevated thymidylate synthase activity and be cross-resistant to 5-fluorouracil (5FU)<sup>455</sup> and that c-fos may play a role in the cellular response to Pt agent damage by mediating DNA repair pathways.<sup>456–458</sup>

Although it is clear that each of these mechanisms can be associated with the resistance of tumor and normal cells to the platinum agents, the relative roles of these mechanisms in the resistance of tumors to treatment in patients have not been established. Such studies and attempts to overcome resistance with BSO and inhibitors of DNA repair are currently in progress.

**CLINICAL PHARMACOLOGY Analogues in Clinical Use.** Cisplatin and carboplatin are licensed in the United States and internationally and are used extensively. Since the primary toxicity of carboplatin is hematopoietic, it has replaced cisplatin for use in many patients and is being used particularly in situations where nonhematopoietic toxicity should be avoided, such as high-dose treatment with bone marrow support<sup>459,460</sup> or with hematopoietic stimulatory factors. There is no evidence for cross-resistance between these two agents. Iproplatin has

been evaluated in phase II trials but was found to be no more or less effective than carboplatin and produced more hematopoietic and gastrointestinal toxicity.<sup>461–465</sup> Tetraplatin (ormaplatin) produced severe neurotoxicity in initial clinical trials<sup>466</sup> but is still being evaluated in phase I trials.<sup>467</sup> Oxaliplatin is similar to tetraplatin in its preclinical toxicity.<sup>468</sup> However, this compound has shown promising activity in gastrointestinal tumors, especially in combination with 5-FU and leucovorin.<sup>469–471</sup> Oxaliplatin has also demonstrated significant activity in patients with ovarian cancer who have previously received cisplatin.<sup>472,473</sup> Oxaliplatin demonstrated a modest effect (15% PR) in advanced, cisplatin-resistant non-small-cell lung patients.<sup>474</sup>

A lipid-soluble platinum compound, JM216,<sup>475</sup> which can be administered orally, is now being evaluated clinically.<sup>476–478</sup> The discovery that oxaliplatin and JM216 are active against tumors lacking mismatch repair (see above) has stimulated interest in these and related compounds, and some of the new platinum analogues may not be totally cross-resistant with cisplatin due to differences in either cellular uptake<sup>402</sup> or cellular detoxification.<sup>479</sup>

**Pharmacokinetics.** Platinum antitumor compounds have been measured in human plasma and other human tissues as total platinum, as ultrafilterable platinum, and as the specific parent compounds. Total platinum can be measured by using compounds containing the radioactive <sup>193</sup>Pt or <sup>195</sup>Pt isotopes<sup>480,481</sup> by trapping the platinum with an ultraviolet absorbing ligand, such as diethyldithiocarbamate,<sup>482</sup> or by flameless atomic absorption spectroscopy.<sup>482,483</sup> Ultrafiltration of plasma and other biologic fluids separates the free platinum compounds from those bound to protein. The protein-bound species are biologically inactive and essentially irreversibly bound to the protein.<sup>484</sup> Both cisplatin and carboplatin have been measured specifically by separation from other species on HPLC columns and detection by electrochemical detection or by collecting fractions and quantifying the total platinum in each fraction.<sup>485,486</sup> The cisplatin concentration has been found to be consistently between 60 and 80% of the ultrafilterable platinum and to follow the same kinetics as the ultrafilterable platinum.<sup>487,488</sup> Carboplatin represents a higher percentage of the ultrafilterable platinum and follows kinetics similar to the ultrafilterable platinum. Because of the sensitivity, accuracy, and convenience of the method, flameless atomic absorption spectroscopy is the most common technique used to measure the platinum agents. Furthermore, since measuring filterable species appears to measure the reactive compounds and to approximate closely the measurement of the parent compounds, measurement of ultrafilterable platinum is most commonly used in pharmacokinetic studies.

