

University of Kerbala

College of veterinary medicine

Pharmacology Lect. # 4

Antineoplastic Drugs

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Introduction: The practice of cancer medicine has changed dramatically as curative treatments have been identified for many previously fatal malignancies such as testicular cancer, lymphomas, and leukemia. Chemotherapy is also employed as part of the multimodal treatment of locally advanced head and neck, breast, lung, and esophageal cancers, soft-tissue sarcomas, and pediatric solid tumors, thereby allowing for more limited surgery and even cures in these formerly incurable cases.

The compounds used in the chemotherapy of neoplastic disease are quite varied in structure and mechanism of action, including: alkylating agents; antimetabolite analogs of folic acid, pyrimidine, and purine; natural products; hormones and hormone antagonists; and a variety of agents directed at specific molecular targets. Figure 1 depicts the cellular targets of chemotherapeutic agents.

The strategy for the discovery of anticancer has undergone a dramatic transformation in the past 15 years, based largely on advances in understanding the molecular basis of malignant transformation. In prior years, cancer drugs were discovered through the large-scale testing of synthetic chemicals and natural products against rapidly proliferating animal tumor systems. Most of the agents discovered in these screens interacted with DNA or its precursors, inhibiting the synthesis of new genetic material and causing broad-based damage to DNA in both normal and malignant cells. The rapidly expanding knowledge of cancer biology has led to the discovery of entirely new and more cancer-specific targets (e.g., growth factor receptors, intracellular signaling pathways, epigenetic processes, tumor vascularity, DNA repair defects, and cell death pathways). Figure 1 outlines the common targets of cancer chemotherapeutic agents.

