Chemotherapy and Drug Resistance

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The thought of having chemotherapy frightens many people. Almost everyone has heard stories about someone who was "on chemo." But we believe that knowing what chemotherapy is, how it works, and what to expect can often help calm your fears and give you more of a sense of control.

Chemotherapy and Drug Resistance

- **History**
- Principles
- Side effects
- Categories of chemotherapeutics
- Drug resistance

What is chemotherapy?

History:

Started after World War II (mustard gas)

1950's-1970's

e.g., lymphoma/ALL, germ cell tumors >>> effective

solid tumors (>90%) >>> resistant

1970's- research on drug resistance

palliative >>> aggressive (control, cure)

e.g., pre- and post-treatment of breast cancer surgery

combination with radiotherapy of osteosarcoma

- **Why chemotherapy is different from other treatments? (systematic)**

- **Chemotherapy in clinical trials (depending on drugs)**

Cancer response to anticancer drugs

- *High responsiveness*:

  HLL, lymphoma

- *Partial responsiveness*:

  breast and ovarian cancer

- *Poor responsiveness*:

  melanoma, small cell lung cancer

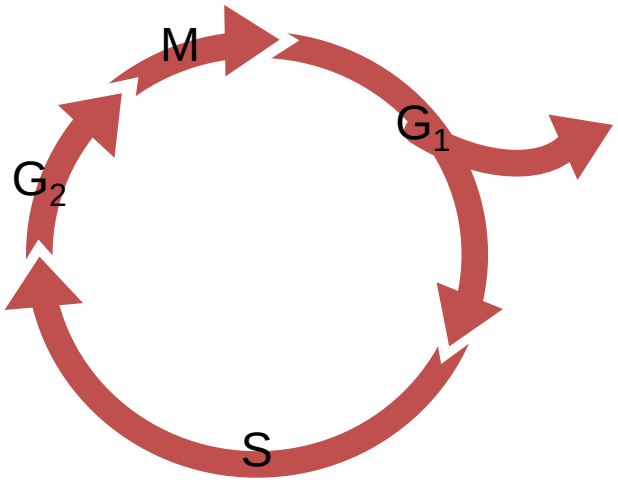
- “Heterogeneous drug sensitivity“ in same type of cancers

Chemotherapy and Drug Resistance

- History
- **Principles**
- Side effects
- Categories of chemotherapeutics
- Drug resistance

How does chemotherapy work?

Proliferating cells



Growth arrest
Differentiation
Apoptosis

Cell Cycle Phases

G1 phase. Metabolic changes prepare the cell for division. At a certain point - the restriction point - the cell is committed to division and moves into the S phase.

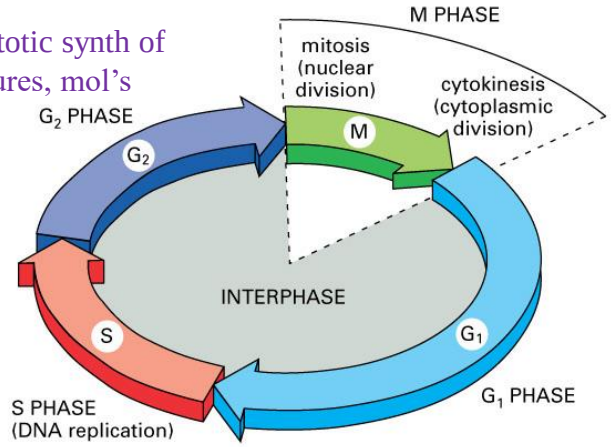
S phase. DNA synthesis replicates the genetic material. Each chromosome now consists of two sister chromatids.

G2 phase. Metabolic changes assemble the cytoplasmic materials necessary for mitosis and cytokinesis.

M phase. A nuclear division (mitosis[1]) followed by a cell division (cytokinesis [2]).

The period between mitotic divisions - that is, G1, S and G2 - is known as interphase.

Premitotic synth of structures, mol's

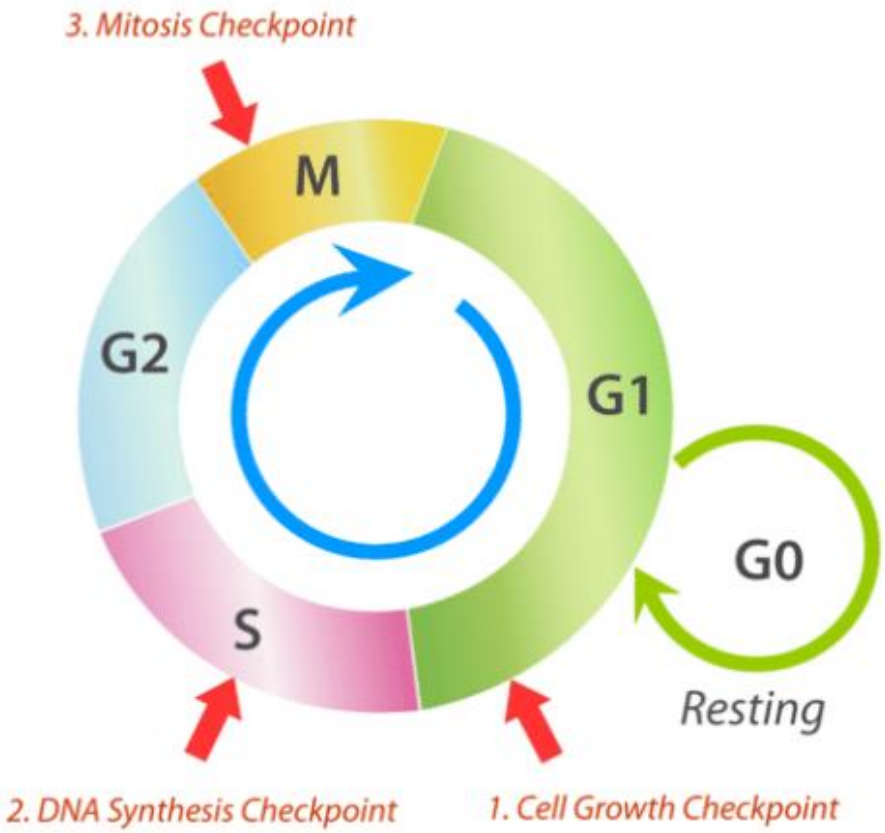


Synth DNA precursors, proteins, etc.

[1] a type of cell division that results in two daughter cells each having the same number and kind of chromosomes as the parent nucleus, typical of ordinary tissue growth.

[2] the cytoplasmic division of a cell at the end of mitosis or meiosis, bringing about the separation into two daughter cells.

Cycle Checkpoints



1. Cell Growth Checkpoint

- Occurs toward the end of growth phase 1 (G1).
- Checks whether the cell is big enough and has made the proper proteins for the synthesis phase.
- If not, the cell goes through a resting period (G0) until it is ready to divide.

2. DNA Synthesis Checkpoint

- Occurs during the synthesis phase (S).
- Checks whether DNA has been replicated correctly.
- If so, the cell continues on to mitosis (M).

3. Mitosis Checkpoint

- Occurs during the mitosis phase (M).
- Checks whether mitosis is complete.
- If so, the cell divides, and the cycle repeats.

What are the goals of treatment with chemotherapy?

- Cure
- Control
- palliation

How to choose drugs?

Factors to consider in choosing drugs:

- Type of cancer
- Stage of the cancer
- The age
- General state of health
- Other serious health problems (*e.g.*, liver & kidney diseases)
- Other type of anticancer treatments given in the past

How to choose drugs? (cont'd)

Doctors must also consider

- ✓ Side effects
- ✓ Drug interactions

  e.g., aspirin may lower blood platelets
- ✓ Vitamins

  e.g., antioxidant vitamins (A, E & C) vs. drugs

How to choose drugs? (cont'd)

Alternative way to consider ---

- The cell target of the drug
- The cancer cell condition
- The best way to deliver the drug
- The side-effect of the drug

Cell target of anticancer drugs

- DNA metabolism
- Cell division machine
- Cell membrane structure
- Cell energy plant……

Mechanisms of chemotherapy

Damage the DNA of the affected cancer cells. It is not always possible to be selective, but selectivity is the ultimate goal of any drug. *e.g.*, cisplatin (Platinol®), daunorubicin (Cerubidine®), doxorubicin (Adriamycin®), and etoposide (VePesid®).

