

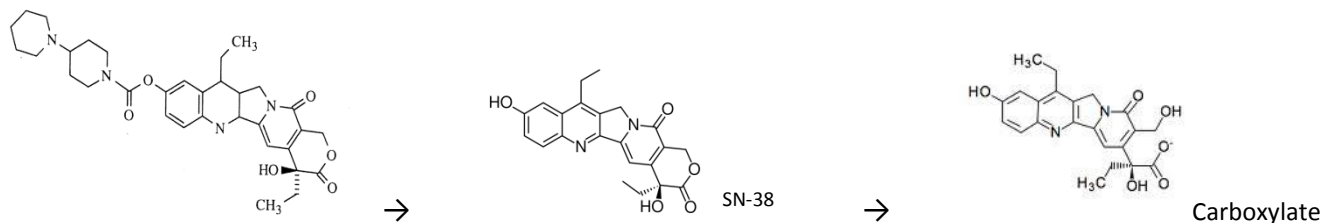
Lecture 5: Topoisomerase inhibitors

Topoisomerase enzymes (Topo I and II) are very important in the process of DNA replication. They relieve torsional strain that would otherwise build up in the unwinding and rewinding of DNA. Unfortunately, the initial high expectations from targeting Topo I enzymes as anti-cancer agents has not been realized. Only two Topo I agents have been approved (irinotecan and topotecan). Other Topo I inhibitors have been in clinical development, and several still are so we must wait for the final verdict on these agents.

Topo I inhibitors – Cell cycle specific (S and G2)

1. Camptothecins:

Irinotecan (Camptosar):



Uses: Colorectal cancer as a component of the FOLFIRI (folinic acid/5-fluorouracil/irinotecan). Also, a component in the experimental regimen (FOLFIRINOX) for pancreatic cancer, but very toxic. Administered IV only. The agent contains a tertiary amine and is formulated as the HCl salt.

Mechanism: Irinotecan is a prodrug of SN-38. The dipiperidyl moiety allows for salt formation (HCl salt) in the parent molecule (irinotecan). SN-38 is very insoluble with no ionizable groups. Enzymatic hydrolysis of irinotecan by esterases converts irinotecan into SN-38 (see above). In addition, the closed ring (lactone) form SN-38 hydrolyses to the open ring (carboxylate) form which is inactive. SN-38 (lactone) is the molecule that binds reversibly in the ternary complex that includes DNA and Topo I (see Figure 1 below). Data indicate that more of the esterase enzymes are present in tumor tissues than plasma and liver.

Toxicity: Myelosuppression (especially neutropenia), severe/unpredictable diarrhea with neutropenia which can lead to sepsis and even death. Early diarrhea is believed to be associated with the cholinergic symptoms caused by the dipiperidyl moiety and this can be treated with atropine. Late diarrhea is less treatable but loperimide is believed to help.

ADME: Terminal t_{1/2} of irinotecan is about 10 hrs, while that of SN-38 is 10-20 hrs. CYP enzymes participate minimally in the metabolism and clearance of irinotecan. Hydrolysis of irinotecan by carboxylesterases and glucuronidation of SN-38 are much more important (see Figure 2 below).

There is an important polymorphism exists in the elimination of SN-38. SN-38 is extensively glucuronidated by UGT1A1. However, about 10-15% of Caucasians and African Americans are homozygous for the *28 allele (7/7 genotype) which express low levels of enzyme and glucuronidate SN-38 poorly. The normal genotype is the 6/6. These numbers reflect the number of TA repeats in the promoter region of the UGT1A1 gene. Therefore, after a given dose, the AUC for SN-38 is higher for 7/7 genotypes relative to 6/6 genotypes. The AUC value is “in between” for heterozygous patients (6/7). The frequency of the 7/7 genotype is lower in Asians.

A genetic assay exists for determining the UGT1A1 status in patients. This assay can identify patients who are at risk of higher than expected AUC values and potential toxicities. Their initial doses of irinotecan can be lowered so that toxicities can be avoided.

Note(s): Camptothecin was originally an ancient Chinese medicine that was isolated from the Camptotheca “Happy Tree” in China.

Figure 1. The ternary complex of (1) camptothecin, (2) DNA, and (3) Topo I.

