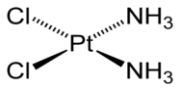
Lecture 2: DNA cross-linkers and alkylating agents – regarded as non-cell cycle specific

Remember that DNA is "electron rich" and these agents are "electron poor"; hence, they react.

1. Platinum agents

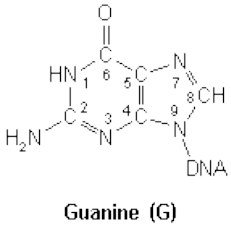
Cisplatin (Platinol): Among oldest, of anti-cancer agents. Everyone should be able to draw cisplatin. Discovered prior to 1900, and utility as an anticancer agent realized about 1960. Light sensitive.



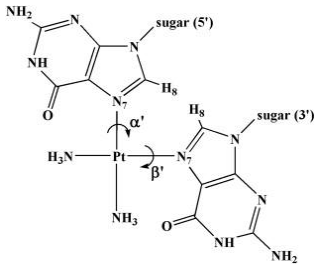
Note the square planar geometry, not tetrahedral.

Uses: Lung cancer, testicular cancer, ovarian cancer, bladder cancer. Administration is IV only. Dose ranges 20-100 mg/m^2 but dependent on disease and renal function. All patients should be adequately hydrated prior to and after receiving cisplatin (see toxicities below).

Figure 1: Structure of guanine (left) and cisplatin adduct with two adenines in DNA (right).



Guanine (G)



Mechanism: Reacts with DNA bases, especially nitrogen of guanine (see Figure 1 above). The cis geometry is critical to this molecule; the trans isomer (transplatin) is much less effective.

Toxicities: Nephrotoxicity, ototoxicity, myelosuppression, severre nausea and vomiting (N/V). Warning on vials not to exceed 100 mg/m2 for any single cycle of cisplatin. Caution with other drugs that are nephrotoxic (ifosfamide, cyclophosphamide). Sodium thiosulfate can be administered to reduce nephrotoxicity. Amifostine can be used to reduce ototoxicity.

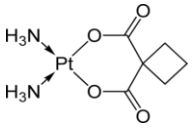
ADME: Plasma t1/2 is very short (less than 30 minutes). No real metabolism but "aquation" (see below). The high renal excretion of cisplatin predisposes to renal toxicity. Renal toxicity can impact other drugs that are excreted by the kidneys.

Note(s): Aluminum reacts with cisplatin so take precautions with certain syringes or infusion equipment. Also, an "aquation equilibrium" occurs with cisplatin. Reconstitution prior to administration should not occur with D5W only – diluent must include some saline (NaCl).

Reason: Outside cells, cisplatin remains largely intact due to high chloride concentration (~100 mM). Therefore the cisplatin molecule remains intact and neutral, and can penetrate cells. However, inside cells the chloride concentration is much lower (~10 mM). The equilibrium drives water molecules to replace the chloride atoms and the aquated forms are charged and are "locked" inside cells. The aquation steps occur as follows:

1. $Pt^{II}(NH_3)_2Cl_2 + H_2O \rightarrow [\ Pt^{II}(NH_3)_2Cl(H_2O)]^+ + Cl^-$
2. $[Pt^{II}(NH_3)_2Cl(H_2O)]^+ + H_2O \rightarrow [Pt^{II}(NH_3)_2(H_2O)_2]^{2+} + Cl^-$

Carboplatin (Paraplatin):



Square planar geometry.

Uses: Mainly ovarian cancer (often with cyclophosphamide); experimental use in prostate cancer with doxetaxel, and lung cancer (often with paclitaxel).

Mechanism: Similar to cisplatin but chemically more stable and less active; also less toxic.

Toxicities: Myelosuppression, but less than cisplatin. Nephrotoxicity and ototoxicity can occur but rare. N/V but less than cisplatin. Main route of excretion is still renal, dosage adjustments are needed for patients with renal impairment.*

ADME: Plasma t1/2 longer (about 3 hrs) than for cisplatin. Although more lipophilic than cisplatin it is still excreted renally.