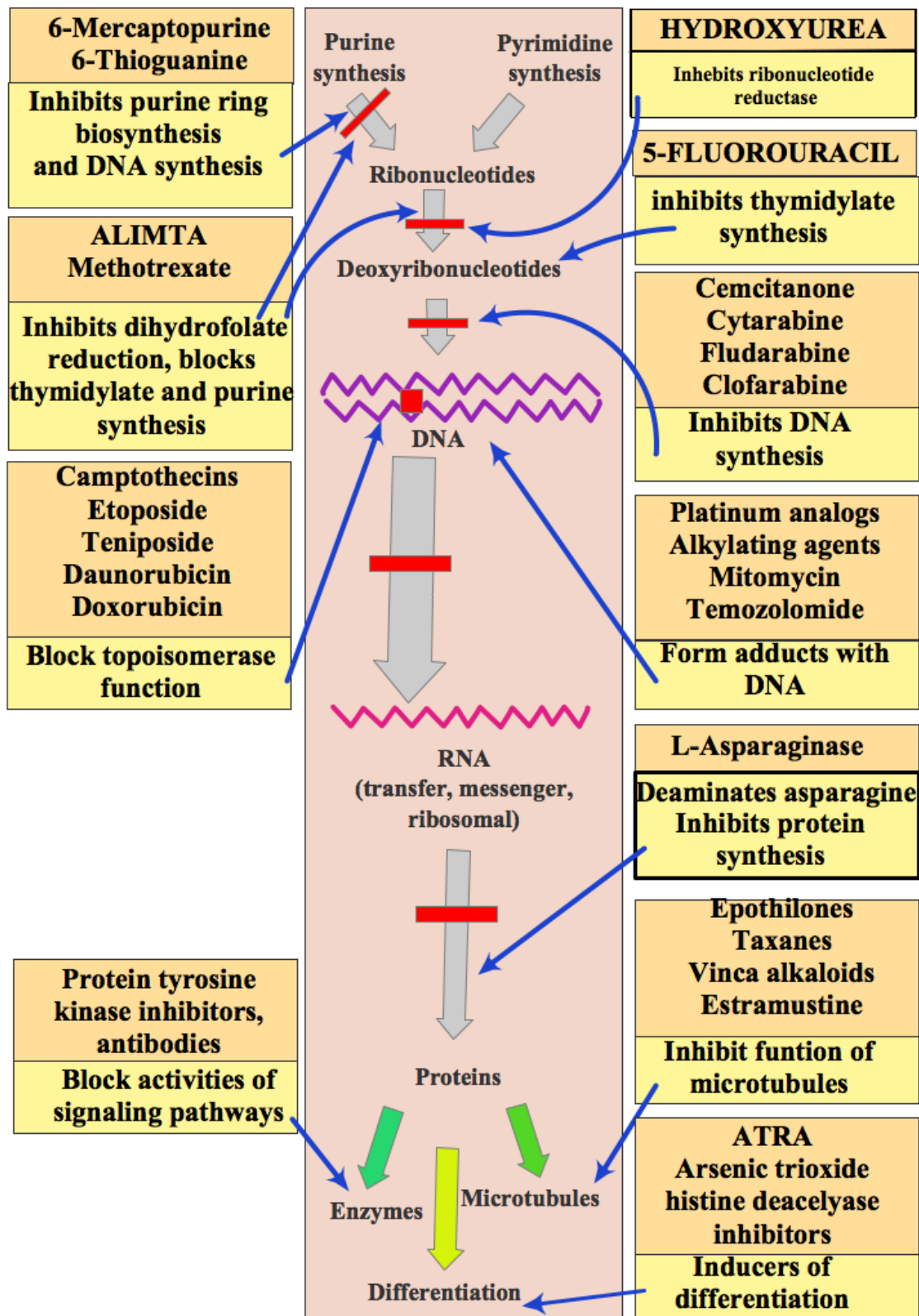


Figure 1. Schematic representation for mechanisms and sites of action of some chemotherapeutic agents

General principles of cancer chemotherapy:

1. The goal of treatment with chemotherapy in veterinary medicine is to increase the length and quality of life of patients based on an accurate histologic diagnosis of the tumor and the clinical stage or extent of the neoplastic process.
2. Chemotherapy is best used as treatment for systemic disease, palliation for metastatic, or nonresectable (cannot be removed completely by surgery) tumors, large tumor size reduction, making them more amenable to surgery and /or radiation therapy.
3. The kinetic of chemotherapy drug-induced cell kill is first-order; a constant percentage of cells is killed with each dose. Antineoplastic drugs are most effective when the tumor is small and is rapidly growing.
4. Because tumor cells undergo a high spontaneous mutation rate, up to 1 in every 10,000 tumor cells may have acquired mutation that can confer resistance at the time of diagnosis, even in the absence of previous exposure to chemotherapeutic agents. Thus, multimodality and multiagent therapy administered as early in the course of disease as is possible, is likely to be the most helpful.
5. Drug resistance develops in neoplastic cells with mechanisms similar to those observed in antibiotic resistant bacteria. These include the following:
 - a. Decreased cell permeability or uptake, or increased efflux of drugs
 - b. Increased production of enzymes which degrade the drug
 - c. Increased capacity to repair or bypass the effects of the drug
 - d. Decreased binding of drug to receptors or target enzymes
6. Multidrug protocols are more efficacious than single drug protocols. They are designed using drugs with different mechanisms of action to augment cell kill and slow the development of resistance.
7. Most drugs are dosed on the basis of body surface area (BSA) in square meters, because of the narrow therapeutic index of antineoplastic drugs.
8. Rapidly multiplying and growing cells are most susceptible to drug effects. These include the normal cells of the hair follicles, gastrointestinal tract, and bone marrow. Thus, hair loss, vomiting and diarrhea, and leucopenia and thrombocytopenia are common side effects of therapy.

9. Most chemotherapeutic drugs have the potential to be mutagenic, embryotoxic, teratogenic, carcinogenic, and cytotoxic.

The cell cycle:

An understanding of the life cycle of tumors is essential for the rational use of antineoplastic agents (Figure 2). Many cytotoxic agents act by damaging DNA. Their toxicity is greatest during the S phase, or DNA synthetic, phase of the cell cycle. Others, such as the vinca alkaloids and taxanes, block the formation of a functional mitotic spindle in the M phase. These agents are most effective on cells entering mitosis, the most vulnerable phase of the cell cycle. Accordingly, neoplasms most susceptible to chemotherapeutic measures, including leukemia's and lymphomas, are those having a high percentage of proliferating cells. Normal tissues that proliferate rapidly such as bone marrow, hair follicles, and intestinal epithelium are thus highly susceptible to damage from cytotoxic drugs.