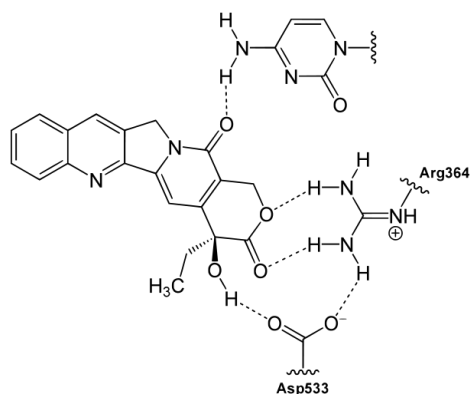
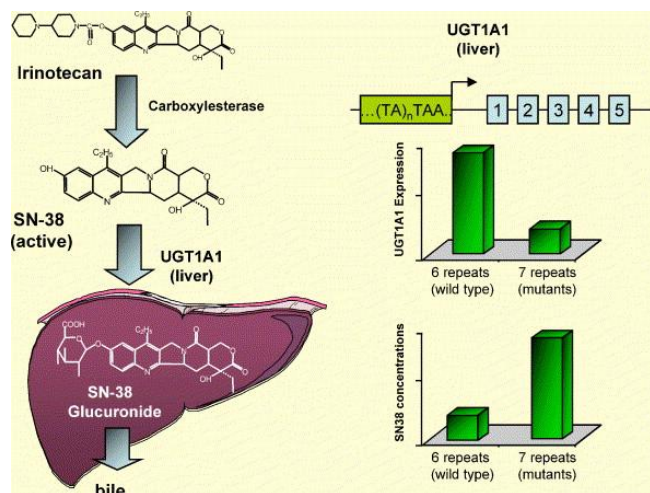
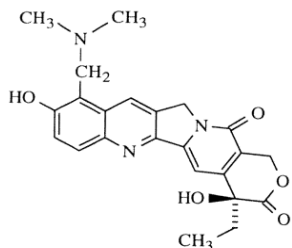


Figure 2. Formation of SN-38 and its glucuronidation by UGT1A1.



Topotecan (Hycamtin):



Uses: Small-cell lung cancer and ovarian cancer. Administration by IV and PO. Because this agent contains a tertiary amine, it is formulated as the HCl salt. Unlike irinotecan, topotecan is not a prodrug and does not require activation. Typical dose of topotecan is about 2 mg/m², compared to >100 mg/m² for irinotecan.

Mechanism: Topotecan binds directly to the Topo I enzyme. Also because of structural differences, topotecan is not glucuronidated like SN-38. Therefore, there is no complicating factor by the involvement of UGT1A1 in its metabolism and excretion. Topotecan is a substrate of several cytochrome P450 enzymes, but no single isoform dominates its metabolism. Therefore, drug interactions involving P450 are not an issue for topotecan dosed IV. However, see oral dosing below.*

Toxicity: Myelosuppression (especially leukopenia, neutropenia), thrombocytopenia, diarrhea (not as bad as irinotecan), nausea, vomiting. Leukocytes decrease proportionately with dose of topotecan and there is a good correlation between topotecan AUC and leukocyte drops. Hence, the dose can be adjusted using AUC measurements of topotecan.

ADME: Bi-phasic elimination with terminal t_{1/2} of about 3 hrs. Metabolism by CYP enzymes occurs but is minor. Like irinotecan, the closed (lactone) ring form undergoes hydrolysis to the open (carboxylate) form which is inactive. Renal excretion of topotecan is the major route of elimination.

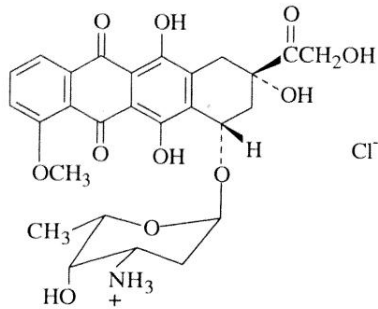
*If dosed orally, bioavailability is only about 40%. However, co-administration of oral cyclosporine A (an inhibitor of transporters ABCB1 (P-gp) and ABCC1 (MRP-1) as well as the metabolizing enzyme CYP3A4, can increase the AUC of total topotecan up to 2-3 fold.

Note(s): Caution encouraged when fever accompanies neutropenia. Combination (febrile neutropenia) can lead to sepsis which can be fatal.