*For patients with renal impairment, dosing of carboplatin can be guided by creatinine clearance as follows:

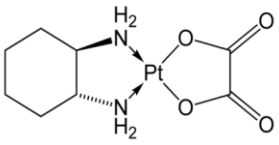
Normal dose of 300 mg/m2 if creatinine clearance is >60 mL/min.
Reduce dose to 250 mg/m2 if creatinine clearance is 41-59 mL/min.
Reduce dose to 200 mg/m2 if creatinine clearance is 16-40 mL/min.

Carboplatin can also be dosed to achieve a target AUC value of 4 -6 mg/mL x min. In this case, a formula (Calvert formula) is used to calculate the appropriate dose (mg) as a function of the glomerular filtrate rate (GFR):

Total Dose (mg) = (target AUC) x (GFR + 25), and GFR can be measured by Cr-EDTA excretion.

Note(s): Aluminum reacts with carboplatin so take precautions with certain syringes or infusion equipment. Carboplatin can be diluted with D5W or normal saline.

Oxaliplatin (Eloxatin):



Deviates slightly from square planar geometry.

Uses: Mainly colorectal cancer; part of the FOLF**OX** regimin (folinic acid/fluorouracil/oxaliplatin); was evaluated in a clinical trial for pancreatic cancer as component in the FOLFIRIN**OX** regimen (folinic acid/fluorouracil/irinotecan/oxaliplatin) – effective but highly toxic regimen.

Mechanism: Similar to cisplatin and carboplatin, but forms conformationally different adducts; also gets aquated in solution to active form; more lipophilic so more penetration into tissues. The need for prehydration and evaluating renal function is less critical.

Toxicities: Peripheral neuropathy is major toxicity; myelosuppression but less than cisplatin. The neuropathy might be caused by chelation of Ca^{+2} ions in sensory nerves by the oxalate moiety that is released from oxaliplatin upon aquation.

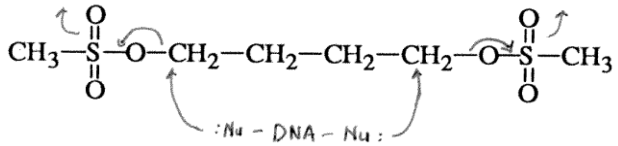
ADME: Triphasic elimination with elimination t1/2 of about 17 hrs; elimination still largely renal and clearance correlates with GFR.

Note(s): Aluminum reacts with carboplatin so take precautions with certain syringes or infusion equipment. Final dilution of oxaliplatin should not include any saline (chloride ions) or agents that make the solution alkaline.

Picoplatin and **Satraplatin** discussed in the text book but these have failed in recent clinical trials.

2. Methyl sulfates, aziridines, and hydrazines

Busulfan (Busulfex or Myleran):



Uses: Mainly IV for myeloablation prior to bone marrow or stem cell transplantation for CML; has been used in combination with cyclophosphamide for this purpose (called BuCy regimen). Administered PO for palliative treatment of CML. Administered IV or PO.

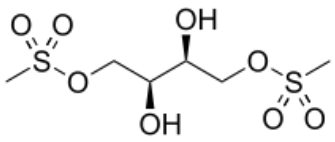
Mechanism: Powerful myelosuppression; bifunctional and direct alkylator of DNA (shown above).

Toxicities: Extreme myelosuppression, hepatotoxicity (veno occlusive disease), seizures (see notes).

ADME: Busulfan is lipophilic and can penetrate the CNS; metabolism occurs mainly by conjugation with glutathione; elimination t1/2 from plasma about 2 hrs; about 30% of the dose excreted renally with much of the drug bound to cellular components. Because busulfan can cross the blood-brain-barrier and induce seizures, patients can be pretreated with phenytoin to prevent seizures. But phenytoin reduces the plasma AUC of busulfan; other anticonvulsants can be used but they can increase the AUC of busulfan. Therefore, pharmacokinetic monitoring of busulfan level is common and the best way to deliver the most precise dose to patients.

Note(s): Syringes or syringe filters that contain polycarbonate are not to be used as they react with busulfan.