Inhibit the synthesis of new DNA strands to stop the cell from replicating, because the replication of the cell is what allows the tumor to grow. *e.g.*, methotrexate (Abitrexate®), mercaptopurine (Purinethol®), fluorouracil (Adrucil®), and hydroxyurea (Hydrea®).

Stop the mitotic processes of a cell. Stopping mitosis stops cell division (replication) of the cancer and may ultimately halt the progression of the cancer. *e.g.*, Vinblastine (Velban®), Vincristine (Oncovin®) and Pacitaxel (Taxol®).

Cancer Cell Staging

- Restricted
- Invasive
- Metastasized

Cancer cell biology

- *Skipper Law* (animal leukemia cell growth)

  cell killed by first-order kinetics

  efficient killing at micrometastasis

- *Gompertzian growth curve* (human tumor cell growth)

  lag-exponential-platau

- *Goldie-Coldman hypothesis*
  spontaneous mutation (10^{-5}) is 10x > normal cell
  gene mutation/amplification, chromosome aberration
  visible tumor contains ~10^9 cells (= 1 gm)
  i.e., 10^4 resistant cells

If drug knows the target, it should kill.

In fact, few cancer cells escape from killing.

i.e., Drug >> select resistant cells

Genome is fluid in cancer cells,

mutation rate: 10^{-5} (10x > normal cells)

According to Goldie-Coldman hypothesis

mutation to one-drug resistance ~ 10^{-5}

mutation to two-drug resistance: 10^{-5} x 10^{-5} = 10^{-10}

>>> combination therapy should give cancer cells

much less chance to survive!

Planning drug doses and schedules

Doses

- based on body surface area
- differ between children and adults
- adjusted for people who are elderly, have poor nutritional status, have already taken or are currently taking other medications, have already received or are currently receiving radiation therapy, have low blood cell counts, or have liver or kidney diseases

Planning drug doses and schedules

Schedule (Cycles)

- A cycle = one dose followed by several days or weeks without treatment for normal tissues to recover from the drug's side effects

- The number of cycles = based on the type and stage of cancer, and side effects

Where are chemotherapy given?

- Hospital
- Doctor’s office
- Outpatient clinic
- Home
- Workplace

What are the ways to take chemotherapy?

- Oral (by mouth)
- Topical (on top of the skin as a cream or lotion)
- Intravenous (into a vein or IV)
- Intramuscular (into a muscle or IM)
- Subcutaneous (under the skin or SQ)
- Intraarterial (into an artery)
- Intrathecal (into the central nervous system via the cerebrospinal fluid)
- Intrapleural (into the chest cavity)
- Intraperitoneal (into the abdominal cavity)
- Intravesical (into the bladder)
- Intralesional (into the tumor)

Some chemotherapy drugs are never taken by mouth because the digestive system cannot absorb them or because they are very irritating to the digestive system.

e.g., some people with certain digestive system symptoms (vomiting, diarrhea, or severe nausea) cannot swallow liquids or pills, or cannot remember when or how many pills to take.

Safety precautions for professionals

Many chemotherapy drugs are dangerous :

- ✓ They can cause abnormal changes in DNA (mutagenic).
- ✓ They may be able to alter development of a fetus or embryo, leading to birth defects (teratogenic).
- ✓ They may be able to cause another type of cancer (carcinogenic).
- ✓ Some may cause localized skin irritation or damage

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Cells attacked by chemotherapeutic agents

- Cancer cells.
- Skin cells and hair follicle cells causing loss of hair (alopecia).
- Gastrointestinal epithelium causing nausea and vomiting.
- Bone marrow depression, causing problems of the immune system and therefore, possibly infections. The formation of platelets is also affected leading to problems with blood clotting.
- Testes or ovaries leading to sterility (either temporary or permanent).

What are the side effects?

Although chemotherapy is given to kill cancer cells, it also can damage normal cells. Most likely to be damaged are normal cells that are rapidly dividing:

- Blood cells
- Cells of hair follicles
- Cells in the reproductive and digestive tracts

Damage to these cells accounts for many of the side effects of chemotherapy drugs. Side effects are different for each chemotherapy drug, and they also differ based on the dosage, the route the drug is given, and how the drug affects you individually.

What are the side effects? (cont'd)

Bone marrow suppression

The bone marrow is the tissue inside some bones that produces white blood cells (WBCs), red blood cells (RBCs), and blood platelets. Damage to the blood cell-producing tissues of the bone marrow is called bone marrow suppression, or myelosuppression, and is one of the most common side effects of chemotherapy.

What are the side effects? (cont'd)

Bone marrow suppression(cont'd)

The decrease in blood cell counts does not occur immediately after chemotherapy because the drugs do not destroy the cells already in the bloodstream. Instead, the drugs temporarily prevent formation of new blood cells by the bone marrow.

Each type of blood cell has a different life span:

White blood cells average a 6-hour lifespan

Platelets average 10 days

Red blood cells average 120 days

What are the side effects? (cont'd)

Bone marrow suppression (cont'd)

The lowest count that blood cell levels fall to is called the *nadir*. The nadir for each blood cell type will occur at different times but usually WBCs and platelets will reach their nadir within 7-14 days. RBCs live longer and will not reach a nadir for several weeks.

What are the side effects? (cont'd)

Bone marrow suppression (cont'd)

Knowing what the 3 types of blood cells normally do can help you understand the effects of low blood cell counts.

White blood cells help the body resist infections.

Platelets help prevent excessive bleeding by forming plugs to seal up damaged blood vessels.

Red blood cells bring oxygen to tissues so cells throughout the body can use that oxygen to turn certain nutrients into energy.

What are the side effects? (cont'd)

Bone marrow suppression-- Low WBC counts:

Even though the WBC count or the neutrophil count is low, it does not mean you will have an infection. But you need to watch for these signs and symptoms of an infection:

- Fever
- Sore throat
- New cough or shortness of breath
- Nasal congestion
- Burning during urination
- Shaking chills
- Redness, swelling, and warmth at the site of an injury

What are the side effects? (cont'd)

Bone marrow suppression-- Low RBC counts:

With anemia, you may have the following symptoms:

- Fatigue
- Dizziness
- Headaches
- Irritability
- Shortness of breath
- An increase in heart rate or rate of breathing or both

What are the side effects? (cont'd)

Bone marrow suppression-- Low platelet counts:

If your platelet count is low, you may show these signs:

- Bruise easily
- Bleed longer than usual after minor cuts or scrapes
- Have bleeding gums or nose bleeds
- Develop ecchymoses (large bruises) and petechiae (multiple small bruises)
- Have serious internal bleeding if the platelet count is very low

What are the side effects? (cont'd)

Other side effects

Hair loss

Appetite loss and weight loss

Taste changes

Reproduction & sexuality

Nausea and vomiting

Constipation

Diarrhea

Fatigue

Heart damage

Nervous system changes

Lung damage

Liver damage

Kidney & urinary system damage

What are the side effects? (cont'd)

Long-term side effects:

Side effects related to specific chemotherapy drugs can continue after the treatment is completed. These effects can progress and become chronic, or new side effects may occur. Long-term side effects depend on the specific drugs received and whether you received other treatments such as radiation therapy.

- Permanent organ damage
- Delayed development in children
- Nerve damage
- Infections
- Blood in the urine
- Another cancer

What questions should I ask about chemotherapy?

- What chemotherapy medications will I be given?
- How will I take these drugs (by mouth or through a vein)?
- How frequently will I need to take chemotherapy?
- How long will I be receiving chemotherapy treatments?
- What side effects might I experience?
- What activities should I do or not do to take care of myself?
- What long-term effects might I expect?
- How can I contact you after office hours if I have signs or symptoms that you need to know about?

What's new in chemotherapy research?

- New chemotherapy medications.
- Novel approaches to targeting drugs more specifically at the cancer cells (like attaching drugs to monoclonal antibodies or packaging them inside liposomes) to produce fewer side effects.
- Drugs to reduce side effects such as colony-stimulating factors and chemoprotective agents (such as dexrazoxane and amifostine).
- Hematopoietic stem cell transplantation.
- Agents that overcome multidrug resistance.