Topo II inhibitors – Not cell cycle specific (but S phase sensitive)

1. Anthracyclines:

Doxorubicin (Adriamycin):



Uses: Many. Acute lymphoblastic leukemia, acute myeloblastic leukemia, soft tissue and bone sarcomas, Wilm's tumor, neuroblastoma. Also for breast, ovarian, bladder, thyroid, gastric cancers, Hodgkin's lymphoma. Administration by IV only (rapidly flowing infusion). This agent is light sensitive.

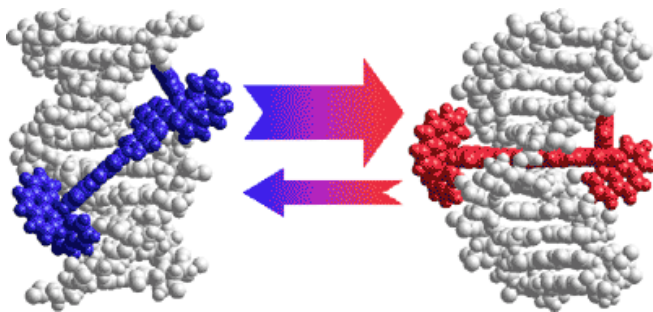
Mechanism: Intercalates into DNA and inhibits Topo II (see Figure 3), and the sugar moiety fits into the minor groove. It can also generate reactive oxygen species (see Figures 4 and 5 below), which is believed to contribute to its efficacy but especially to its cardiotoxicity.

Toxicity: Myelosuppression, cardiotoxicity; the risk of developing congestive heart failure (CHF) increases rapidly with increasing total cumulative doses of doxorubicin in excess of 300 mg/m². Other toxicities include nausea and vomiting, alopecia, peripheral neuropathy. Secondary acute myelogenous leukemia (AML) or myelodysplastic syndrome (MDS) can occur (several years later). Also if extravasation occurs, severe tissue necrosis can occur at injection site.

ADME: Doxorubicin distributes quickly into tissues and then slow elimination from the tissues leads to a long elimination t_{1/2} of about 30-40 hrs. The reduction of the 7 position (by oxidoreductase) along with hydrolytic removal of the aminoglycone are major routes of metabolism. These metabolites remain active and can contribute to cardiotoxicity. P450 reductase can serve as a source of electrons in the bioactivation of doxorubicin (Figures 4 and 5 again). Finally, another mechanism to explain cardiotoxicity involves the rubicinol metabolite and its perturbation of Ca²⁺ channels in the heart.

Note(s): Made from daunorubicin. A very useful but toxic drug. It is red-orange and nickname is "red death" or "red devil". Many deaths occurred prior to the understanding that the agent causes cumulative cardiotoxicity leading to CHF. Now that this is understood, it is less common. Dexrazoxane can be used to minimize cardiotoxicity.

Figure 3. Some modes of DNA intercalation; DNA in grey and intercalators in blue and red.



Doxil: A pegylated liposomal formulation of doxorubicin. Extravasation and cardiotoxicity issues less than the adriamycin formulation but can cause palmar-plantar erythrodysesthesia (hand-foot syndrome).

Myocet: A non-pegylated formulation of doxorubicin that is also believed to be less cardiotoxic than doxorubicin but does not cause hand-foot syndrome. Not yet approved in the U.S. but might be soon.

Figure 4. How molecular oxygen is reduced by stepwise addition of electrons.

Formation and Elimination of Reactive Oxygen Species (ROS)

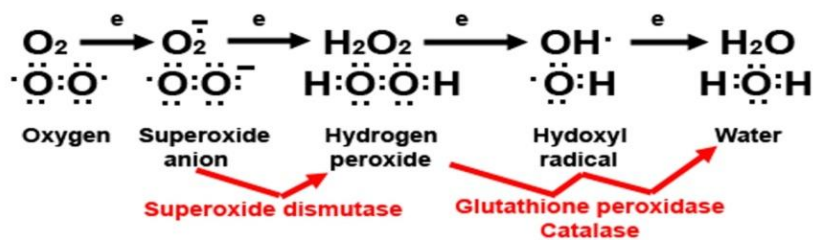
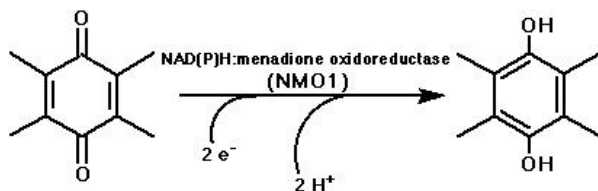
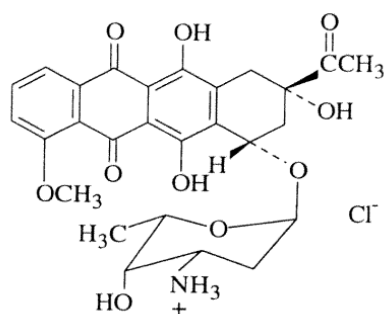