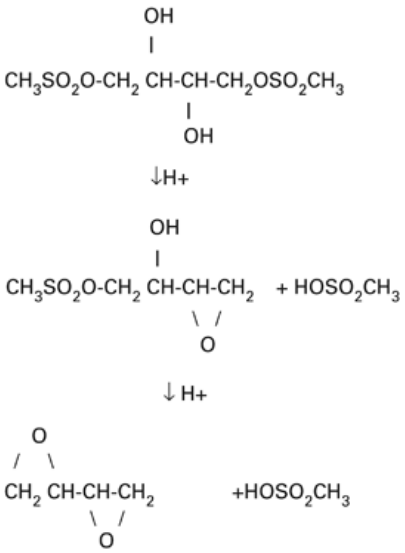
Treosulfan (approved in Europe but not in the U.S.; being studied in many clinical trials):



Uses: Historically for ovarian cancer in EU but now being studied for myeloablation prior to allogeneic stem cell transplantation, in place of busulfan.

Mechanism: Powerful myelosuppression; alkylator of DNA as for busulfan, however it forms epoxides intramolecularly prior to reacting with DNA. Also, more potent immunosuppressant than busulfan so very attractive to replace busulfan in allogeneic transplants.

Figure 2. Formation of epoxides intramolecularly in treosulfan.

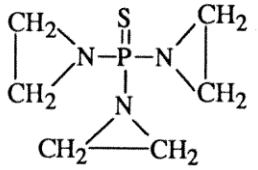


Toxicities: Similar to busulfan but less toxic to CNS.

ADME: Elimination t1/2 similar to busulfan (1-2 hrs); more polar than busulfan so less penetration into CNS.

Note(s): Because less toxic than busulfan, decreased need (or perhaps no need) for pharmacokinetic monitoring.

Thio-triethylenephosphoramide (Thiotepa):



→ Tepa (more active metabolite)

Uses: Breast cancer, ovarian cancer; also myeloablative agent (like busulfan) prior to allogeneic and autologous stem cell transplantation; Administered IV. Also used for bladder cancer but administration is intravesically.

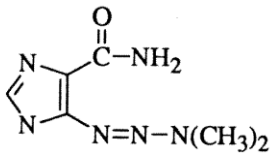
Mechanism: A direct alkylator of DNA but more active following conversion to TEPA (see above); active parent and metabolite.

Toxicities: Severe myelosuppression, infections, N/V.

ADME: Elimination t1/2 of thiotepa is about 2 hrs, that of Tepa is nearly 20 hrs. Both molecules can penetrate the CNS and cause dizziness, headaches, etc. Most excretion is renal.

Note(s): Administered as initial dose, followed by maintenance doses every 1-4 weeks. A delayed or slow response does not necessarily indicate a lack of effect. Increasing the frequency of dosing may only increase toxicity. After maximum benefit is obtained by initial therapy, maintenance doses should not be administered more frequently than weekly in order to preserve correlation between dose and blood counts.

Dacarbazine (DTIC):



Uses: Hodgkin's lymphoma (part of ABV**D** regimen: adriamycin, bleomycin, vinblastine, dacarbazine): also used for melanoma. Administered IV only.

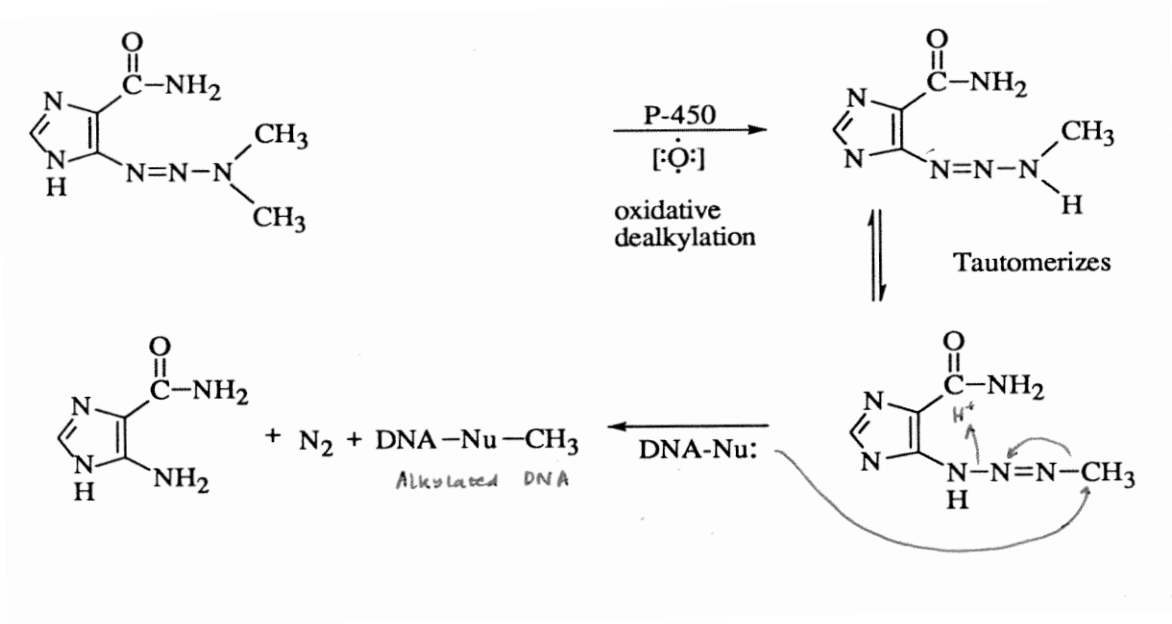
Mechanism: A prodrug; following N-dealkylation by cytochrome P450, the molecule tautomerizes to more reactive form which reacts with DNA, specifically the O-6 position on guanine (see Figure 3 below).