What's new? (cont'd)

- Liposomal therapy using chemotherapy drugs (synthetic fat globules). The liposome, or fatty coating, helps them penetrate the cancer cells more selectively and decreases possible side effects (such as hair loss and nausea and vomiting). *e.g.*, Doxil (the encapsulated form of doxorubicin) and DaunoXome (the encapsulated form of daunorubicin).
- Monoclonal antibodies (or proteins) that bind to tumor-associated cell surface antigens and cause the destruction of tumor cells through a variety of methods. Monoclonal antibodies, a special type of antibody produced in laboratories, can be designed to guide chemotherapy medications directly to the tumor. Monoclonal antibodies (without attached chemotherapy) can also be used as immunotherapy drugs, to strengthen the body's immune response against cancer cells.

Chemotherapy and Drug Resistance

- History
- Principles
- Side effects
- **Categories of chemotherapeutics**
- Drug resistance

Categories of chemotherapeutic agents

- **Alkaloids**
- Alkylating agents
- Antibiotics
- antimetabolites
- Enzymes
- Hormones
- Platinum compounds
- New anticancer drugs

Catagories of chemotherapeutic agents

- Alkaloids:

  Microtubule inhibitors

  - vinca alkaloids (*e.g.*, vincristine, vinblastine)
  - paclitaxel (taxol) and docetaxel

  Chromatin function

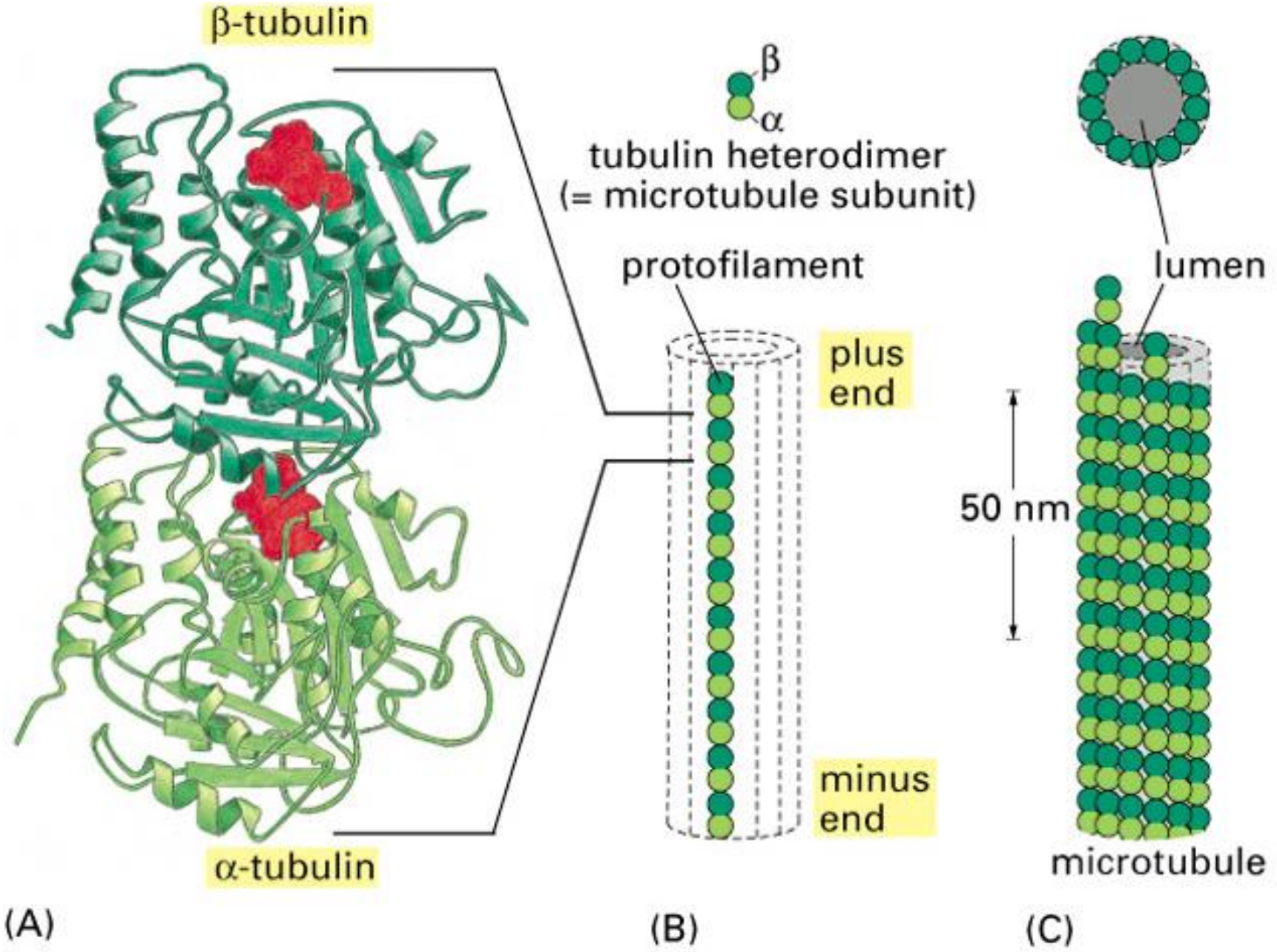
  - epipodophyllotoxins = topoII inhibitor (*e.g.*, etoposide VP-16, teniposide VP-26)
  - camptothecin = topo I inhibitor

Taxus brevifolia

Catagories of chemotherapeutic agents

- Alkaloids: Microtubule inhibitors: vinca alkaloids

Action mechanism: The vinca alkaloids are cell specific agents and block cells in mitosis. Their biological activity is explained by their specific binding to tubulin. Upon binding to vinca alkaloids, tubulin dimers are unable to aggregate to form microtubules. This effectively decreases the pool of free tubulin dimers available for microtubule assembly, resulting in a shift of the equilibrium toward disassembly. Formation of paracrystalline aggregates by vinca-bound tubulin dimers shifts the equilibrium even further toward disassembly and microtubule shrinkage. They block mitosis with metaphase arrest.

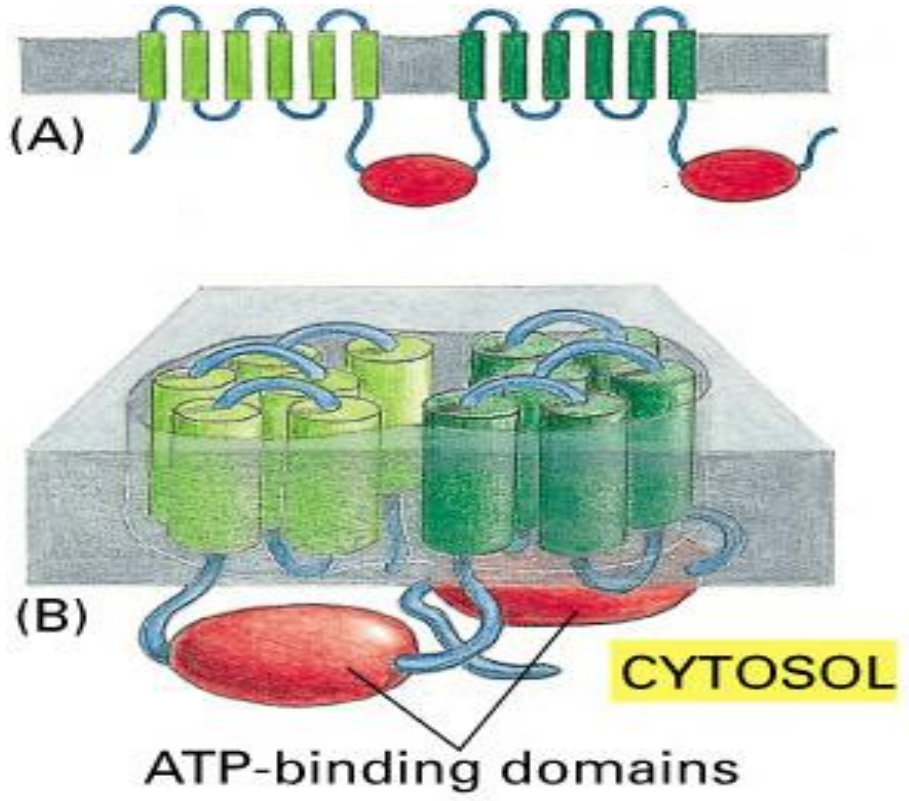

β-tubulin
α-tubulin
β
α
tubulin heterodimer
(= microtubule subunit)
protofilament
plus
end
minus
end
lumen
50 nm
microtubule
(A)
(B)
(C)

Catagories of chemotherapeutic agents

- Alkaloids: Microtubule inhibitors: vinca alkaloids

Resistance: Drug resistance is due primarily to decreased drug accumulation and results from overexpression of the P-glycoprotein, an ABC transporter (shown on next slide). Note that this is one of the drug classes that may show MDR-mediated cross-resistance with multiple other natural products. Resistance can also be due to alterations in tubulin structure resulting in changes in drug binding to the tubulin.

