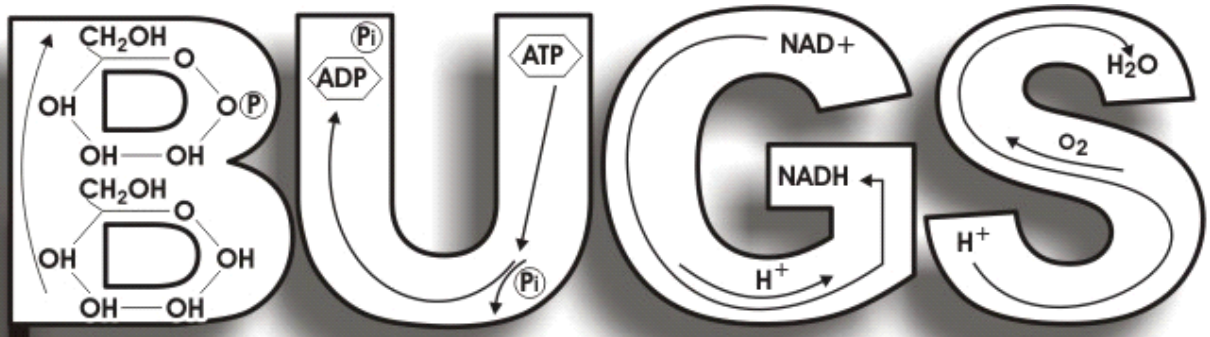
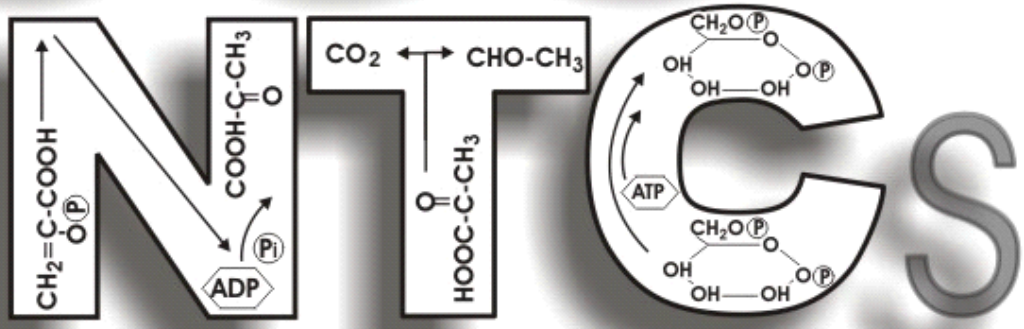




PHAR 301

SET #6 Lectures 14-16

ANNOUNCEMENTS

The BUGS office will be closing Thurs. April 5th. Pick up your NTCs, or anything else you need from BUGS before this date!

BUGS Merck Frosst Tour: Thurs. March 29th, 1:00pm-3:00pm, Only 30 spots, Cost $20. Bus provided from McGill and back. Sign Up at the BUGS office!

The Princeton Review

MERCK FROSST

< Biochemistry Undergraduate Society - 5th Floor McIntyre rm. 511 >
< bugs@sus.mcgill.ca - (514) 398-5247>

Tuesday, March 6th, 2007
Dr Hales - Lecture #14

Cancer & Chemotherapeutic Drugs

Sections in book to read: Brody, Chapters 42 & 43

- Cancer is mainly a disease in older people (50+; rate of incidence goes up around this age - but there are also cancers that occur in the young - tend to be specific types like testicular)

Probability of Developing/Dying from cancer

- On the basis of current incidence rates, 38% of Canadian women and 44% of men will develop cancer during their lifetimes (huge! Cancer is a huge problem)
- On the basis of current mortality rates, 24% of women and 29% of men, or approximately 1 out of 4 Canadians, will die from cancer (thus in terms of treatment, quite a long way to go!)
- Mortality: 37% CV, 28% cancer (significant cause of mortality)
- Many types of cancer

Most common cancer in males (new diagnostic cases):

1. prostate
2. lung
3. colorectal

Most common cancer in females (new diagnostic cases):

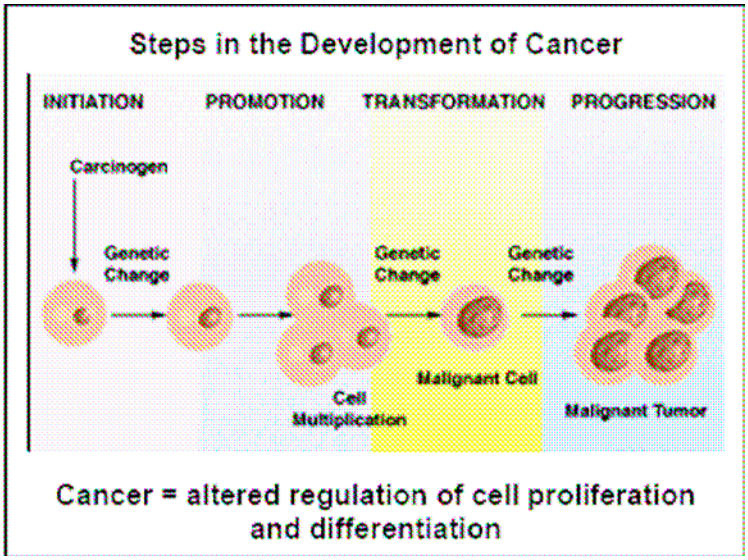
1. breast
2. lung
3. colorectal

Deaths for men: 1) lung, 2) colorectal, 3) prostate (even though prostate is the highest incidence, it is not the one that causes the most mortality)

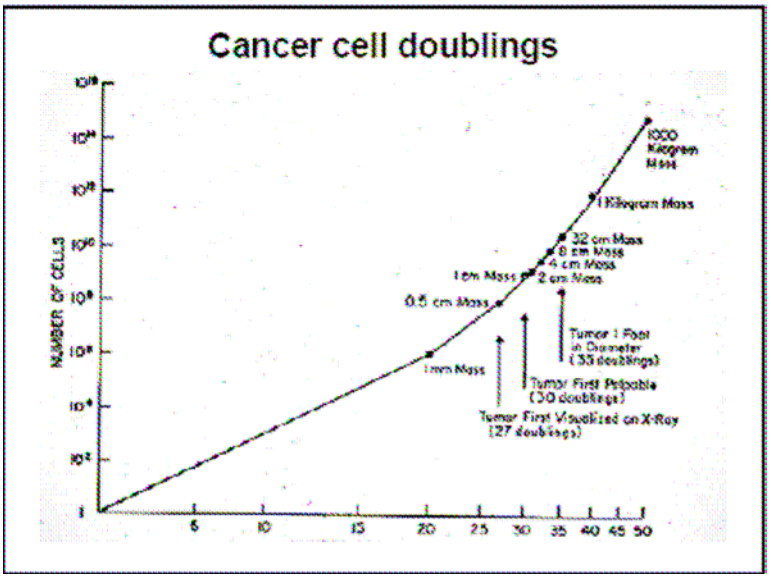
Deaths for women: 1) lung, 2) breast, 3) colorectal