Toxicities: Myelosuppression, hepatotoxicity, N/V.

ADME: Biphasic elimination from plasma; terminal elimination t1/2 of about 5 hrs; metabolized by the liver, especially CYP1A, but nearly half the drug excreted unchanged.

Note(s): The hepatotoxicity of DTIC can be extreme and has caused some deaths.

Figure 3. Dacarbazine activation by P450.

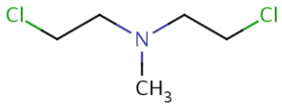


The activation of dacarbazine is shown in the text (Foye's p.1216) but the details are less clear. The final methylation of O-6 of guanine is clear. Note that the activation of temozolomide (non-enzymatic) is also shown in the figure, so don't be confused.

Procarbazine and **Temozolomide** are also discussed in the text. Again, methylation of O-6 guanine occurs. Patients who are able to repair this damage respond less to these agents than patients who cannot readily repair.

3. Alkylating agents - Nitrogen mustards

Mechlorethamine (Mustargen):



→ Aziridine

Uses: Formerly for various leukemias, less use today; palliative treatment for late stage Hodgkin's lymphoma. The prototype of all alkylating agents. Formulated as the HCl salt. Administered IV, IP.

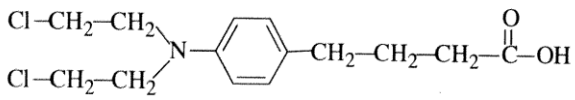
Mechanism: Binds to DNA after formation of reactive aziridine, which binds to guanine at N7 position.

Toxicities: Highly toxic; myelosuppression, severe N/V; severe vesicant and local toxicity if extravasation occurs; hepatotoxic; rarely but also secondary cases of hemolytic anemia or leukemia many years later.

ADME: Actually so reactive that it reacts with water and biomolecules very quickly after administration. Some inactive diol metabolite recovered in the urine.

Note(s): Originally developed as a mustard agent for chemical warfare. Results of medical experiments were initially secret and released later. In most cases, better agents are available.

Chlorambucil (Leukeran):



Uses: Chronic lymphocytic leukemia, lymphosarcoma, Hodgkin's lymphoma. Administered PO.

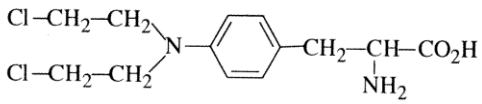
Mechanism: Active intact but relative to mechlorethamine (above) the electrons on the aromatic nitrogen are not as basic and the formation of the aziridinium ion is slower; can be activated by N-dealkylation and then formation of reactive aziridine.

Toxicities: Myelosuppression, hepatotoxicity (patients with impaired liver function should be closely monitored). CNS toxicities: seizures, tremors, hallucinations.

ADME: Oral absorption is good due to lack of charge; molecule is rapidly metabolized and the elimination t1/2 is a little over 1 hr; beta oxidation is important in the metabolism to form the chain shortened phenylacetic acid metabolite which retains antitumor activity.

Note(s): CNS toxicities can occur because the drug is non-polar (lipophilic) and can enter the brain.