A typical ABC transporter



ABC transporters are a transport system superfamily that is one of the largest and possibly one of the oldest gene families

Categories of chemotherapeutic agents

- Alkaloids: Microtubule inhibitors: Taxol

Action mechanism: In contrast to other microtubule antagonists, taxol disrupts the equilibrium between free tubulin and microtubules by shifting it in the direction of assembly, rather than disassembly. As a result, Taxol treatment causes both the stabilization of microtubules and the formation of abnormal bundles of microtubules. Studies have shown that Taxol binds to microtubules at a ratio of about one drug molecule per molecule of polymerized tubulin dimer. The binding site for Taxol is apparently distinct from the binding sites for colchicine, vinblastine , podophyllotoxin and GTP.

Categories of chemotherapeutic agents

- Alkaloids: Microtubule inhibitors: Taxol

Resistance: Resistance to these drugs arises through mechanisms similar to how it arises to the vinca alkaloids, mainly overexpression of P-glycoprotein. Those are multidrug resistance and mutation of the gene coding for one of the tubulin subunits.

Categories of chemotherapeutic agents

- Alkaloids: Chromatin function inhibitors

Action mechanism: In eukaryotic nucleus, topoisomerases are needed to permit selected regions of DNA to become sufficiently untangled and relaxed to allow transcription, replication, and other essential functions to proceed. To do this topoisomerases have the ability to break DNA strands and then to reseal these breaks after the topological changes have occurred.

The clinically useful drugs in this class are inhibitors of topoisomerase II as they break both strands of DNA. Several inhibitors of the type I enzyme are in early clinical trials but look promising as anticancer agents.

Catagories of chemotherapeutic agents

- Alkaloids: Chromatin function inhibitors

Resistance: Resistance to these drugs is commonly accompanied by cross resistance to several drugs. Resistant cells demonstrate overexpression of the MDR gene that encodes the p- glycoprotein drug efflux transporter . In addition an "atypical " multidrug resistant phenotype has been identified in which cells that are resistant to topoisomerase-II inhibitors retain normal drug transport characteristics. These cells have altered or decreased topoisomerase activity.

Categories of chemotherapeutic agents

- Alkaloids
- **Alkylating agents**
- Antibiotics
- antimetabolites
- Enzymes
- Hormones
- Platinum compounds
- New anticancer drugs

Categories of chemotherapeutic agents

- Alkylating agents
  - nitrogen mustards
    (*e.g.*, mechlorethamine, cyclophosphamide)
  - nitrosoureas
  - other alkylating agents
    (*e.g.*, dacarbazine, mitomycin C)

Categories of chemotherapeutic agents

- Alkylating agents: Nitrogen mustards

Action mechanism: All of the alkylating agents form strong electrophiles through the formation of carbonium ion intermediates. This results in the formation of covalent linkages by alykylation of various nucleophiles moieties. The chemotherapeutic and cytotoxic effects are directly related to the alkylation of DNA mainly through the 7 nitrogen atom of guanine although other moieties are also alkylated.

Catagories of chemotherapeutic agents

- Alkylating agents: Nitrogen mustards

Resistance: Cross-resistance between different alkylating agents often occurs. Resistance may represent the summation of a series of changes, none of which by itself confers significant resistance. Several biochemical mechanisms have been implicated as a cause of resistance to these drugs including decreased permeability of the drug into the cells, increased production of nucleophiles such as glutathione and increased repair of DNA. Two mechanisms of resistance that are probably of clinical importance are increased drug inactivation and decreased drug uptake.

Categories of chemotherapeutic agents

- Alkylating agents: Nitrosoureas

Action mechanism: This class of alkylating agents appear to function as bifunctional alkylating agents but differ in both pharmacological and toxicological properties from the other alkylating agents. The nitrosoureas are converted nonenzymatically into a carbonium ion and an isothiocyanate molecule. The carbonium ion acts as a typical alkylating agent and is probably responsible for the cytotoxic action of the nitrosoureas. The isothiocyanate may interact with proteins and account for some of the toxic effects of these drugs.

Categories of chemotherapeutic agents

- Alkaloids
- Alkylating agents
- **Antibiotics**
- antimetabolites
- Enzymes
- Hormones
- Platinum compounds
- New anticancer drugs

Categories of chemotherapeutic agents

- Antibiotics
  - Dactinomycin (Actinomycin D)
  - Anthracycline antibiotics (*e.g.*, adriamycin)
  - Anthragenediones (Mitoxantrone)
  - Bleomycin
  - Plicamycin (Mithramycin)

Categories of chemotherapeutic agents

- Antibiotics: Dactinomycin (Actinomycin D)

Action mechanism: At low concentrations dactinomycin inhibits DNA directed RNA synthesis and at higher concentrations DNA synthesis is also inhibited. All types of RNA are affected, but ribosomal RNA is more sensitive. Dactinomycin binds to double stranded DNA, permitting RNA chain initiation but blocking chain elongation. Binding to the DNA depends on the presence of guanine. It appears that the phenoxazone chromophore region of the drug intercalates between bases in the DNA and that the 2-amino group of the guanine is important in the formation of a stable drug -DNA complex. This blockade is responsible for the cytotoxic effect.

Categories of chemotherapeutic agents

- Antibiotics: Dactinomycin (Actinomycin D)

Resistance: Resistance results mainly from decreased retention of the drug in the cancer cell. Cells resistant to dactinomycin show cross-resistance to anthracyclines and vinca alkaloids as a result of amplification of the gene for P-glycoprotein. In addition resistance can be reversed by competition with verapamil and a variety of other lipophilic compounds.

Categories of chemotherapeutic agents

- Antibiotics: Anthracycline antibiotics

Action mechanism: The anthracyclines all bind to DNA, by intercalation, and their cytotoxicity largely results from this binding. They bind to double stranded DNA. If the structure of the anthracyclines is modified so that the binding to DNA is altered their is usually a decrease or loss of antitumor activity. Inhibition of DNA and RNA synthesis is not though to be critical for cytotoxicity as it only occurs at high drug concentration.

Categories of chemotherapeutic agents

- Antibiotics: Anthracycline antibiotics

  Resistance: The most common mechanism of resistance to the anthracyclines is increased drug efflux due to amplification of the gene for P-glycoprotein, the multidrug transporter. However, two other mechanisms of resistance have been reported. These are decreased topoisomerase II activity and increased glutathione peroxidase activity. The latter is consistent with free radical formation being important in the mechanism of action of these drugs.

Categories of chemotherapeutic agents

- Antibiotics: Anthragenediones (Mitoxantrone)

Action mechanism: Mitoxantrone interacts with DNA by a high-affinity intercalation. It also produces a lower affinity binding as a result of electrostatic interactions. Intercalation of mitoxantrone into DNA interferes with the strand-reunion reaction of topoisomerase II, resulting in production of protein-linked double-strand DNA breaks. Cells in late S phase are more sensitive. Tumor cells resistant to mitoxanthone may show cross resistance to other natural products.

Categories of chemotherapeutic agents

- Antibiotics: Bleomycin

Action mechanism: Bleomycin has been found to profoundly inhibit DNA synthesis while RNA and protein synthesis are much less affected. Bleomycin usually produces a block in the early G2 phase of the cell cycle. The cytotoxic activity results from their ability to cause fragmentation of DNA. Single strand breaks occur predominantly but double strand breaks occur also. Bleomycin has two major domains in it's structure. One portion interacts with DNA and one binds iron. Both iron and oxygen are required for bleomycin to degrade DNA. The drug binds Fe^{2+}, and binds DNA by intercalation between GT or GC bases, and acts as a ferrous oxidase (Fe^{2+}, Fe^{3+}) resulting in production of oxygen free radicals that cleave DNA.