In pharmacokinetic studies after cisplatin administration, total platinum in the plasma follows a triphasic pattern, with the first phase  $t_{1/2}$  about 30 minutes, the second phase  $t_{1/2}$  about 60 minutes, and the third phase  $t_{1/2}$  greater than 24 hours.<sup>487,488</sup> Measurements of the ultrafilterable platinum indicate that the initial, more rapid clearance phases are due to the renal clearance of filterable platinum, the majority of which is the parent compound.<sup>488</sup> Carboplatin exhibits similar pharmacokinetics, except that the initial half-lives are somewhat longer, less of the total platinum is protein bound, and a greater percentage of the agent is excreted by the kidneys.<sup>486,489</sup> The pharmacokinetics of total and filterable platinum after iproplatin administration appears to be similar to those of carboplatin.<sup>490</sup> Decreased creatinine clearance results in higher plasma levels of both cisplatin and carboplatin and potentially greater toxicity.

After bolus administration of 100 mg/m<sup>2</sup> of cisplatin, initial peak plasma concentrations of 3 to >5 microgram/ml are achieved,<sup>485</sup> with this value decreasing to less than 0.2 microgram/ml at 2 hours. After the usual clinical dose of about 300 mg/m<sup>2</sup> of carboplatin, peak plasma levels of about 30 microgram/ml are reached, declining to about 5 microgram/ml at 2 hours.<sup>486,489</sup>

In typical clinical use, usually in combination with other agents, the platinum antitumor agents are given intravenously, either as a single dose or daily for several days, with repeat courses at 3 to 4 weeks. The agents are given as an infusion over several hours rather than as a bolus dose and, especially with very high doses, may be given as 24-hour or longer infusions. Because of the close relationships between plasma AUC of carboplatin and renal function and between AUC of

carboplatin and toxicity, dosing algorithms based on renal function have been established and are now widely used in the dosing of carboplatin.<sup>491–493</sup>

Cisplatin and carboplatin have also been administered regionally. There has been considerable experience with the intraperitoneal route, particularly in the treatment of ovarian cancer.<sup>494–496</sup> Very high intraperitoneal concentrations can be obtained, and systemic toxicities can be reduced by the concomitant systemic administration of thiosulfate.<sup>497,498</sup> Cisplatin has also been administered intra-arterially for the treatment of tumors in the extremities,<sup>499–502</sup> brain tumors,<sup>503–505</sup> carcinoma of the head and neck,<sup>506,507</sup> carcinoma of the liver,<sup>508</sup> and carcinoma of the bladder.<sup>509,510</sup> Intravesicular instillation of cisplatin has been used for the treatment of superficial cancers of the bladder.<sup>511–513</sup> Cisplatin has also been instilled into the pericardial sac for the treatment of malignant pericardial effusions.<sup>514,515</sup>

**TOXICITIES Renal.** The most serious, and usually dose-limiting, toxicity of cisplatin is renal.<sup>516,517</sup> This toxicity is manifested clinically by elevated BUN and creatinine, is cumulative with continued cisplatin exposure, and is potentiated by other nephrotoxins.<sup>518</sup> Decreases in serum electrolytes have been associated with platinum renal toxicity, including symptomatic hypomagnesemia.<sup>519</sup> Although the toxicity may remain subclinical, or the renal function return to normal, significant pathologic damage appears to persist.<sup>520</sup> The pathology of the renal damage is characterized by focal acute tubular necrosis, dilatation of convoluted tubules, thickened tubular basement membranes, formation of casts, and epithelial atypia of the collecting ducts.<sup>520,521</sup> High fluid intake with forced diuresis<sup>522,523</sup> can reduce the incidence and severity of the renal toxicity. Systemic administration of thiols can reduce renal toxicity of cisplatin in animal models, and in a clinical trial, systemic diethyldithiocarbamate appeared to reduce nephrotoxicity without affecting ototoxicity or myelosuppression.<sup>524</sup> The nephrotoxicity of the second-generation platinum complexes, such as carboplatin and iproplatin, is markedly less than that of cisplatin.