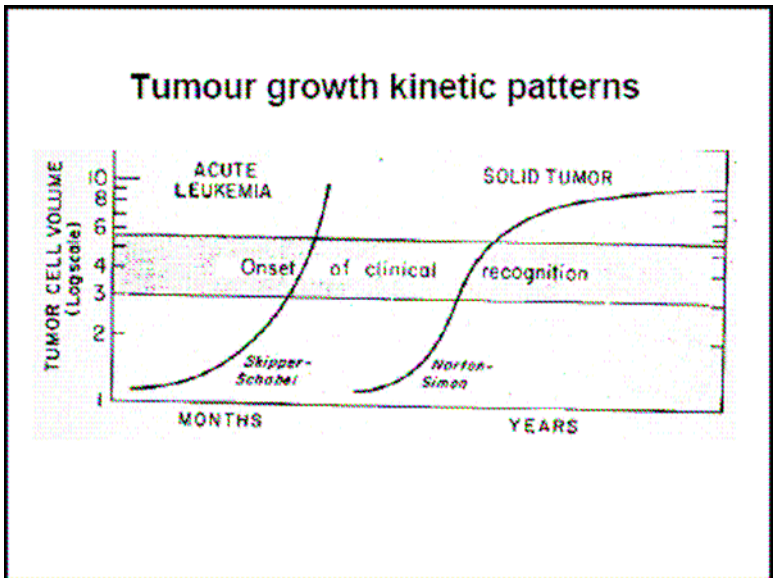
Steps in the Development of Cancer

- some form of change (maybe a carcinogen, hormone, etc.) that causes change in cells that leads to proliferation without control
- we know some of the specific genes involved in genetic changes
- **Key point**: regulation of proliferation and differentiation is altered
- way to hit cancer: kill the cell (so that they are not proliferating) and/or affect differentiation (make them differentiate so that they might not proliferate)



- most of the time, we only diagnose cancer late in its development
- by the time you get a 1mm mass, you've already had 20 cell doublings (but at this point, usually don't see it; the first time it is visualized on an X-ray is when it reaches ~0.5cm)
- 0.5cm is equal to 27 cell doublings; at this point you already have a significant number of cells (10^9 cells that are transformed and are rapidly dividing) by the time you detect it
- By 35 cell doublings, you get a one foot mass! We do not have much time - you must treat it when you see it and by this point it is already the "tip of the iceberg"





- kinetics are different for different types of tumors
- **acute leukemia** (diffuse); time-frame is months - implies that there is nothing limiting the growth of the tumor cells so that the leukemia can develop
- **solid tumor** (lung, prostate or colorectal), starts off the same but then the tumor growth "plateaus" off, the tumour is not growing exponentially anymore
- **Reason**: because it is a solid tumor, cells in middle of the tumour have less access to nutrients, oxygen, and blood; therefore the growth becomes slower or they are removed from the cell cycle so that they are just sitting there.

Question: Does this mean that these cells are not a problem?

- If most drugs are directed at cell proliferation, then we cannot kill those cells very easily – this is probably why solid tumors have highest incidence of death.

- Most drug treatments are for acute leukemia type (also lymphomas); harder to effectively use drugs to treat for solid tumors
- if tag DNA, look at how much gets incorporated - tag cells
- labelling index: how many cells have DNA synthesis going on
  - high index: most cells are dividing
  - solid tumors: lower labelling index
  - leukemias and lymphomas: higher labelling index

Cancer treatment:

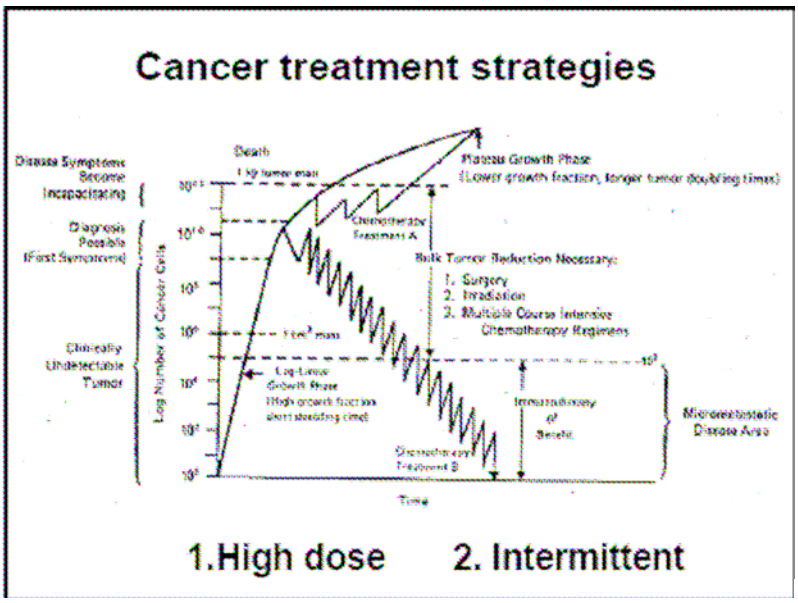
- destroy neoplastic cells (focus **radiation** on a specific area),
- drugs (what we will talk about today)
- modify host immune-defences systems (make person react against transformed cells)
- removal via surgery (only possible for solid tumours with cells that haven't spread)

- Want to eradicate all the cancer cells without harming the normal cells; around 50% can be cured - it is very cancer specific though. Chemotherapy contributes to 17-20% of the types of cancer.

Goal in chemotherapy: get rid of all the cancerous cells

- problem: even if we have a super efficient drug and we get rid of 99% of cancer cells, we will get symptomatic improvement but we are still left with one billion cancer cells! It is difficult to eradicate the last few cancer cells - the cell kill is **first order**.

- Try to design cancer treatments that are as effective as possible at killing the largest percentage of cancer cells.



At plateau: longer tumor doubling time and less cells dividing

- Can do surgery, irradiation, or multiple course intensive chemotherapy regime.