Slowly growing tumors with a small growth fraction (e.g., carcinomas of the colon or non-small cell lung cancer) are less responsive to cycle-specific drugs. Many drugs are cell cycle specific in that they kill tumor cells in specific phase of the cell cycle (Figure 2).

G₁Phase: in this phase there is synthesis of proteins and RNA required for DNA replication in the S phase. The duration of the G₁ phase varies from hours to days depending on the cell type.

S Phase: DNA synthesis occurs in the S phase. Its duration usually is 2-4 hours. Many drugs act at this phase of the cycle.

G₂ phase: The G₂ phase is characterized by the synthesis of proteins and RNA required for mitosis and cell division. Its duration usually is 3-8 hours.

M phase: Mitosis, its duration is 1 hour.

G₀ Phase: It is the resting phase and cells at this phase are resistant to the cytotoxic action of drugs. Most normal cells are found in this phase.

Classification of antineoplastic drugs:

- I. Alkylating agents:** These agents contain one or more alkyl groups (R-CH₂-CH₂-X), which are converted to reactive intermediates to form covalent bonds with

compounds containing hydroxyl, amino, phosphate, sulfhydryl, or other nucleophilic groups.

Mechanism of action:

- Alkylating agents cross-link DNA and inhibit replication
- Alkylation labelizes DNA and increases breakdown of the molecule
- Alkylation of proteins and RNA may also occur