Melphalan (Alkeran):



Uses: Multiple myeloma, palliative treatment of non-resectable ovarian cancer. Administered PO and IV.

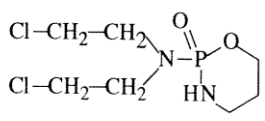
Mechanism: Active intact but relative to mechlorethamine (above) the electrons on the aromatic nitrogen are not as basic and the formation of the aziridinium ion is slower; can be activated by N-dealkylation and then formation of reactive aziridine. Note that the molecule contains an amino group on a chiral carbon. This was done intentionally to incorporate the L-phenylalanine to attempt to exploit specific amino acid transport into cells. It turns out the facilitated diffusion is more important for uptake of the agent.

Toxicities: Myelosuppression, hepatotoxicity, and N/V but certainly reduced; rare but serious pulmonary fibrosis and interstitial pneumonitis can occur.

ADME: Absorption can be variable when dosed orally; elimination t1/2 is 1-2 hrs; beta oxidation does not participate in metabolism, nor do CYP enzymes; modification of the parent molecule appears to be through chemical hydrolysis to mono and dihydroxy melphalan.

Note(s): CNS toxicities are low because the drug is polar (ionized) and cannot enter the brain.

Cyclophosphamide (Cytoxan):



Uses: Lymphomas, leukemias, multiple myeloma, breast cancer, myeloablation prior to transplantation. Administered IV (PO occasionally).

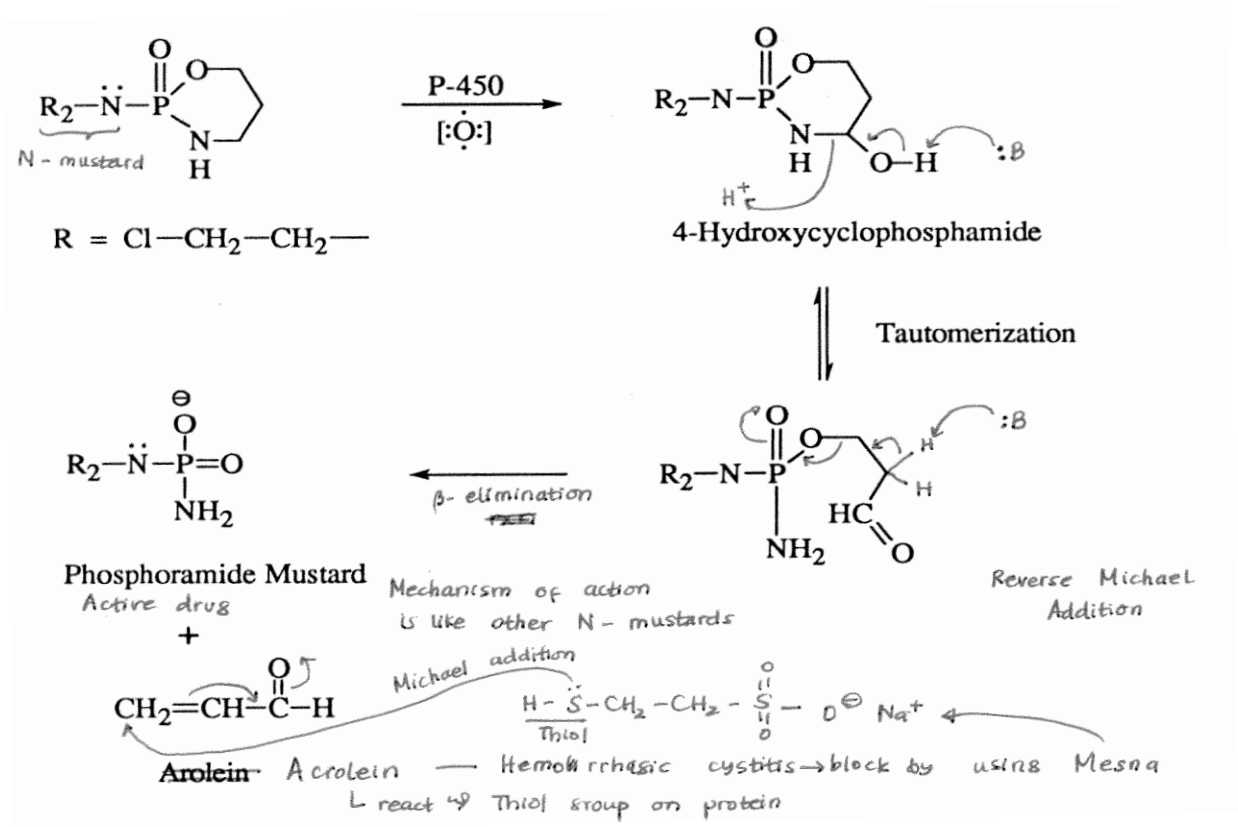
Mechanism: A prodrug: Following dealkylation by cytochrome P450 (CYP2B6 mainly, CYP3A4 less), the 4-hydroxy metabolite tautomerizes and eliminates acrolein to form a phosphoramide mustard which forms an aziridine which will react with DNA (see Figure 4). Metabolism by CYP enzymes is also important in the formation toxic metabolites, chloroacetaldehyde and acrolein.