**Ototoxicity.** Ototoxicity has been a significant problem with cisplatin. This toxicity is characterized by tinnitus and hearing loss.<sup>366,367,525</sup> The hearing loss is usually in the high-frequency range, 4,000 to 8,000 Hz, but may occur in the lower ranges, which include the speech frequencies.<sup>525,526</sup> Since the higher frequencies are usually involved, the hearing loss may not be symptomatic. Vestibular toxicity does not usually occur but can be seen.<sup>527,528</sup> The ototoxicity of cisplatin is dose related and is usually cumulative with subsequent courses of the agent.<sup>529, 530</sup> Radiation prior to or simultaneous with the cisplatin administration enhances the toxicity<sup>531,532</sup> but this additive effect may be less if the cisplatin precedes the radiation.<sup>526</sup>

The pathologic findings associated with ototoxicity, in both experimental animals and patients, are selective damage to the outer hair cells of the cochlea and lesions in the organ of Corti, the spiral ganglion and cochlear nerve, and the stria vascularis.<sup>533–536</sup> In studies of organ cultures of the cochlear structures, the hair cells are very sensitive to very low concentrations of cisplatin.<sup>537</sup> Vestibular toxicity is associated with degeneration of the maculae and cristae.<sup>528</sup>

**Neurotoxicity.** The neurotoxicity seen with the administration of cisplatin consists principally of peripheral neuropathy involving both the upper and lower extremities, with paresthesias, weakness, tremors, and loss of taste.<sup>538</sup> Seizures and leucoencephalopathy have also been described.<sup>539–542</sup> The neurotoxicity may be persistent<sup>543</sup> and may progress after cessation of cisplatin therapy.<sup>542</sup> The quantitative determination of vibratory perception threshold has been reported to correlate with cisplatin neurotoxicity.<sup>544</sup>

Particularly severe neurotoxicity has been reported after intra-arterial infusions of cisplatin, with cranial nerve paralysis occurring after intra-arterial infusions for head and neck cancer,<sup>541,542</sup> and severe peripheral neuropathy after lower limb perfusion.<sup>545</sup> In experimental animals, severe CNS toxicity was seen when compounds that open the blood-brain barrier were administered prior to systemic cisplatin treatment, and intracarotid cisplatin produced damage to the blood-brain barrier and severe neurotoxicity.<sup>546</sup> However, severe neurotoxicity was not seen in patients treated with intracarotid cisplatin for primary brain

tumors.<sup>547</sup> The neurotoxicity of ifosfamide has been reported to be enhanced by prior treatment with cisplatin.<sup>548</sup>

Since various pharmacologic maneuvers have been able to control or reduce the nephrotoxicity and severe nausea and vomiting produced by cisplatin, neurotoxicity has become the dose-limiting toxicity of cisplatin.<sup>549</sup> An interesting observation is that treatment of animals with an ACTH analogue will prevent neurotoxicity from cisplatin and will facilitate the recovery of established neurotoxicity<sup>550,551</sup> but will not interfere with the antitumor effect of the agent. In a randomized, placebo-controlled clinical trial, this compound appeared to prevent or ameliorate the neurotoxicity of cisplatin.<sup>551</sup> Neither carboplatin or iproplatin appear to produce significant neurotoxicity with the doses used thus far with autologous bone marrow transfusion.<sup>552-554</sup>

**Gastrointestinal Toxicity.** Severe nausea and vomiting have been a significant problem with cisplatin, occurring in almost all patients receiving the drug.<sup>517,555</sup> The cause of this toxicity is not firmly established. Work in animal models indicates that abdominal visceral innervation and 5-hydroxytryptamine receptors on visceral afferent nerves play a role in mediating this toxicity,<sup>556</sup> but there is also evidence that the chemoreceptor trigger zone in the medulla plays a role.<sup>557,558</sup> The use of a dopamine antagonist, metoclopramide, prior to and during cisplatin administration has been effective in controlling this toxicity,<sup>559,560</sup> and the steroids dexamethasone or methylprednisolone alone or in combination with metoclopramide have also been useful.<sup>561-563</sup> More recently, antiserotonin analogues such as ondansetron and granisetron have proven highly effective in controlling nausea and vomiting after platinum administration. The gastrointestinal toxicities of carboplatin and iproplatin are much less than those of cisplatin.<sup>564-566</sup>

**Immune Effects.** In contrast to the alkylating agents, many of which are significantly immunosuppressive, cisplatin appears to have no immunosuppressive effect at the usual clinical doses and may even augment immune function at these doses.<sup>567</sup> Monocyte-mediated cytotoxicity was found to be increased in ovarian cancer patients after cisplatin treatment,<sup>568</sup> and OKT81 cytotoxic cells were increased in patients after cisplatin therapy.<sup>569</sup>

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