Categories of chemotherapeutic agents

- Antibiotics: Bleomycin

Resistance: Several mechanisms of resistance have been described. One of the most common is an increased degradation of bleomycin by certain amidase enzymes. Low levels of this enzyme are found in tumors sensitive to the drug and high levels in many tumors resistant to it. However, some studies have not been able to correlate tumor responsiveness with levels of degradative enzymes. Changes in transport have also found in some resistant tumors.

Categories of chemotherapeutic agents

- Alkaloids
- Alkylating agents
- Antibiotics
- **antimetabolites**
- Enzymes
- Hormones
- Platinum compounds
- New anticancer drugs

Categories of chemotherapeutic agents

- Antimetabolites
  - Antifolates (*e.g.*, methotrexate)
  - Purine antimetabolites (*e.g.*, 6-thioguanine)
  - Pyrimidine antagonists
    (*e.g.*, 5-fluorouracil, cytosine arabinoside)

Catagories of chemotherapeutic agents

- Antimetabolites: Antifolates (*e.g.*, methotrexate)

Action mechanism: Folic acid is an essential growth factor from which is derived a series of tetrahydrofolate cofactors that provide single carbon groups for the synthesis of RNA and DNA precursors such as thymidylate and purines. Folic acid must be reduced in two successive steps by dihydrofolate reductase (DHFR) before it can function as a coenzyme. The fully reduced form in the one that picks up and delivers single carbon units in various metabolic processes. The enzyme dihydrofolate reductase is the primary site of action of most folate analogs such as methotrexate. Inhibition of this enzyme leads to toxicity through partial depletion of cofactors required for the synthesis of purines and thymidylate.

Catagories of chemotherapeutic agents

- Antimetabolites: Antifolates (*e.g.*, methotrexate)

Resistance: Several biochemical mechanisms of resistance have been demonstrated. The major mechanisms are decreased drug uptake, amplification of the dihydrofolate reductase gene and thus an increase in the target enzyme, mutations in the DHFR gene, and decreased ability to form methotrexate polyglutamate inside cells.

Categories of chemotherapeutic agents

- Antimetabolites: Purine antimetabolites

  Action mechanism: Most studies indicate that the thiopurines work at multiple sites and that their mechanism of action is a result of combined effects at these different sites. The thiopurines must first be converted into the nucleotide form in order to be active. This conversion is catalyzed by phosphoribosyltransferase enzymes. The thiopurine nucleotide forms inhibit the first committed step in the de novo purine synthesis pathway (PRPP amidotransferase) and the key step in guanine nucleotide biosynthesis, IMP dehydrogenase. This latter site is the branch point where IMP is channeled towards either guanine nucleotide synthesis or adenine nucleotide synthesis. The mononucleotide derivatives are ultimately converted to triphosphates which can be incorporated into RNA and DNA.

Categories of chemotherapeutic agents

- Antimetabolites: Purine antimetabolites

  Resistance: For these antipurines to work, efficient generation and maintenance of the nucleotide forms is necessary. In experimental tumors, lack of an altered phosphoribosyltransferase enzyme is the most commonly encountered mechanism of resistance. This enzyme is primarily responsible for forming the nucleotide. A different pattern is seen in humans receiving thiopurine therapy where increased alkaline phosphatase activity seems to be a major cause of resistance. This enzyme catalyzes the breakdown of the nucleotide form and could protect tumor cells by antagoning the accumulation of thiopurine nucleotides.

Categories of chemotherapeutic agents

- Antimetabolites: Pyrimidine antagonists *(e.g.*, 5-Fu)

Action mechanism: Members of this group are direct inhibitors of thymidylate synthetase the key enzyme in thymidylate synthesis. 5-FU must first be converted to the nucleotide form to be active as a cytotoxic agent. FUMP can be incorporated into RNA and also can be converted to the deoxynucleotide(F-dUMP). This latter reaction is crucial to the cytotoxic effects of 5-FU. FdUMP inhibits the enzyme thymidylate synthetase which leads to deletion of TTP, a necessary constituent of DNA. DNA synthesis is inhibited until the drug is removed and new enzyme synthesis occurs. Incorporation into RNA has resulted in observed effects on the function of both rRNA and mRNA.

Categories of chemotherapeutic agents

- Antimetabolites: Pyrimidine antagonists

  Resistance: A number of biochemical mechanisms have been identified that are associated with resistance to 5- FU. The major ones include decreased conversion to the nucleotide form and increased breakdown of the nucleotide. For each of these mechanisms changes in several different enzymes might account for resistance.

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- New anticancer drugs

Catagories of chemotherapeutic agents

- Enzymes

  L-asparaginase was developed after it was noted that guinea pig serum suppressed the growth of lymphosarcomas in mice. The active serum component was found to be L-asparaginase, an enzyme that hydrolyzes L-asparagine to L-aspartate. The enzyme is effective because a few neoplastic cells have low levels of asparagine synthetase activity and require L-asparagine for growth. Resistance rapidly develops to in most cancer cells.

Categories of chemotherapeutic agents

- Enzymes

Hydroxyurea inhibits DNA synthesis without inhibiting the incorporation or precursors into RNA or protein. Specifically, it inhibits ribonucleotide reductase to block deoxyribonucleotide formation and DNA synthesis. This enzyme is closely related to proliferative status in cancer cells. It is involved in the de novo synthesis of all the precursors used in DNA synthesis. It converts ribonucleotide diphosphates to deoxyribonucleotides. Hydroxyurea is an S phase specific drug. Resistance is due to changes in the ribonucleotide reductase.

Categories of chemotherapeutic agents

- Hormones: (*e.g.*, Tamoxifen)

  Action mechanism: Tamoxifen is a competitive inhibitor of estradiol binding to the estrogen receptor. It acts as a complete antagonist in some systems and as an antagonist with partial agonist activity in other systems. By binding to the receptor it competes with the binding of endogenous estradiol and its major therapeutic effect reflects this antiestrogenic mechanism. It induces a change in the 3 dimensional shape of the receptor inhibiting its binding to the estrogen response element on DNA.

Categories of chemotherapeutic agents

- Alkaloids
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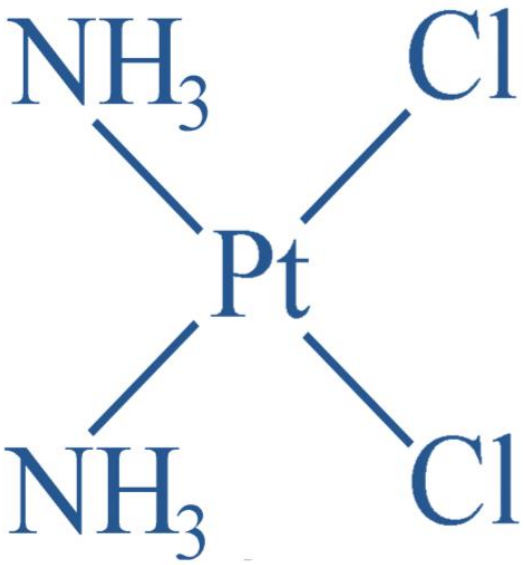
Categories of chemotherapeutic agents

- Platinum compounds: (*e.g.*, cisplatin, carboplatin)

Action mechanism: The platinum compounds are DNA cross-linking agents similar to but not identical to the alkylating agents. The platinum compounds exchange chloride ions for nucleophilic groups of various kinds. Both the cis and trans isomers do this but the trans isomer is known to be bioligically inactive for reasons not completely understood. To possess antitumor activity a platinum compound must have two relatively labile cis-oriented leaving groups. Cross-resistance between the two groups of drugs is usually not seen.