Toxicities: Myelosuppression, hemorrhagic cystitis (bladder toxicity). CNS toxicities. Because of potential for bladder toxicity, all patients should be well hydrated prior to administration to cyclophosphamide.

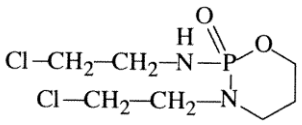
ADME: CYP enzymes are extremely important in the metabolism with less than 15% of the dose unmetabolized; the parent molecule has a terminal t1/2 of about 5-10 hrs; excretion of metabolites is mainly renal.

Note(s): Co-administration of an agent (MesNa) which concentrates in the urine, will bind to and inactivate the acrolein. Conjugation with the endogenous thiol glutathione (GSH) helps mitigate the nephrotoxicity of chloroacetaldehyde.

Figure 4. Activation of cyclophosphamide by P450.



Ifosfamide (Ifex):



Uses: Mainly testicular cancer; experimental use in other cancers. Administered IV.

Mechanism: Like cyclophoshamide it is a prodrug that is converted to the active from by CYP450 (CYP3A4 mainly, CYP2B6 less). Note that the chloroethly groups are on different nitrogen atoms and steric hindrance slows hydroxylation at C-4.

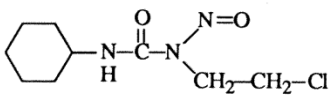
Toxicities: Myelosuppression, nephroroxicity, hemorrhagic cystitis (bladder toxicity), CNS toxicities. Overall formation of chloroacetaldehyde is higher than from cyclophosphamide. Actually higher potential for kidney, bladder, and CNS toxicities than for cyclophosphamide; all patients should be well hydrated prior to administration to ifosfamide. The enhanced CNS toxicity of the agent can induce a coma and even death.

ADME: Similar to cyclophosphamide but overall CYP metabolism is slower so higher doses needed; displays dose-dependent pharmacokinetics; elimination t1/2 about 15 hrs, shorter at low doses; excretion is mainly renal.

Note(s): As a measure of protection from bladder toxicity, patients should be well hydrated and the protective agent (MesNa) should also be dosed prior to administration of ifosfamide. The sulfur agent N-acetylcysteine may provide some protection from nephrotoxicity. No agent for protection against CNS toxicities.

4. Alkyating agents – Nitrosoureas

Lomustine (CeeNU):



Uses: Brain cancer (glioblastoma), Hodgkin's lymphoma. Administered PO.