Figure 5. How a simple quinone (menadione) can accept or release electrons.



Daunorubicin (Cerubidine):



Uses: Remission induction for acute non-lymphocytic leukemia and acute lymphocytic leukemia. Commonly used in combination with other agents. Administration by IV only (rapidly flowing infusion).

Mechanism: Intercalates into DNA and inhibits Topo II; it can also generate reactive oxygen species, which is believed to contribute to its efficacy but especially to its cardiotoxicity.

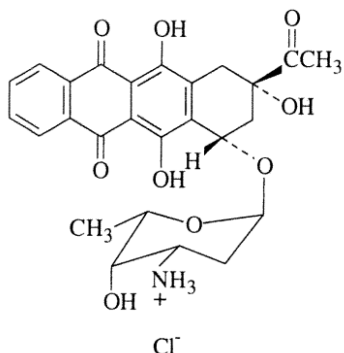
Toxicity: Similar to doxorubicin but believed to be less toxic: Myelosuppression, cardiotoxicity; the risk of developing CHF increases rapidly with increasing total cumulative doses of daunorubicin in excess of 450 mg/m². Other toxicities include nausea and vomiting, alopecia, peripheral neuropathy. Secondary acute myelogenous leukemia (AML) or myelodysplastic syndrome (MDS) can occur (several years later). Also severe tissue necrosis can occur at injection site if extravasation occurs.

ADME: Like doxorubicin, daunorubicin distributes quickly into tissues and slow elimination from the tissues leads to an elimination t_{1/2} of about 20 hrs. Metabolism is quite similar to that for doxorubicin. However, reduction to the rubicinol metabolite occurs faster for daunorubicin than for doxorubicin, so it is more cardiotoxic. P450 reductase can serve as a source of electrons in the bioactivation of daunorubicin.

Note(s): Daunorubicin was actually discovered before doxorubicin. Initially isolated from a bacterium. In the 1950s, Farmitalia Research Laboratories (an Italian research company), began a research effort to isolate anticancer compounds from soil bacteria. A sample was isolated from the area surrounding the Castel del Monte which was a 13th century castle in Apulia. A red pigment was isolated from a new strain of *Streptomyces peucetius*, and an antibiotic was produced from this bacterium. It was also found to have good activity against murine (mouse) tumors. A group of French researchers was doing similar research and discovered the same compound at about the same time. So the two teams named the compound daunorubicin. They combined the name Dauni, the name of a pre-Roman tribe that occupied the area of Italy where the bacterium was found, with the French word for ruby (*rubis*), which described its red color. Like doxorubicin, daunorubicin is light sensitive.

DaunoXome: A liposomal formulation of daunorubicin that is less cardiotoxic. Used mainly for Kaposi's sarcoma associated with HIV.

Idarubicin (Idamycin):



Uses: Acute myeloid leukemia, commonly in combination with other agents (e.g. cytarabine). Also used in breast cancer. Administration by IV only (rapidly flowing infusion).

Mechanism: Intercalates into DNA and inhibits Topo II; it can also generate reactive oxygen species, which is believed to contribute to its efficacy but especially to its toxicity.

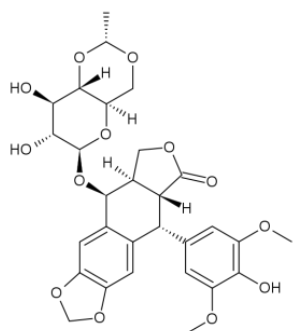
Toxicity: Myelosuppression, cardiotoxicity; the risk of developing congestive heart failure (CHF) increases with increasing total cumulative doses of idarubicin. Total allowable cumulative dose not well defined but higher than for doxorubicin and daunorubicin. Other toxicities include nausea and vomiting, alopecia, peripheral neuropathy. Secondary acute myelogenous leukemia (AML) or myelodysplastic syndrome (MDS) can occur (several years later). Also, severe tissue necrosis can occur at injection site if extravasation occurs. Use extra caution when combining anthracycline agents with other cardiotoxic drugs (e.g. Herceptin or Avastin). More on this later.