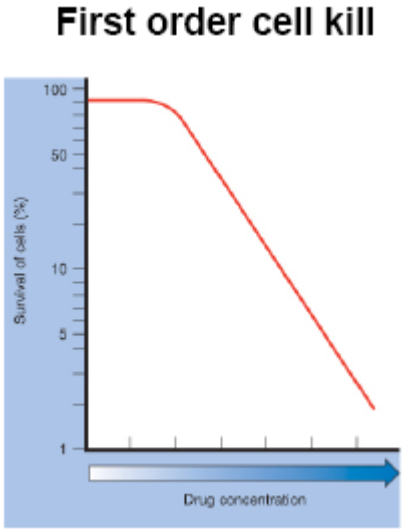
Chemotherapy treatment A: dramatic decrease in number of cells in tumour - cells that are left will divide though and we will have to treat again – eventually they will be back and we will have a lot of tumor cells

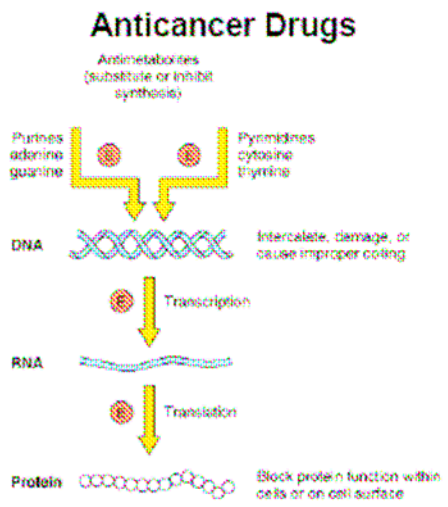
To be effective: need to get rid of all of the cancer cells; C**hemotherapy treatment B.**

Last cancer cells: immunotherapy might also help. After 5 years, if you have survived, chances are you have killed most or all of the cancer cells.

Principles of "classical" cancer chemotherapy

1. cure = death of every malignant cell
2. do not rely on host mechanisms to eliminate moderate numbers of cancer cells (although now we try to help immune system to amplify differences between immune cells and normal cells)
3. cell-kill follows first order kinetics, i.e. a constant % of cells are killed (constant proportion so it is difficult to kill the last cells)





What is the limiting factor? - The toxicity to normal cells so we have to compromise… must use as high a dose as possible without killing the person.
- We worry most about bone marrow (WBCs, RBCs, immune system), GI cells and germ cells in males.

<u>Chemotherapeutic drugs</u>

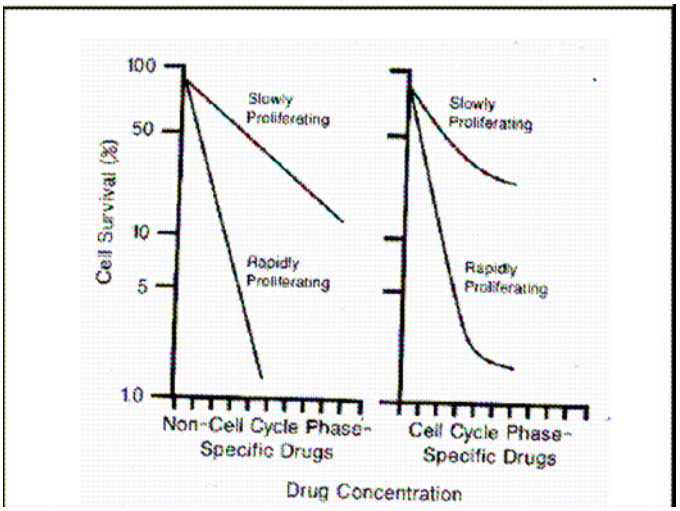
- most drugs that we have are anti-metabolites or directly hit DNA (in way that is not repairable)
- can target transcription – not a major target though
- can target translation – again not a very specific way to kill cells and therefore not a major target
- alkylating agents: cyclophosphamide, cisplatin
- anti-metabolites - block synthesis of precursors to DNA, ex. Methotrexate
- we also have nucleotide analogs that arrest DNA synthesis ex. 5-fluorouracil
- anti-tumour antibiotics (natural products - synthesized by an organism and they react against mammalian cells) ex. vinca alkaloids, react against microtubules
- topoisomerase inhibitors: ex. Doxorubicin (affect ability of DNA to wind/unwind and religate)

Role of cell cycle kinetics

- S: DNA synthesis (e.g.: anti-metabolites, block synthesis of nucleotide building blocks or are nucleotide analogs)
- G2: pre-mitosis
- M: mitosis and cell division (think of role of microtubules, so target them and target cells in M phase)
- G1: pre-DNA synthesis
- G0: Resting phase (not many drugs target this stage; problem in solid tumors)

<u>Cell cycle phase specificity</u>

- We can have drugs killing dividing cells but not at a specific time in cell cycle: ex. alkylating agents (kill rapidly when cells are dividing rapidly)
Reach plateau if only killing in a specific phase - if not in that phase, they do not die (we often use high doses and in combination - hit as many cells as possible - there are criteria to follow to see which drugs to combine)



Chemotherapeutic drugs

- alkylating agents, largest class: nitrogen mustards (first ones used)
- mechloroethamine: still used in Hodgkin's disease

- oldest group of drugs: WWI discovery – soldiers exposed to nitrogen mustard as a weapon; it was found that those soldiers exposed to it had a decrease in WBC count. Some were exposed to sulfur mustard – **incredibly reactive**

Polyfunctional alkylating agents
Historical notes: in the early 1900s, the only treatments available for tumorous cancers were surgical excision and radiation therapy. The discovery of the first chemotherapy drug came about serendipitously. During the 1940s, researchers reviewing records from WW1 noticed that Allied soldiers who were exposed to the chemical weapon nitrogen mustard gas had a decreased level of WBCs. It was thought that this might be used as a therapeutic effect in patients with leukemia whose white cell counts are elevated. Nitrogen mustard and its derivatives are still used to treat leukemia today.
Can control reactivity of nitrogen mustard by changing methyl group.
Use nitrogen mustards to treat leukemia; proliferation of WBCs

When you lose the chlorine groups, we get an electron-deficient active intermediate; it will look for electrons to share from other molecules inside the cell.

- will find this in the N7 of guanine – we will get the formation of a covalent bond
- form an adduct, modifying G in DNA

Question: Is it specific?

- No! It will react wherever there are electrons that can be shared (will act on adenine, different molecules in RNA, proteins, and small molecules that can share electrons)! DNA crosslinking is thought to be the major reason why cells die.

Two potential sites where you can alkylate - second chlorethyl can lose chlorine and form another covalent linkage with another guanine molecule or a guanine protein or some different combination.

- what you are doing is cross-linking - these are difficult to repair so if the cells are rapidly replicating, sometimes the processes that repair this DNA damage does not have time to do the repair before the cell has to decide whether it can proliferate or die – therefore cause the cells to kill themselves because they don't have time to repair their DNA.

Alkylation of DNA:

- Miscoding → when make adducts, change properties of the base, so now it miscodes, causing mutations
- depurination
- strand breaks
- cross links

Cytotoxicity of a monofunctional vs. bifunctional alkylating agents → two chlorethyl groups;
Question: Is the fact that there are two chlorethyl groups important in the ability to kill the cell?