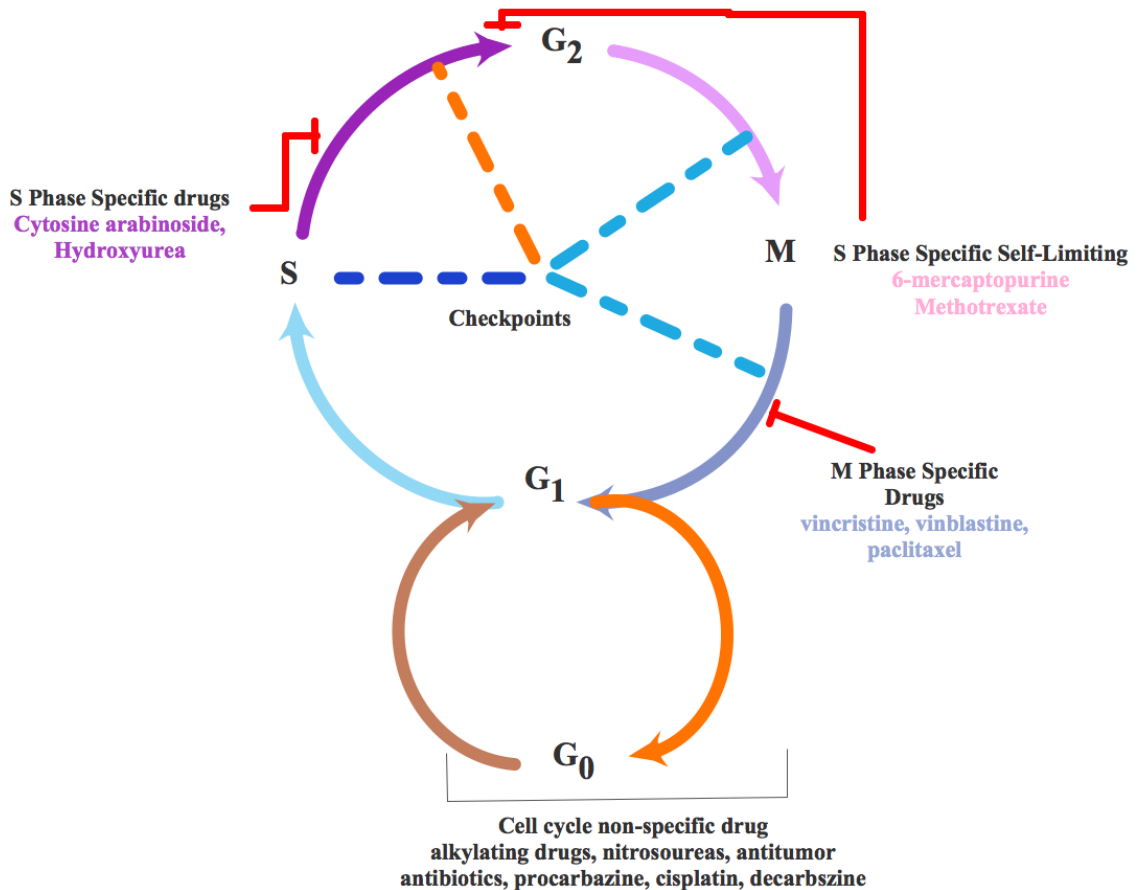


Figure 2. Cell cycle specificity of antineoplastic agents. Mitosis (M) is followed by the first growth phase (G₁), or by a resting phase (G₀). During the S phase, DNA synthesis takes place. The S phase is followed by a second growth phase (G₂).

A. Nitrogen mustards: See Figures 1 and 2

- 1) **Cyclophosphamide (Cytosan®):** is the most commonly used alkylating agent in veterinary medicine. It is used for immunosuppression and alone or in combination with other agents for lymphoreticular neoplasms, mammary gland and other carcinomas.

Pharmacokinetics: cyclophosphamide is inactive until being hydroxylated in the liver by microsomes as the first step in conversion to phosphoramidate mustard and acrolein, the active metabolite. It is very well absorbed orally, widely distributed except to CNS. The metabolites and the parent compound are excreted in the urine.

Administration: Cyclophosphamide may be administered orally or IV at intervals which vary with the type of cancer.

Adverse effects:

- i. Myelosuppression (white blood cells)
- ii. Mild alopecia
- iii. GI disturbances (nausea, vomiting, and diarrhea)
- iv. Cystitis is most common in dogs and is due to the irritant effects of acrolein and other metabolites

- 2) **Chlorambucil (Leukeran®):** It is the most commonly used as an immunosuppressive agent and for treatment of lymphocytic leukemia in cats and multicentric lymphoma in dogs and cats. Chlorambucil is administered orally every 2 days

Pharmacokinetics: It is slow-acting nitrogen mustard, which is well tolerated following oral administration. It is metabolized by the liver to form active metabolite, phenylacetic acid mustard. Metabolites are excreted in urine.

Adverse effects: It is very well tolerated with rare adverse such as myelosuppression with chronic administration

- 3) **Melphalan (Alkeran®):** It has been used predominantly in the treatment of multiple myeloma and other lymphoreticular neoplasms, as well as anal sac adenocarcinoma. It is administered orally. Melphalan does not require hepatic metabolism for activity like cyclophosphamide. Adverse effects include myelosuppression as the most common adverse effect.

- 4) **Mechlorethamine HCL (Mustargen®):** This agent is the most commonly used for treatment of relapsed lymphoreticular neoplasms. Although available in an ointment, it is highly immunosuppressive and topical administration is not recommended. IV is the most common route of administration. It is widely distributed in tissues despite rapid deactivation in tissues.
- 5) **Nitrosoureas:** These agents are lipid soluble and cross the BBB. Lumustine and Carmustine are examples for these agents. **Lumustine** is useful in the management of CNS neoplasms. It is very well tolerated when administered orally. It is readily absorbed following oral administration and metabolized by the liver into active metabolites that are excreted in urine. In dogs, lumustine may cause delayed, cumulative dose-related, chronic, irreversible hepatotoxicity and should not be used in patients with preexisting hepatic disease.
- 6) **Procarbazine (Matulane®):** It is used in combination with other drugs for treatment of relapsed and CNS lymphoma as well as granulomatous meningoencephalitis. It is very well absorbed after oral administration. Procarbazine is an alkylator that crosses the BBB and it is also a monoamine oxidase inhibitor. It is metabolized in liver and kidney. Metabolites are cytotoxic and excreted in the urine.
- 7) **Streptozocin (Zanosar®):** Its mechanism of action is not well known, but it is thought to alkylate and thus, inhibits DNA synthesis. It is selectively, typically, and irreversibly destroys the pancreatic β -cells in dogs resulting in diabetes mellitus. After IV administration, streptozocin distributed to most tissues; concentration in the pancreas is higher than those found in the plasma. It is metabolized in liver and both metabolites and parent compound are excreted in the urine.