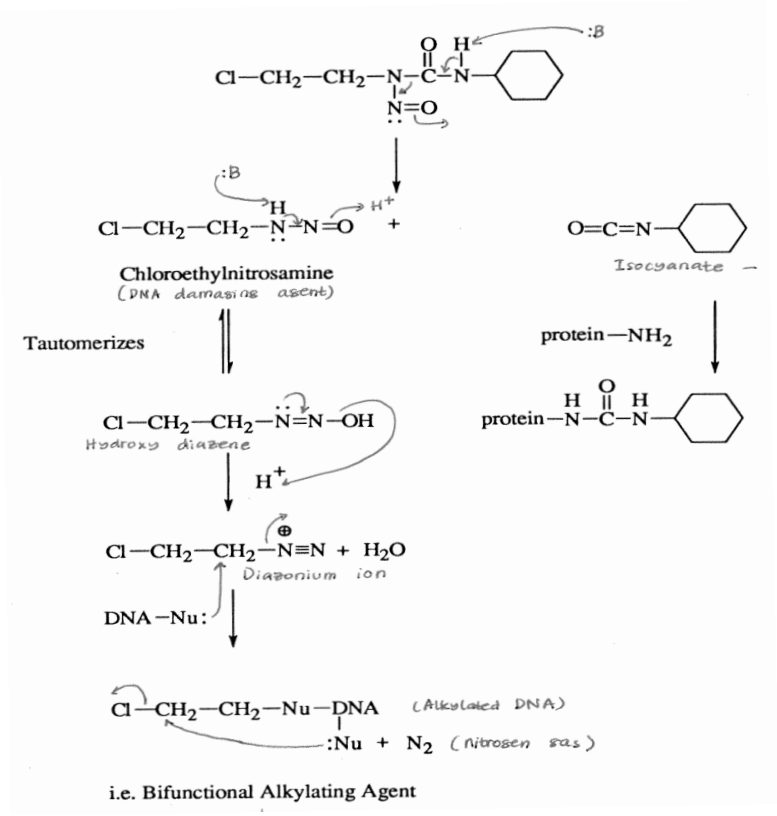
Mechanism: A prodrug and ultimately forms a diazonium ion that reacts with DNA (see Figure 5). Reacts with proteins and RNA in addition to DNA; might have some G1 or S phase specificity.

Toxicities: Myelosuppression, hepatotoxicity, pulmonary toxicity, seizures. Leukemias and myelodysplasias have occurred years later.

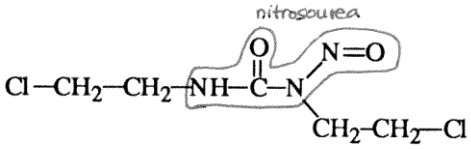
ADME: Rather unstable structure that begins to fragment in aqueous solution immediately. Metabolites (fragments appear in the urine) and can be eliminated over 2 days. The drug is non-polar (lipophilic) and therefore enters the CNS readily. This helps explain both its efficacy for brain cancer and CNS toxicities. Drug levels in CSF can reach 50x the levels in blood.

Note(s): Somewhat more stable than carmustine.

Figure 5. Lomustine activation.



Carmustine (BiCNU):



Uses: Brain cancer (glioblastoma), multiple myeloma (often in combination with other agents); also NHL. Administered IV. Formulated is lyophilized powder that is reconstituted prior to use.

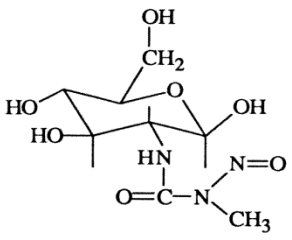
Mechanism: Like CeeNU (see Figure 5) but can lead to additional electrophiles.

Toxicities: Myelosuppression, hepatotoxicity, acute pulmonary toxicity (pulmonary fibrosis), seizures. Leukemias and myelodysplasias have occurred years later. Also pulmonary fibrosis can appear many years later.

ADME: Unstable structure that begins to fragment in aqueous solution immediately; no intact molecule in plasma after 15 minutes; metabolites (fragments) mainly appear in the urine. The drug is non-polar (lipophilic) and therefore enters the CNS readily. This helps explain both its efficacy for brain cancer and CNS toxicities. Drug levels in CSF can reach 50x the levels in blood.

Note(s): Discard if contents of vial are an oil as this indicates decomposition.

Streptozocin (Zanosar):



Uses: Pancreatic cancer (islet cell). Administered IV.

Mechanism: A prodrug and ultimately forms a diazonium ion that reacts with DNA; the glucopyranose moiety helps confer islet cell specificity.

Toxicities: GI toxicity (nausea, vomiting); only mild myelosuppression; use of the agent commonly leads to some level of kidney toxicity; rarely but has been associated with diabetes insipidus. Local irritation if extravasation occurs.

ADME: Agent degrades rapidly and distributes to both the liver and kidney; elimination from the plasma occurs within minutes; substantial excretion by the kidneys.

Note(s): A drug interaction with doxorubicin can occur which can increase the t1/2 and toxicity of dox. Does not penetrate the CNS well, too polar.