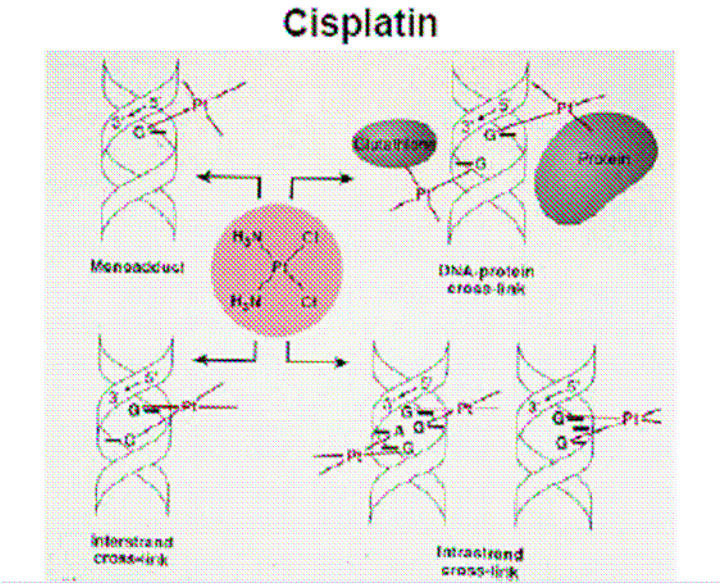
- Yes!

Monofunctional: replace a chlorethyl group with a hydroxyl group
Bifunctional, has 2 chlorethyl groups → **much** more cytotoxic than the monofuctional alkylator; therefore the monofunctional compound is much less active. Sometimes drugs we use are trifunctional; that they can often crosslink is important for cytotoxicity.

Alkylating agents: proliferation specific (dependent) but **not** cell cycle phase specific

- We already talked about cyclophosphamide while discussing immunosuppressants
- cyclophosphamide is a nitrogen mustard

- Methyl group in mechlorethamine is replaced by phosphoramide group
    - When they synthesized cyclophosphamide and tested it as an anti-cancer drug (it was a designer drug) – they thought that cancer cells have phosphoramidase so they should be able to specifically cleave this bond and liberate the nitrogen mustard inside the cancer cells. You would only have nitrogen mustard inside your cancer cells and your normal cells would be fine.
        - It did not work this way though; however, since the ring can share electrons it does stabilize the drug a lot. While mechlorethamine has to be injected IV – it is so active that at the site of injection, you can cause cell death and damage to your cells and veins - the half-life is minutes, cyclophosphamide can be given orally and has a longer duration of action.



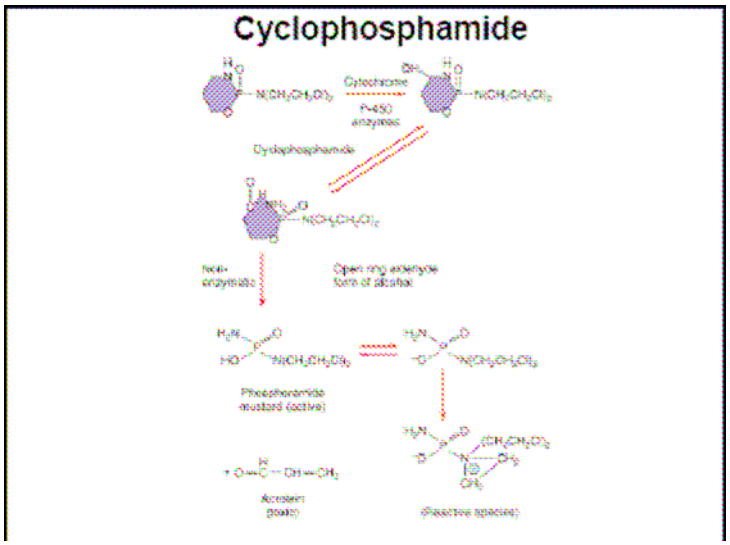
        - Cyclophosphamide can actually be given orally (it is stable)
        - It's activated inside cells, but not activated by phosphoramidase but by CYP450- hydroxylation → equilibrate with an aldehyde form of the drug and then it breaks down to form phosphoramide mustard (anti-tumor activity) and acrolein (also reactive and can interact with sulfhydryl groups and proteins and can cause damage); sometimes try to inactivate acrolein but keep phosphoramide mustard active so as to decrease damage to things such as bladder that is caused by acrolein.

Toxicity of alkylating agents - to rapidly proliferating cells – some of the normal cells affected include:
- hematopoietic system
- G1 tract
- Gonads

- Cyclophosphamide damages your hair cells; consequence of cancer treatment is that people lose their hair

There are hundreds of different anticancer drugs which act as alkylating agents.

Cisplatin:
- discovered serendipitously also (like cyclophospamide)
    - more like an accident though
- also an alkylating agent
- people were using metals for electrodes; bacteria which were in contaminated solutions were killed; organo-platinum compounds are used as cancer drugs
- Has chlorine, platinum and amine groups



- Basically it forms DNA -DNA and DNA-protein crosslinks and it can form inter-strand crosslinks or within/between strands of DNA

– you lose chlorine groups and form a covalent adduct with DNA or protein - get crosslinking

- Used for **solid tumors**

Resistance to alkylating agents:

- we are always looking for compounds that are more toxic to cancer cells and less for normal cells (SELECTIVITY)

- increase inactivation of drug (non-specific, just looking for electrons to share); → If we put in a lot of sources of electrons we could inactivate the drug.
    - One of the things that could happen is we could add nucleophilic "trapping agents" – molecules that can donate electrons and can inactivate the alkylating agent
        - One of the endogenous ones that we have in all of our cells is **glutathione**→ it has an electron it can share, it is composed of three amino acids: glycine, cysteine and glutamic acid.
        - Cysteine can share the electron with alkylating agent.
- If increase DNA repair; tumor cells could develop resistance
- If decrease activation of the drug;
    - ex. cyclophosphamide needs to be activated by cyt-P450 reaction then you will get resistance

Tumor cells have thought of all these things; some have modified things like glutathione, some have increased DNA repair, others have decreased cyt-P450 activity.