ADME: Idarubicin also distributes quickly into tissues with slow elimination from the tissues leads to an elimination $t_{1/2}$ of about 22 hrs. The reduction of the 7 position (by cytosolic reductases) to form the diol metabolite is the major route of metabolism. The diol metabolite has a terminal $t_{1/2}$ that is even longer ~45 hrs. Some hydrolytic removal of the aminoglycone occurs. The metabolites remain active and can contribute to cardiotoxicity. P450 reductase can serve as a source of electrons in the bioactivation of doxorubicin. Minor metabolites of idarubicin are formed by CYP2D6 and CYP2C9.

Note(s): Note lack of methoxy group on this agent relative to doxorubicin and daunorubicin. This makes this agent even more lipophilic and it is believed to make the drug penetrate tissues faster and more completely than the other anthracyclines. Activity is about 5-6 times greater than that of daunorubicin. Also light sensitive.

2. Podophyllotoxins:

Etoposide:



Etoposide (VePesid, Etopophos, VP-16):

Uses: A first line treatment of small cell lung cancer. Also, sarcomas (Kaposi's and Ewing's), testicular cancer, lymphomas, non-lymphocytic leukemia, and glioblastoma multiforme (brain cancer). Often given in combination with other drugs (e.g. bleomycin for testicular cancer). Administered IV or PO. When administered IV, care must be taken to avoid hypotension (see Toxicities below).

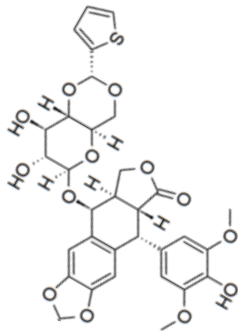
Mechanism: Binds to topoisomerase II. Unlike the anthracyclines, the podophyllotoxins do not possess a quinone moiety. Therefore do not generate reactive oxygen species – and also no cardiotoxicity.

Toxicity: Myelosuppression, nausea and vomiting, alopecia, potential serious hypotension if given IV too quickly. Because the drug and its metabolites are quite polar, considerable excretion (>50%) occurs through the kidneys and bladder. Therefore, it is important that patients drink plenty of water to avoid renal and bladder toxicity. Caution with cisplatin. Secondary leukemias can occur with this agent, as for the anthracyclines.

ADME: Elimination $t_{1/2}$ of about 5-10 hrs. Excreted both in the feces and urine. About 50% of drug is excreted unchanged. Total clearance of etoposide correlates well with creatinine clearance and can be used as a guide in dose adjustment. Interaction with cisplatin use can occur by reduction of renal excretion. O-Demethylation by CYP3A4 to form the catechol metabolite can occur but this is minor so CYP interaction unlikely.

Note(s): Synthesized from a natural product originally from the Mayapple plant that is native to eastern North America.

Teniposide (Vumon, VM-26):



Uses: Childhood acute lymphoblastic leukemia, often combined with cytarabine. Administered IV only. Formulated in Cremophor/ethanol (see Toxicities below).

Mechanism: Binds to topo II. Unlike the anthracyclines, the podophyllotoxins do not possess a quinone moiety. Therefore do not generate reactive oxygen species – and also no cardiotoxicity. Teniposide possesses a thiophene ring off the glycone moiety which makes it more lipophilic than etoposide – less water soluble. Cremophor formulation causes hypersensitivity reactions*.

Toxicity: Myelosuppression, mucositis, hypersensitivity*, nausea and vomiting, alopecia, potential dramatic drop in blood pressure if given IV quickly. Because the drug is less polar than etoposide there is less excretion (~10%) through the kidneys and bladder. Therefore, hydration of patients is not necessary. Secondary leukemias can occur with this agent, as for the anthracyclines.

ADME: Elimination from plasma is biphasic; terminal $t_{1/2}$ is about 10 hrs; because teniposide is less polar than etoposide there is less renal excretion.

Note(s): Like etoposide, synthesized from a natural product originally from the Mayapple plant that is native to eastern North America.