II. Platinating agents: like alkylating agents, cisplatin and carboplatin act similarly since they cross-link DNA and prevent replication of DNA. **They are cell cycle nonspecific antineoplastic agents.**

1) **Cisplatin (cis-diaminodichloroplatin, CDDP, Platinol-AQ®):**

Mechanism of action: It acts like a bifunctional alkylating agent producing inter- and intrastrand crosslinks in DNA through binding to guanine residues.

Therapeutic uses: In dogs, cisplatin is administered IV alone or in combination with other antineoplastic agents to treat carcinomas and sarcomas.

Pharmacokinetics: Cisplatin is not absorbed orally. Following parenteral administration, it accumulates in kidneys, liver and the GI tract.

Administration: No aluminum needles should be used for administration. In dogs, saline should be given four hours before and two hours after IV cisplatin. It is given once every three weeks. In horses, intratumoral injection of cisplatin in sesame oil once every two weeks is the usual protocol for sarcoids, squamous cell carcinomas/papilloma, and melanomas.

Adverse effects: Nausea and vomiting is the most common acute toxicity. Renal toxicity (due to accumulation of platinum), and myelosuppression (thrombocytopenia and/or neutropenia) are more common side effects.

Note: *Cisplatin causes fatal pulmonary toxicity in cats and should not be used.*

2) Carboplatin (Paraplatin®)

Mechanism of action: Carboplatin acts like a bifunctional alkylating agent causing inter- and intrastrand crosslinks.

Therapeutic uses: Similar to cisplatin (carcinomas, sarcomas), except carboplatin can be safely administered to cats. It is more effective against malignant melanoma.

Pharmacokinetics: It is well distributed throughout the body tissue after IV administration. It accumulates in liver, kidneys, skin, and tumor tissues. The parent drug is degraded into platinum and platinum-complex compounds that are excreted in urine.

Administration: In dogs, cisplatin is given by slow IV infusion without saline diuresis every 21 days and every 28 days in cats. Intratumor, cisplatin may be mixed with sterilized sesame oil and give intratumorally every four weeks to cats with nasal cutaneous squamous cell carcinoma.

Adverse effects: It is less emetogenic and nephrotoxic when compared to cisplatin. The most common adverse effects still include G₁ disturbances and myelosuppression. Neutrophil nadir (changes in white blood cells) is 14 days in dogs and 14-21 days in cats.

III. Antimetabolites: (See Figures 1 and 2): They are structural analogs of normal cellular molecules such as folic acid, purine, or pyrimidines; they impaired DNA synthesis and are primarily effective during S phase.

1. Methotrexate:

Mechanism of action: Methotrexate inhibits dihydrofolate reductase and prevents conversion of dihydrofolic acid (DHFA) to tetrahydrofolic acid (THFA), which is essential for purine and pyrimidine synthesis.

Therapeutic uses: Methotrexate is used as immunosuppressive agents and it is previously was used in treatment of lymphomas, carcinomas, and sarcomas. Usually, methotrexate is given IV or orally alone or in combination with other drugs.

Pharmacokinetics: It exhibits dose-dependent oral absorption with variability in peak concentration and bioavailability. It is widely distributed except to the CNS. Almost all is eliminated via renal tubular secretion and glomerular filtration without hepatic metabolism.

Adverse effects: Include nausea, vomiting, and diarrhea most commonly and less frequently, myelosuppression.