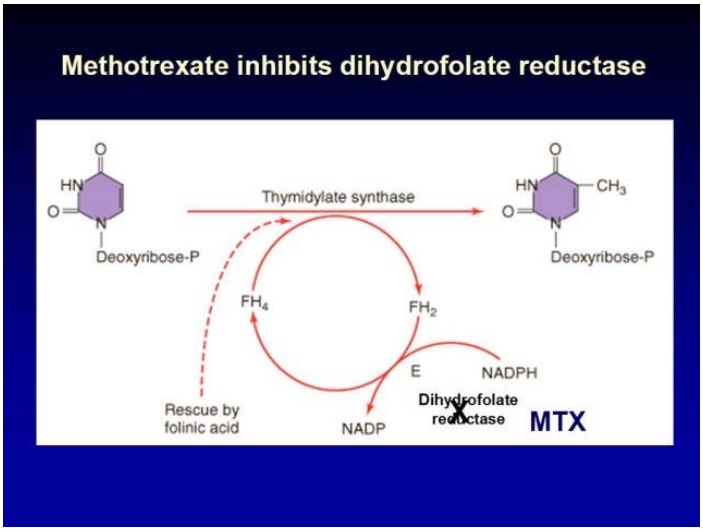
Thursday, March 8th, 2007.
Dr. Hales - Lecture #15

Cancer & Chemotherapeutic Drugs II

Sections in book to read: Brody, Chapters 42 & 43

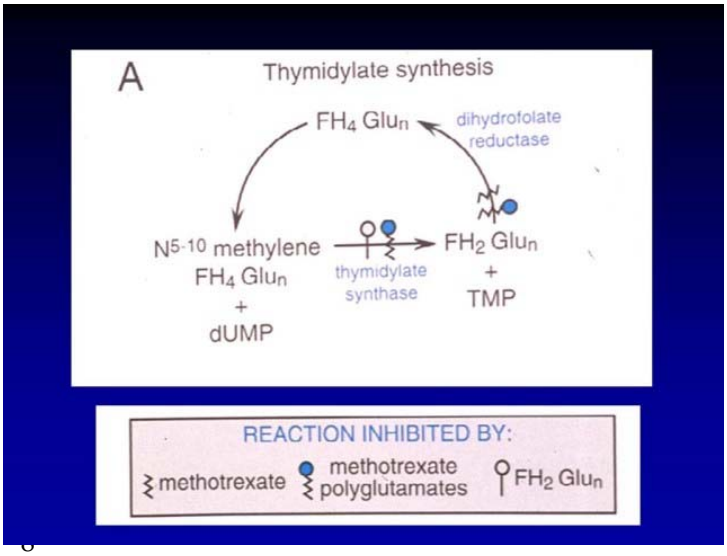
Recall from Tuesday: Introduction to anti-cancer drugs that target proliferating cells, but need to be more selective

Today's lecture:
- Alkylating agents (Cyclophosphamide, Cisplatin)
- Anti-metabolites (Methotraxate, 5-Fluorouracil)
- Anti-tumor antibiotics (Vinca, alkaloids)
- Topoisomerase inhibitors (Doxorubicin)
- Hormonal agents
- Other



Antimetabolites
Drugs that substitute or inhibit DNA synthesis

Methotrexate (MTX)
- folic acid analog (similar structure and elements)
- can be taken into the cell through the same active transport process as folic acid
- interacts with many of the same enzymes as folic acid
- inhibits mammalian **dihydrofolate reductase** (this enzyme is essential to reduce dihydrofolate into tetrahydrofolate)
- tetrahydrofolate (activated folate) is needed for methyl-transfer reactions in the synthesis of thymidine
- very potent, low amount of MTX are needed to inhibit dihydrofolate reductase
- **Trimethoprim** is an antibacterial drug (but not very effective on mammalian enzyme)
- **Pyrimethamine** is a very good inhibitor of the protozoal dihyrofolate reductase enzyme – hence, it is used in the treatment of malaria
- specificity based on the affinity of the drug to bind one specific enzyme
- once in the cell, MTX gets metabolized (so we can add some glutamic acid residues on the drug to make it larger, more charged and less able to get out of the cell = drug is active for a longer period of time)
- the metabolites (polyglutamates) are active and inhibit not only dihydrofolate reductase but also **thymidylate synthase** (very effective drug)

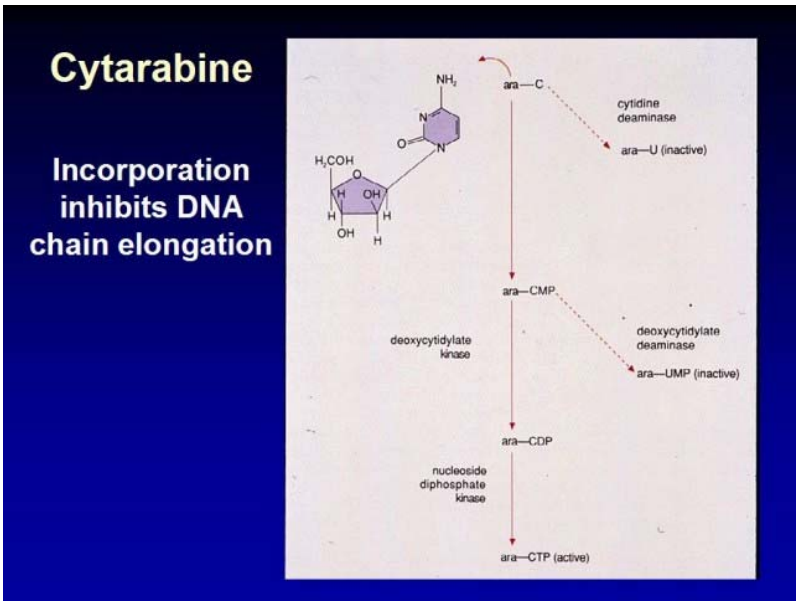


- can also rescue non-cancer cells → can play with folate and add a pre-activated folate to avoid MTX toxicity (based on the differences in ability to uptake and actively transport the drug between cancer and non-cancer cells)
- MTX is potent, but not totally specific: can also affect *de novo* purine synthesis (any methyl transfer reaction can be inhibited)
- Mechanism of action of MTX: starve cells of thymidine so can no longer make DNA, therefore rapidly proliferating cells will die

<u>Mechanisms of Resistance to MTX</u>
- impaired active transport into cells
- impaired polyglutamate formation (less drug is retained inside the cancer cell)
- increased (eg. Gene amplification in some cancer cells – more copies of the gene!) or altered dihydrofolate reductase (alter the affinity)
- decreased thymidylate synthase → if you are not using thymidylate synthase to make thymidine (if you are using another pathway instead – ex. salvage pathway), then you won't have as much of an effect

5-Fluorouracil (5-FU)
- pyrimidine analog (looks like uracil, just add a Fluorine instead of a proton)
- gets incorporated into RNA, DNA
- is metabolized by the same enzymes and the same pathways as uracil

  - So you start with 5-FU, it gets metabolized into FUMP (monophosphate), then to FUDP (diphosphate) from which you can make FUTP (triphosphate) and incorporated into RNA - however, this is not the main target (not the main pathway or mechanism of action).
  - **Main pathway** → can form a deoxy sugar from FUDP (deoxyribose = FdUMP, which is the active metabolite). FdUMP forms a ternary complex with dUMP and thymidylate synthase and freezes the synthesis of thymidine (so the cell can't replicate its DNA).