Cis-diamminedichloroplatinum (II)
"Cisplatin"



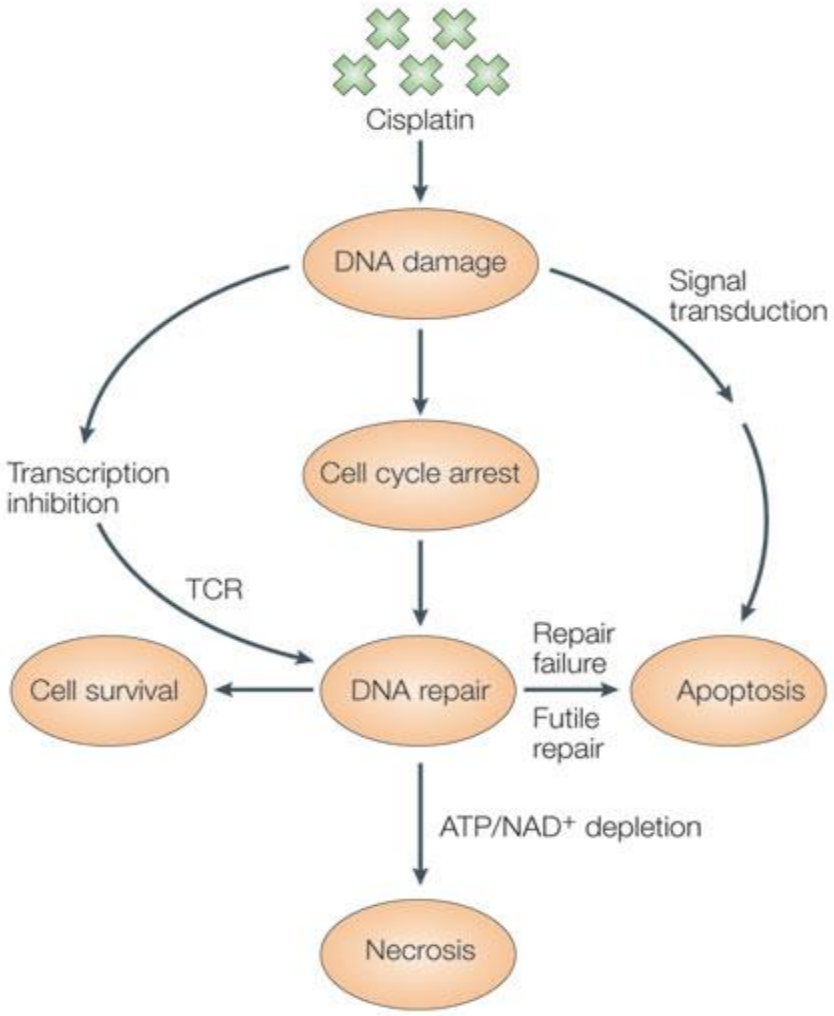
Cisplatin

Cisplatin, **cisplatinum**, **platamin**, **neoplatin**, **cismaplat** or ***cis*-diamminedichloroplatinum(II)** (**CDDP**) is a chemotherapy drug. It was the first member of a class of platinum-containing anti-cancer drugs, which now also includes carboplatin and oxaliplatin. These platinum complexes react *in vivo*, binding to and causing crosslinking of DNA, which ultimately triggers apoptosis (programmed cell death).

Medical use

- Cisplatin is administered intravenously as short-term infusion in normal saline for treatment of solid malignancies.
- It is used to treat various types of cancers, including sarcomas ,some carcinomas (*e.g.*, small cell lung cancer, and ovarian cancer), lymphomas, bladder cancer, cervical cancer, and germ cell tumors.
- Cisplatin is particularly effective against testicular cancer; the cure rate was improved from 10% to 85%.
- In addition, cisplatin is used in Auger therapy.

Cisplatin



Nature Reviews | Drug Discovery

Cisplatin

Side effects

Cisplatin has a number of side-effects that can limit its use:

- **Neurotoxicity** (nerve damage) can be anticipated by performing nerve conduction studies before and after treatment. Common neurological side effects of cisplatin include visual perception and hearing disorder, which can occur soon after treatment begins. While triggering apoptosis through interfering with DNA replication remains the primary mechanism of cisplatin, this has not been found to contribute to neurological side effects. Recent studies have shown that cisplatin noncompetitively inhibits an archetypal, membrane-bound mechanosensitive sodium-hydrogen ion transporter known as NHE-1. It is primarily found on cells of the peripheral nervous system, which are aggregated in large numbers near the ocular and aural stimuli-receiving centers. This noncompetitive interaction has been linked to hydroelectrolytic imbalances and cytoskeleton alterations, both of which have been confirmed in vitro and in vivo. However, NHE-1 inhibition has been found to be both dose-dependent (half-inhibition = 30 µg/mL) and reversible.

Cisplatin

❑**Nephrotoxicity** (kidney damage) is a major concern. The dose is reduced when the patient's creatinine clearance (a measure of renal function) is reduced. Adequate hydration and diuresis is used to prevent renal damage. The nephrotoxicity of platinum-class drugs seems to be related to reactive oxygen species and in animal models can be ameliorated by free radical scavenging agents (*e.g.*, amifostine). Nephrotoxicity is a dose-limiting side effect.

❑Nausea and vomiting: cisplatin is one of the most emetogenic chemotherapy agents, but this symptom is managed with prophylactic antiemetics (ondansetron, granisetron. etc.) in combination with corticosteroids. Aprepitant combined with ondansetron and dexamethasone has been shown to be better for highly emetogenic chemotherapy than just ondansetron and dexamethasone.

❑Electrolyte disturbance: Cisplatin can cause hypomagnesaemia, hypokalaemia and hypocalcaemia. The hypocalcaemia seems to occur in those with low serum magnesium secondary to cisplatin, so it is not primarily due to the cisplatin.

Cisplatin

- **Ototoxicity** (hearing loss): there is at present no effective treatment to prevent this side effect, which may be severe. Audiometric analysis may be necessary to assess the severity of ototoxicity. Other drugs (such as the aminoglycoside antibiotic class) may also cause ototoxicity, and the administration of this class of antibiotics in patients receiving cisplatin is generally avoided. The ototoxicity of both the aminoglycosides and cisplatin may be related to their ability to bind to melanin in the stria vascularis of the inner ear or the generation of reactive oxygen species.

- Myelotoxicit: This agent can also cause profound bone marrow suppression.

- Hemolytic anemia can be developed after several courses of cisplatin. It is suggested that an antibody reacting with a cisplatin-red-cell membrane is responsible for hemolysis.