2. 5-Fluorouracil (5-FU, Adrucil®):

Mechanism of action: 5-FU is a pyrimidine analog. It is phosphorylated in cells to F-dUMP, which blocks thymidylate synthase reactions and thus inhibits DNA synthesis. It is also incorporated into RNA and DNA.

Therapeutic uses: It is used in chemotherapy of carcinoma of the GI tract, mammary gland, liver and lungs. In horses, 5-FU is used for treatment of sarcoids and in dogs, cats, and horses for treatment of cutaneous squamous cell carcinoma by intratumor injection.

Pharmacokinetics: 5-FU is administered parenterally since oral absorption is unpredictable. Distribution is wide and the drug readily enters CSF. Metabolism normally occurs in liver by reduction of the pyrimidine ring.

Administration: In dogs and cats: 5-FU is administered IV once a week. Intratumoral can be injected directly into tumor tissue every two weeks for seven treatments.

Adverse effects: In dogs, the most common adverse effects include diarrhea, and other GI disturbances along with myelosuppression.

3. Cytosine arabinoside (Cytarabine, CytosarU®):

Mechanism of action: Cytosine arabinoside is a pyrimidine analog, which is phosphorylated in cells to Arabinoside CMP and incorporated into DNA, resulting in labile linkage.

Therapeutic uses: It is used in dogs and cats for lymphoreticular neoplasms, myeloproliferative disease, granulomatous meningoencephalitis and CNS lymphoma.

Pharmacokinetics: Cytosine arabinoside is rapidly activated and metabolized by deaminase mainly the liver, but is also converted in the kidneys, and intestinal mucosa into uracil arabinoside (ara-U). 80% of cytosine is excreted in the urine within 24 hours (90% as ara-U and 10% as unchanged cytosine arabinoside).

Administration: It is administered IV or SC once a day to dogs and cats.

Adverse effects: Myelosuppression is the most common toxicity and anemia and thrombocytopenia may also occur.

4. Azathiopurine (Imuran®): It is converted to the active metabolite 6-mercaptopurine (6-MP) in the liver, which will interfere with the de novo synthesis of purine nucleotides. It is used mainly as an immunosuppressive agent in the treatment of immune-mediated diseases and perianal fistulas in dogs.

5. 6-Mercaptopurine (6-MP, Purinethal®): It is a purine analog, which is phosphorylated in cells. It inhibits multiple steps RNA synthesis. It is mainly used in dogs and cats for treatment of acute lymphocytic leukemia, and lymphosarcoma. 6-MP is administered orally once daily.

6. 6-Thioguanine: It is a purine analog with actions and uses that are similar to 6-MP.

IV. Mitotic spindle inhibitors: (see Figures 1 and 2). Vincristine and vinblastine are considered as main representative agents of this group.

1. Vincristine/ Vinblastine:

Mechanism of action: The vinca alkaloids, vincristine and vinblastine bind to tubulin in the mitotic spindle to prevent cell division during metaphase. They are active in the G₂ and M phases of the cell cycle.

Therapeutic uses:

- a. **Vinblastine (Vincasar PFS®):** Is the most commonly used mitotic inhibitor in veterinary medicine. It is employed in the treatment of lymphoreticular neoplasms, carcinomas, and sarcomas in dogs.
 - b. **Vinblastine (Velban®):** Its main use in treatment of lymphomas and mast cell tumors in dogs.
2. **Taxanes:** Paclitaxel (Taxol®) is an example of this group of antineoplastic agents. The mechanism of action of taxol (from the Japanese yew tree) is due to the inhibition of microtubule dissolution (i.e., depolymerization). Since microtubules assembly is essential for vital interphase and mitotic cellular function, normal cell division will not occur, as it should be. Taxol is effective against mammary carcinomas, osteogenic osteosarcoma, and histiocytosis in dogs.