Both Methotrexane and 5-Fluorouracil target cells in S-phase, because they are affecting DNA synthesis.

Cytarabine
- like vidarabine, an antiviral drug
- to form cytarabine instead of changing the nucleotide structure, it is the sugar that is changed. Instead of the ribose sugar, we have the arabinose sugar (ara-C). It then gets phosphorylated by deoxycytidylate kinase (ara-CMP into ara-CDP) and nucleoside diphosphate kinase (ara-CDP into ara-CTP). ara-CTP is the active metabolite.
- Incorporation of ara-CTP inhibits DNA chain elongation. So again, freezing the cancer cells in S-phase.

Mechanism of resistance to Cytarabine

The cancer cell can try to deaminate ara-C, forming inactive metabolites (eg. ara-U, or ara-CMP into ara-UMP) via enzymatic reactions.

6-Mercatopurine

- purine analog (sulfhydryl group, SH-, added on purine)
- inhibits conversion of IMP (inosine monophosphate) to Adenine and Guanine – can inhibit it at lots of different steps!
- has effects on many enzymes in the pathway of A and G synthesis

Pentostatin

- purine analog (very similar structure to adenosine)
- inhibits **adenosine deaminase**
- incorporation results in strand breakage

Overall, all the nucleotides analogues, as well as the sugar analogues, fool the enzymes by getting incorporated and altering DNA synthesis. Even though these anti-cancer drugs target enzymes by inhibiting some of them, we assume the real target is DNA synthesis.

Anti-metabolite Resistance

- Changes in target enzymes (can alter the affinity or the amount of the enzymes so that the drugs don't get metabolized, therefore, they do not get activated. We can by-pass the resistance by giving the active metabolite directly.)
- Decreased activation
- Increased inactivation (Cytarabine)
- Decreased access to target site (Methotrexate)

All work in S-phase!

Targeting Microtubules

Useful as complementary drugs with alkylating agents and anti-metabolites since they have other targets than DNA.

1) Vinca Alkaloids

- from periwinkle leaves (Vinca Rosea), natural products, huge complex molecules
- have many analogues that are used as anti-cancer drugs (Vinblastine, Vincristine)
- form a complex with tubulin, terminate assembly and cause depolymerization of microtubules and mitotic arrest (chromosome assembly and separation for cell division are altered). Cancer cells get stuck in M-phase.
- not totally specific to proliferating cells (because microtubules are also involved in secretion and axonal transport) but pretty specific

2) Taxol

- from the bark of the Pacific yew tree (now, we're able to synthesize it)
- promotes microtubule assembly and inhibits disassembly
- tubulin polymerization is stabilized (eg. paclitaxel)
- toxic, because stabilizes MTs that are NOT suppose to stay stable (have other roles).

Abraxane: Paclitaxel in albumin-coated nanoparticles

- Taxol is now available as a nanoparticle (the first to be packaged in an albumin coated nanoparticle to increase the selectivity, so it gets more specifically into the tumor cells). The drug might not change but the delivery process can.

Anti-tumour antibiotics:

Doxorubicin

- has four ring structure that make it a planar molecule that easily gets intercalated into DNA and RNA.

1. Intercalates, blocking DNA synthesis and affecting RNA synthesis
2. Binds to DNA and topoisomerase II (involved in the rearrangement of DNA → cuts and religates); Doxorubicin prevents resealing breaks
3. Generates free radicals (that cause cell aging) that can be cytotoxic to tumour cells! To reduce toxicity, can give antioxidants.
4. Alters cell membrane fluidity and ion transport (it is toxic even though it doesn't get in the cell)
5. Triggers apoptosis (active cell death process)

Can have bone marrow and cardiac toxicity

Most of the anti-cancer drugs affect bone marrow, but Doxorubicin is even more dangerous since it also affects cardiac cells (dose dependent unwanted effects). Can change ECG (electrocardiogram).

Bleomycin

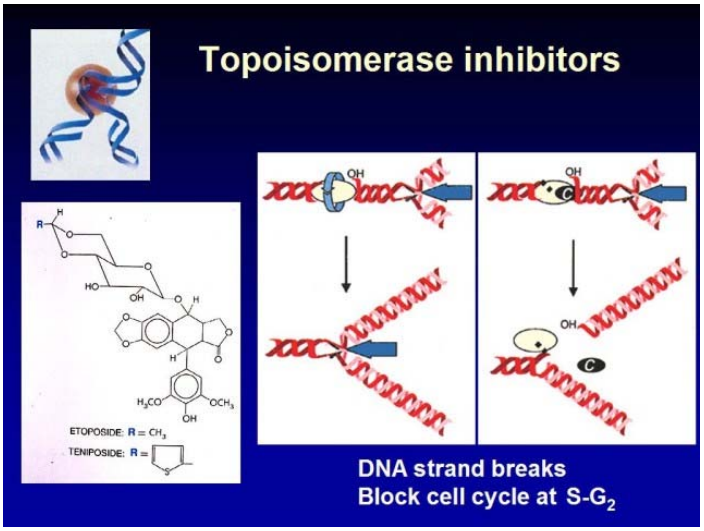
- VERY complex structure, extracted from a natural product
- forms free radicals, damaging DNA (cause DNA breaks)

Dactinomycin

- has a chromophore and a polypeptide structure (huge complicated molecule)
- Intercalates specifically between adjacent G-C base pairs
- Inhibits DNA-dependent RNA synthesis
- Used in experiments in lab to interfere with RNA synthesis - but mostly used as anti-cancer drug

Topoisomerase inhibitors

- topoisomerases are involved in rearranging DNA – one of the steps in rearranging DNA is to cut the DNA (then re-ligate it)
- The cutting works but the re-ligation is blocked, because the drug is there bound to the enzyme.
- Block cell cycle at the interphase of S and G_2 phases



Etoposide and **Teniposide**

- natural products
- many types of topoisomerases, can try to be specific to one

Failure of current drug regiments

- Drug toxicity
- Drug resistance

Undesired effects of anticancer drugs

- Bone marrow ;
- Digestive track; GI epithelium is highly proliferative; can cause ulceration and diarrhea
- Hair root; loss of hair (alopecia)
- Gonads; gonads have proliferating cells
- Tissues undergoing repair; impaired healing
- Tumor mass
- Fetus; teratogenesis (can cause malformations)

Undesired effects of anticancer drugs

Tissue or system affected	*Toxic effects*
Bone marrow	Leukopenia and lymphocytopenia with an increased risk of infection or activation of quiescent infection Immunosuppression Thrombocytopenia leading to hemorrhage Anemia
Digestive tract	Oral ulceration Intestinal ulceration, diarrhea
Hair root	Alopecia
Gonads	Menstrual irregularities, ammenorrhea, infertility Impaired spermatogenesis, sterility
Tissues undergoing repair (surgical wounds, etc.)	Impaired healing
Tumor mass	In the case of leukemias and lymphomas, rapid destruction of the cells of the tumor mass can result in the release of large amounts of nucleic acid breakdown products, and the consequent increase in uric acid can cause renal damage
Fetus	Teratogenesis