Cisplatin

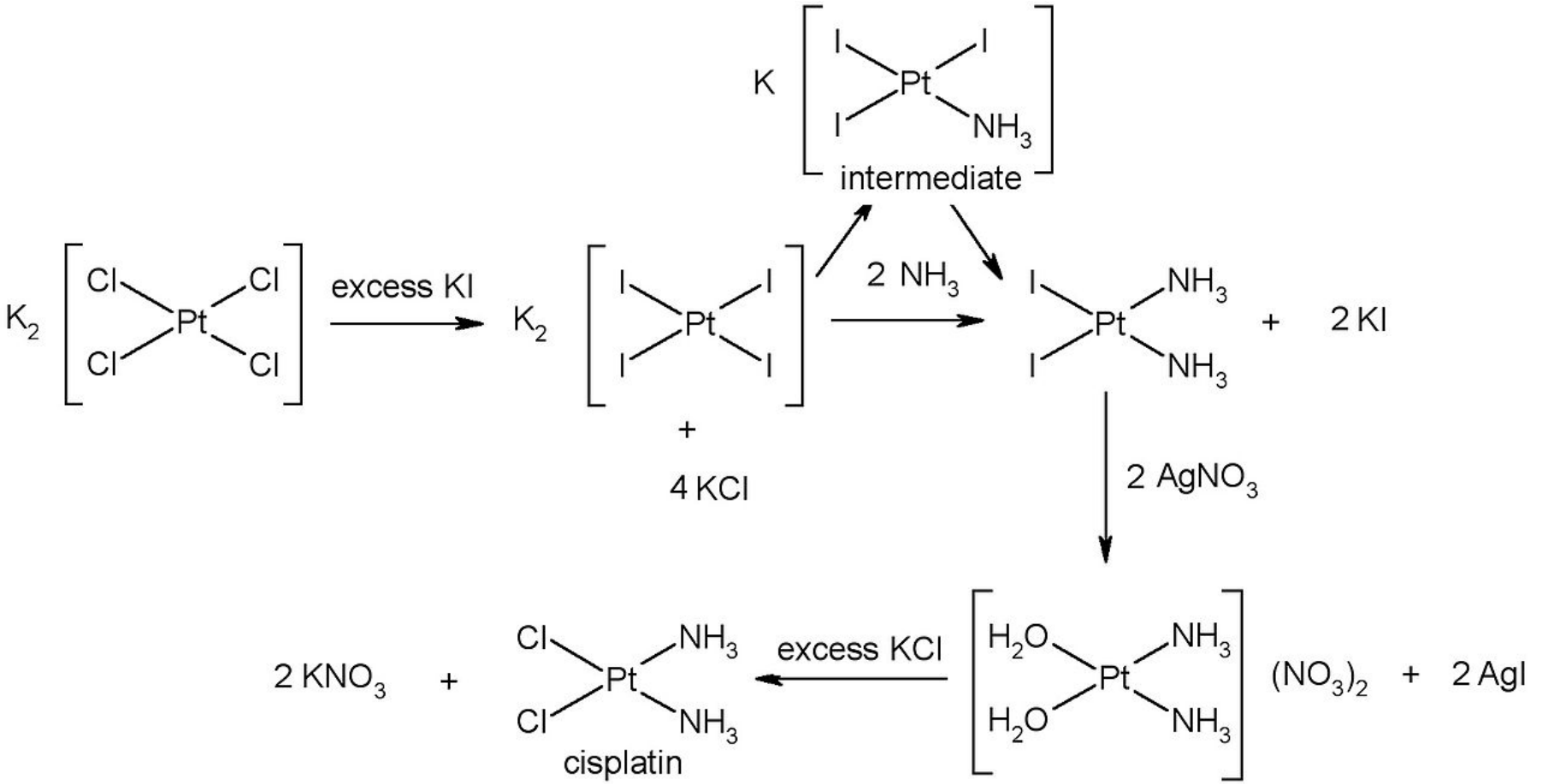
Synthesis

The synthesis of cisplatin starts from potassium tetrachloroplatinate. The tetraiodide is formed by reaction with an excess of potassium iodide. Reaction with ammonia forms $K_2[PtI_2(NH_3)_2]$ which is isolated as a yellow compound. When silver nitrate in water is added insoluble silver iodide precipitates and $K_2[Pt(OH_2)_2(NH_3)_2]$ remains in solution. Addition of potassium chloride will form the final product which precipitates In the triiodo intermediate the addition of the second ammonia ligand is governed by the trans effect.

For the synthesis of transplatin $K_2[PtCl_4]$ is first converted to $Cl_2[Pt(NH_3)_4]$ by reaction with ammonia. The trans product is then formed by reaction with hydrochloric acid.

Cisplatin

Synthesis



Cisplatin

Mechanism of action

Following administration, one of the chloride ligands is slowly displaced by water (an aqua ligand), in a process termed aquation. The aqua ligand in the resulting $[PtCl(H_2O)(NH_3)_2]^+$ is itself easily displaced, allowing the platinum atom to bind to bases. Of the bases on DNA, guanine is preferred. Subsequent to formation of $[PtCl(guanine\text{-}DNA)(NH_3)_2]^+$, crosslinking can occur via displacement of the other chloride ligand, typically by another guanine. Cisplatin crosslinks DNA in several different ways, interfering with cell division by mitosis. The damaged DNA elicits DNA repair mechanisms, which in turn activate apoptosis when repair proves impossible. In 2008, researchers were able to show that the apoptosis induced by cisplatin on human colon cancer cells depends on the mitochondrial serine-protease Omi/Htra2. Since this was only demonstrated for colon carcinoma cells, it remains an open question if the Omi/Htra2 protein participates in the cisplatin-induced apoptosis in carcinomas from other tissues.

Cisplatin

Mechanism of action contd..

Most notable among the changes in DNA are the 1,2-intrastrand cross-links with purine bases. These include 1,2-intrastrand d(GpG) adducts which form nearly 90% of the adducts and the less common 1,2-intrastrand d(ApG) adducts. 1,3-intrastrand d(GpXpG) adducts occur but are readily excised by the nucleotide excision repair (NER). Other adducts include inter-strand crosslinks and nonfunctional adducts that have been postulated to contribute to cisplatin's activity. Interaction with cellular proteins, particularly HMG domain proteins, has also been advanced as a mechanism of interfering with mitosis, although this is probably not its primary method of action.

Note that although cisplatin is frequently designated as an alkylating agent, it has no alkyl group and so cannot carry out alkylating reactions. It is correctly classified as alkylating-like.

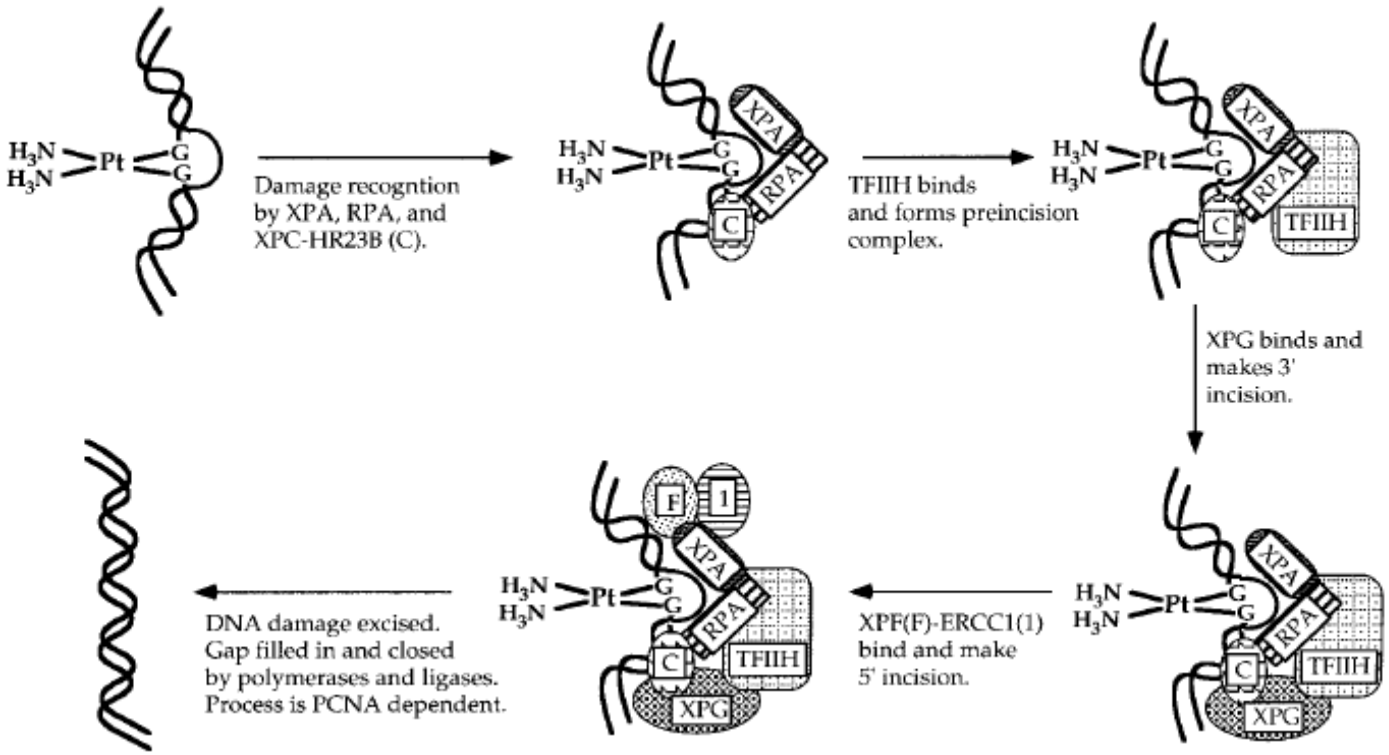
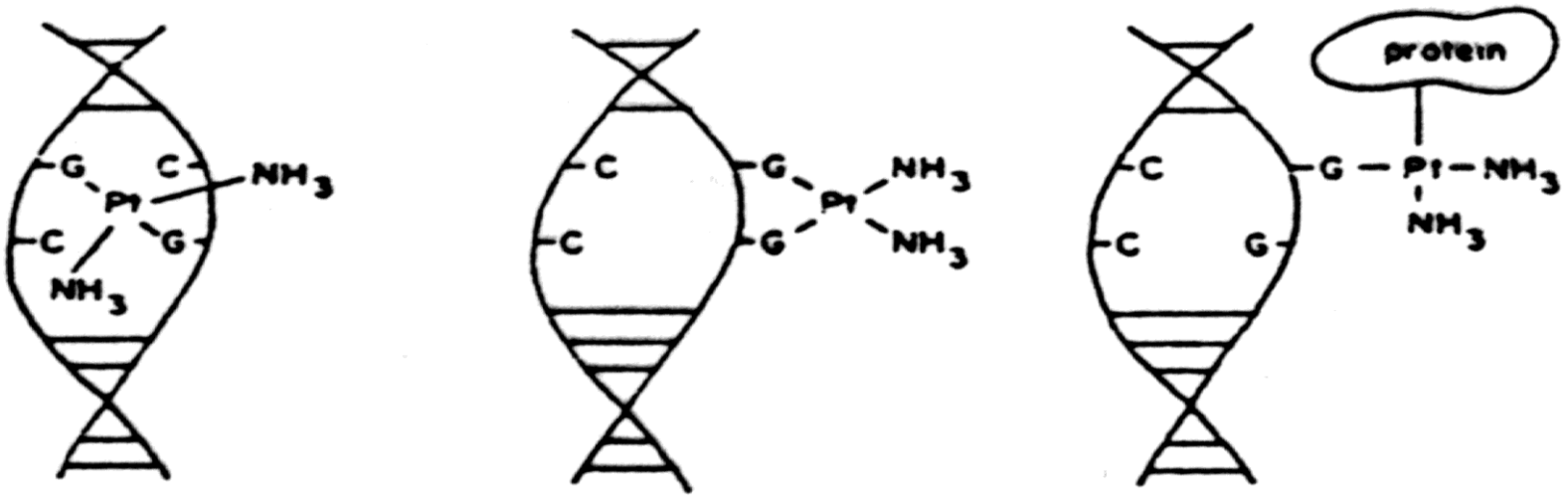