V. Topoisomerase inhibitors of antitumor antibiotics: (see Figures 1 and 2)

1.Doxorubicin (Adriamycin): It is anthracycline antibiotic that:

- Inhibits topoisomerase II, an enzyme is involved in the cleavage, unwinding of segments of DNA;
- It intercalates with DNA (forms a stable complex);
- Inhibits DNA helicase;
- Generates oxygen free radicals leading to oxidative damage to cell membranes and DNA;
- It is non-cell cycle specific (inhibits both DNA and RNA synthesis)
- It is most active in S phase

Doxorubicin is used in the treatment of carcinomas and sarcomas in dogs, especially lymphoma, thyroid and mammary gland carcinoma and osteosarcoma. Doxorubicin is not absorbed orally and causes severe tissue necrosis if given SC or IM. After IV administration, it is rapidly and widely distributed throughout the body except to the CNS. It is mainly metabolized by the liver to active metabolite,

doxorubusinol, and other inactive metabolites that are primarily excreted by bile and feces along with the non-metabolized parent compound.

2. Mitoxantrone (Novantrone®): I

Mechanism of action:

- It intercalates between base pairs and nonintercalative electrostatic interactions;
- It inhibits both DNA and RNA synthesis;
- It may also function as an inhibitor of topoisomerase II;
- It is not considered cell-cycle phase specific, but it is more active during the S phase.

Therapeutic uses: Include lymphoma (naïve and relapsed), squamous cell carcinoma, soft tissue sarcomas, mammary gland carcinoma, transitional cell carcinoma, and other carcinomas/sarcomas.

3. Actinomycin D (Dactinomycin, Cosmegen®): It intercalates with the DNA helix and block transcription by RNA polymerase. It is not cell cycle specific. It has been used for treatment of dogs and cats' lymphoma (naïve and relapsed), soft tissue and bone sarcomas, and some carcinomas. Actinomycin is administered IV once a week in dogs and every 3-4 weeks in cats. It is poorly absorbed when given orally and thus it must be given IV. It is rapidly distributed throughout the body tissues except CNS. It is excreted unchanged in urine and bile.

4. Etoposide (Topscar®): Is semisynthetic analog of the natural product podophyllotoxins, found in mandrake plants. Etoposide is a DNA topoisomerase II inhibitor that stops the action of topoisomerase II after it creates a nick in one strand of the DNA. The nicked DNA is unable to unwind and eventually breaks.

VI. Hormones: Glucocorticoids as an example for this group of antineoplastic agents. Glucocorticoids cause apoptosis of lymphocytes and thus are lymphocytic as a mechanism of action. They are not considered as cell cycle specific. Major use of glucocorticoids is the treatment of dogs and cats lymphoreticular neoplasms including lymphomas of CNS and mast cell tumors. They are

frequently used in cancer therapy with other drugs for their symptomatic improvement of appetite and well-being.

VII. Enzymes

1. L-Asparaginase (Elspar®):

Mechanism of action: L-asparaginase is an enzyme which hydrolysis L-asparagine to deplete circulating levels and thus, inhibits protein synthesis. Normal cells synthesis sufficient L-asparagine for protein synthesis, but certain neoplastic cells require and exogenous source of this amino acid, and its depletion results in cell death. It is G1 phase specific.

Therapeutic uses: Acute lymphoblastic leukemia and multicentric lymphoma in dogs and cats. It is used in multidrug protocols.

Pharmacokinetics: The metabolic fate of asparaginase is not known. It is probably metabolized by proteases in the liver and kidneys. Only trace amount are found in bile and urine of dogs.

Administration: To decrease the incidence and severity of anaphylactic shock to a "foreign protein", L-asparaginase is administered IM or DC once a week.

VIII. Miscellaneous

1. Piroxicam: As NSAID that inhibit both COX-1 and COX-2, it is thought that piroxicam inhibit COX-2 activity expressed on transitional cell carcinoma cells and to inhibitions of tumor angiogenesis.

2. Mitotane: Is a chlorinated hydrocarbon, which is selectively cytotoxic to the cells of the adrenal cortex.

3. Bleomycin: It is a mixture of glycopeptide antibiotics, which bind to DNA and cause chain scission and fragmentation via generation of free radicals. It is a G2 specific antineoplastic agent.