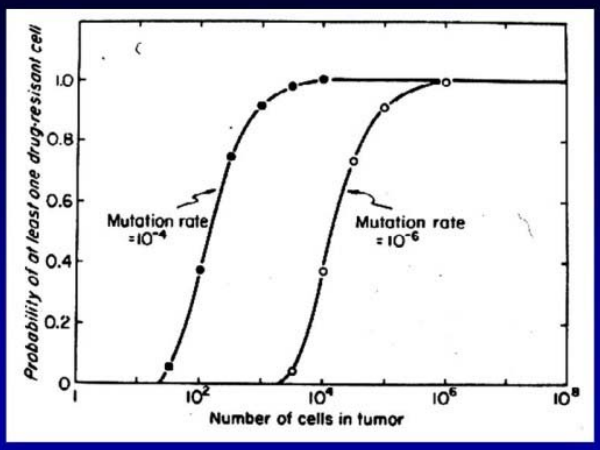
Cancer chemotherapeutic Drug Resistance

- Increased DNA repair (for alkylating agents)
- Formation of "trapping agents" (ex. glutathione → so the cell can have more nucleophiles that would inactivate our electrophilic alkylating agents)
- Changes in target enzymes (ex. alter the dihydrofolate reductase)
- Decreased activation
- Increased inactivation
- Decreased access to target site

Mutations as a mechanism of resistance

- mutations usually don't occur that often, even though you have a lot of cells in tumours.
- Mutation is usually not the only cause/extent of resistance to drugs

Mutations as a mechanism of resistance



There usually something else going on… and researchers may investigate this using cells in culture.

Multi-drug resistance

- Cells selected with C-colchicine or V-vinblastine (both Vinca Alkaloids, both act on tubulin assembly into MTs) so can understand that the cells can be resistant to the first two drugs.
- But Doxorubicin has a totally different mechanism of action (had a list of five different mechanisms, but MT structure was not one of them). We see that cells resistant to vinca alkaloids are also resistant to Doxorubicin. How come?

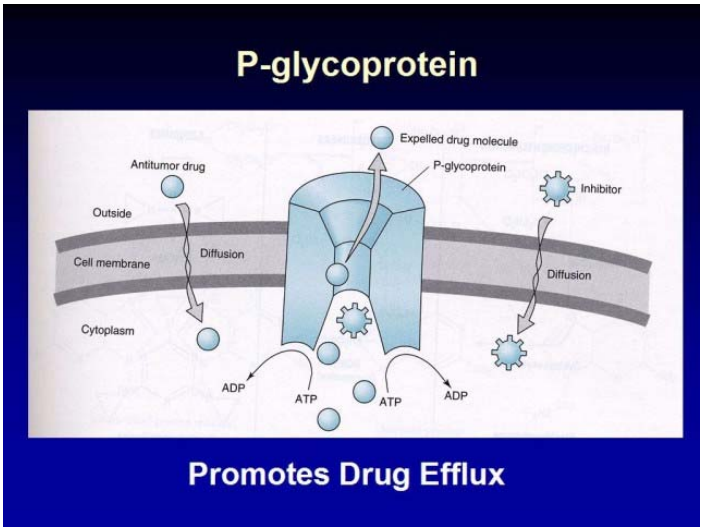
Multi-drug resistance

Table 9-9. Relative Resistance of Human KB Carcinoma Cells to Three Anticancer Drugs

	Relative Resistance		
Cell Line[a]	*Vinblastine*	*Colchicine*	*Doxorubicin*
KB-3-1	1	1	1
KB-C4	254	1750	159
KB-C4-R	3	6	4
KB-V1	213	170	458
KB-V1-R	1	1	1

Cells selected with C- colchicine or V – vinblastine; R- revertant

Because of P-glycoprotein, a transport protein.
This carrier protein was pumping out the drugs, not only selective to vinca alkyloids but also to doxorubicin. Best explanation why a cell would have a resistance to unrelated (different) drugs.



Can add an inhibitor of p-glycoprotein to keep the anti-cancer drug in the cell.

<u>Combination Chemotherapy</u>
Give the drug (usually not alone), always in combination to by-pass resistance and maximize the killing power on the tumour cells without affecting normal cells (like bone marrow cells).
Drugs should be:

1) Effective singly (each has to be able to kill the cells alone)
2) Synergistic (they should have more effect when put together)
3) Have non-cumulative toxicities to the normal cells
4) Be given in maximum tolerated doses – if it's a first order cell kill, want to kill the maximum percentage of tumour cells with each dose of the drug

Example of mixture of drugs used to treat Breast cancer

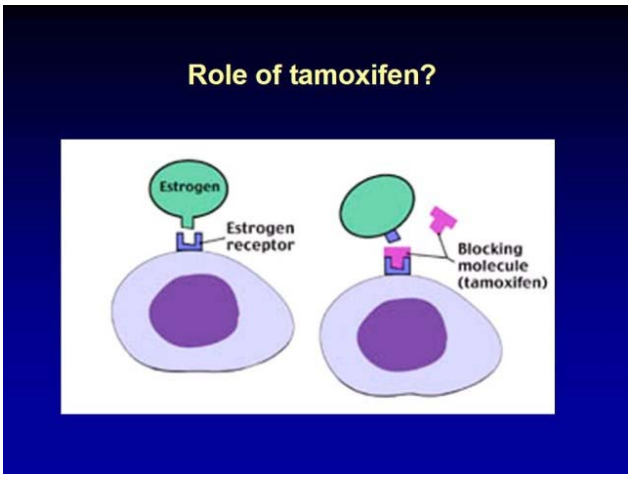
- Vincristine (target MTs)
- Prednisome (glucocorticoid that can be highly toxic to some cells)
- Cyclophosphamide (alkylating agent)
- Methotrexate (folic acid analog)
- Fluorouracil (pyrimidine analog)
    - These all cause bone marrow depression except vincristine (has effect on nerve transport and other things)

<u>Approaches to increase drug selectivity?</u>
<u>Hormonal approaches</u> (more targeted approach)

- Many of the common cancer cells (Breast and Prostate cancer) are hormone-dependent, so it is possible to target them based on this difference (compared to other cells that are not hormone dependent).