Figure . A schematic diagram of nucleotide excision repair of a cisplatin-DNA cross-link. The DNA damage is recognized by XPA, RPA, and XPC-HR23B (C). TFIIH binds forming a pre-incision complex. XPG makes the 3′-incision, and the 5′-incision is made by XPF-ERCC1 ((F) and (1), respectively). Once the damage is excised, the DNA is filled in by polymerases and ligases in a PCNA dependent process

DNA damages caused by cisplatin

The principal sites of reaction are the N7 atoms of guanine and adenine. The main interaction is formation of intrastrand cross links between the drug and neighboring guanines. DNA - protein cross linking also occurs but this does not correlate with cytotoxicity.

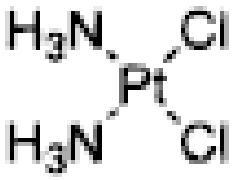


Categories of chemotherapeutic agents

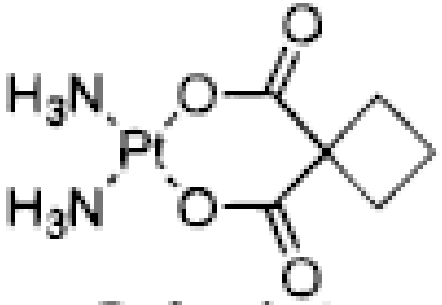
- Platinum compounds: (*e.g.*, cisplatin, carboplatin)

  Resistance: No clear -cut dominant mechanism of resistance to cisplatin has been identified. Often, resistant cells have an increased ability to repair intrastrand adducts but in many cases this is insufficient to explain the extent of resistance. Resistance in some cases has been shown to correlate with the increase in sulfhydryl compounds such as glutathione or the metal binding protein metallothionein. Thus, as with the alkylating agents multiple causes of resistance may occur.

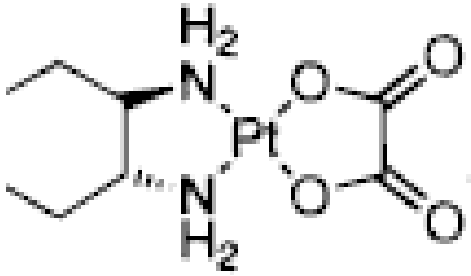
Active platinum compounds



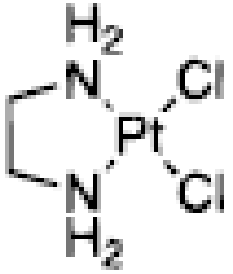
Cisplatin



Carboplatin

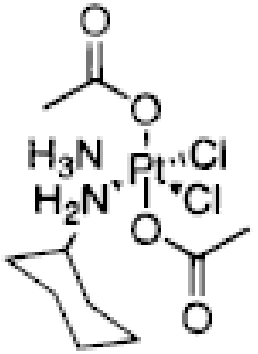


Oxaliplatin

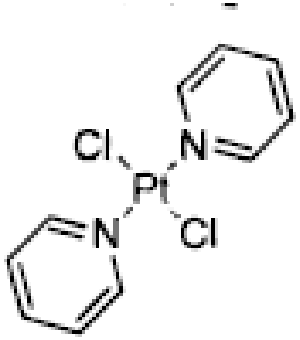


[Pt(en)Cl_2]

Active platinum compounds

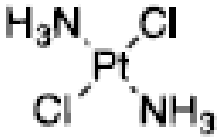


JM 216
Oral Analog

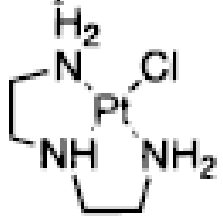


$[Pt(py)_2Cl_2]$
Active trans
compound

Inactive Platinum Compounds



trans-DDP



$[Pt(dien)Cl]^+$

Categories of chemotherapeutic agents

- Alkaloids
- Alkylating agents
- Antibiotics
- antimetabolites
- Enzymes
- Hormones
- Platinum compounds
- **New anticancer drugs**

Categories of chemotherapeutic agents

- New anticancer drugs
  - Monoclonal antibody -drug, -toxin, or –radionuclide conjugates
  - Biological response modifiers (*e.g.*, interferons, interleukin-2)
  - Adoptive immunotherapy
  - Hematopoietic growth factors
  - Induction of tumor cell differentiation
  - Gene therapy

Categories of chemotherapeutic agents

- New anticancer drugs (cont'd)
  - Antisense therapy
  - Tumor vaccines
  - Therapy directed against tumor metastases
  - Inhibitors of angiogenesis

Chemotherapy and Drug Resistance

- History
- Principles
- Side effects
- Catagories of chemotherapeutics
- **Drug resistance**

Mechanism of drug resistance

- Reduced drug accumulation
- Altered drug metabolism
- Enhanced DNA repair
- Altered drug target……

(Pharmacological & biochemical views)

Drug resistance (I)

- *Temporary resistance*:

  reversible change in drug utility/metabolism, cell kinetics/exposure, blood supply *etc*.

- *Permanent resistance*:

  irreversible change in genetic mutation

Drug resistance (II)

- *Intrinsic resistance*:

  cell mutation exists before drug exposure

- *Acquired resistance*:

  cell mutation exists after drug exposure

A majority of anticancer drugs are inhibitors of DNA metabolism

e.g., cisplatin

Anticancer drug cisplatin

Labeled uses: bladder carcinoma, ovarian carcinoma, ovarian germ cell tumor, testicular germ cell tumor, testicular carcinoma

Unlabeled uses: adrenal cortex carcinoma, breast carcinoma, gastric carcinoma, cervical carcinoma, endometrial carcinoma, head & neck carcinoma, lung carcinoma, neuroblastoma, osteosarcoma, prostatic carcinoma

High response rates in cisplatin-containing combination chemotherapy because its lack of hematologic toxicity (but it causes kidney damage)

Cell response to cisplatin

- DNA repair:
  - involves nucleotide excision repair
  - negatively regulated by HMG1 *in vitro (in vivo*?)
- Apoptosis:
  - involves both mitochondria- & membrane-associated caspase activation
  - JNK signaling-mediated

Fate of DNA damage in cells

- DNA repair:

  involves "repairsome" ≥ 16 proteins

  including damage recognition proteins (XPA, XPE)

- Apoptosis:

  involves activation of caspases

[Both processes can be mediated by p53!]