Tamoxifen

- A competitive partial agonist/antagonist (depends on which estrogen receptor it interacts with and where) to target hormone responsive tumours.
- It can block access of estrogen to the estrogen receptor - inhibitor of estrogen action.
- Also has been used to prevent cancer (ex. breast cancer)



Mechanisms of action of Tamoxifen

- Anti-estrogen
- Suppression of IGF-1 (insulin growth factor 1)
- Up-regulation of TGFβ (transforming growth factor beta, up-regulation can lead to apoptosis)

- Tamoxifen has been used in PREVENTING breast cancer, in those individuals that are at high risk of it (genetic predisposition)

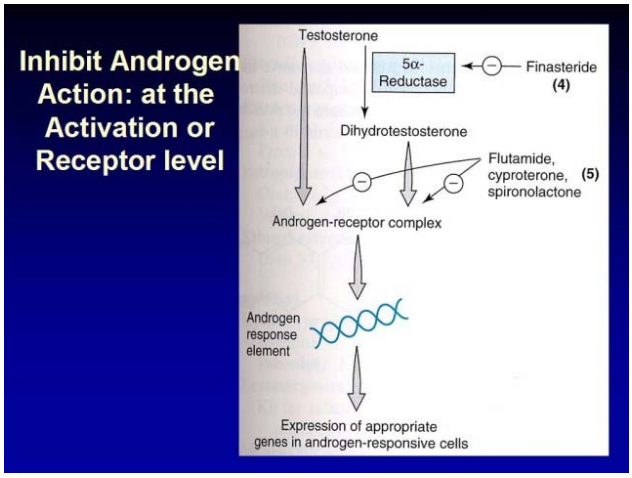
Consequences of estrogen withdrawal? → Menopause…therefore someone on Tamoxifen would have all the symptoms of menopause

Aromatase Inhibitors

- stop synthesis of estrogen from androgens by blocking cyt-P450 aromatase enzyme (enzyme that converts testosterone → estradiol) – impact would be felt most in breast cancer cells where there proliferation is estrogen-dependent
- **Formestane** and Exemestane

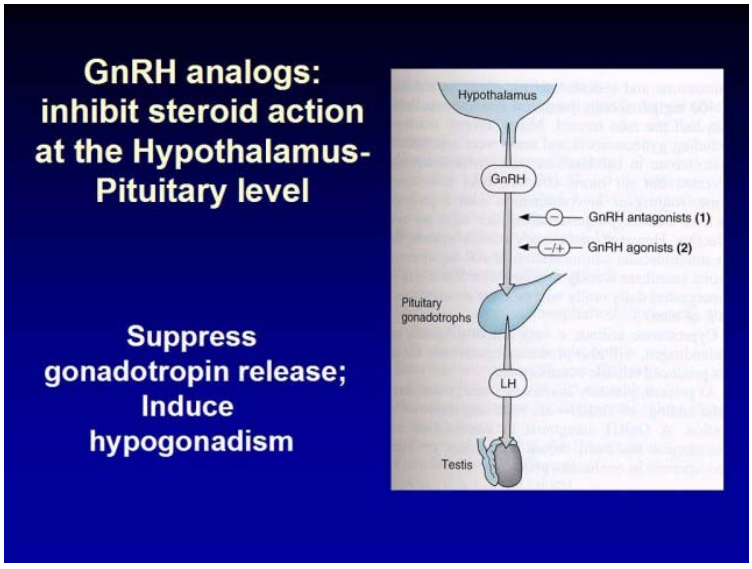
In post-menopausal women

- Aromatase inhibitor activity depends on whether the woman is in menopause or not.
- In post-menopausal woman, if the cells are estrogen receptor positive (determined by biopsy), the cancer is supported by the estrogen, so it grows. So we can give an aromatase inhibitor to block estrogen synthesis, and stop cancer growth.



- In males, prostate cancer cells are also dependent on androgen (have androgen receptors). 5alpha reductase inhibitors stop androgen action: *at the activation* - inhibit conversion of testosterone (by 5α-reductase) into dihydrotesterone (active androgen that binds receptor)
- **Finasteride** blocks 5α-reductase, stopping DHT synthesis
- At the *receptor level* (block the receptor complex; block the receptors ability to bind to DHT).
- **Flutamide**, anti-androgen drug that inhibits androgen action at the receptor level

Net result: less androgen to interact with the receptors to promote growth of the prostate cancer cells.



GnRH analogs

- Can go upstream, and suppress the axis, stop the communication between hypothalamus and pituitary (have less FSH and LH, less synthesis of androgen by the testis, or less synthesis of estrogen by the ovary).

GnRH Agonist/Antagonist

Leuprolide

- GnRH (gonadorelin) analog
- is more potent
- longer lasting

Management of carcinoma of breast cancer

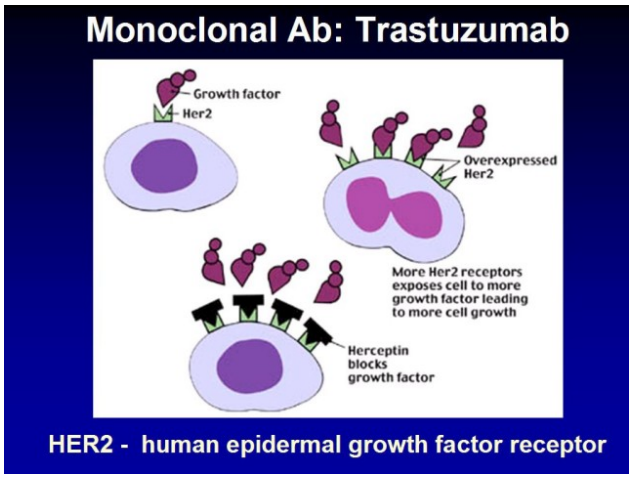
- Early diagnosis
- Surgery

Breast Cancer

- Limited surgery; radiotherapy (early diagnosis is key! That's why there is so much emphasis on mammograms)
- Chemotherapy:
    - ER (estrogen receptor) positive
        - Aromatase inhibitors (after menopause) (hormonal approach)
        - Anti-estrogens: Tamoxifen
    - ER negative (useless to try target hormones, these cells do not have estrogen receptors):
        - Doxorubicin
        - Docetaxel
        - Cyclophosphamide
        - Trastuzumab (monoclonal antibody targeted specifically at the HER-2 + growth hormone receptor) - need to know if the tumor has growth factor receptors

Monoclonal Ab: **Trastuzumab**

- HER-2+ → human epidermal growth factor receptor
- Many tumour cells over-express this receptor, exposing it to more growth factors leading to more cell growth
- Monoclonal Ab can block growth factor receptor.



Carcinoma of the prostate

- is totally age-dependent
- % of US Males with prostate cancer by age
    - 1% for 46-64
    - 6% for 65-74
    - 12% for 75+

Prostate Cancer (PC)

- Watchful waiting (can measure specific protein markers to see if there's evidence of cancer)
- Radical prostatectomy (removal of prostate)
- Radiotherapy
- Hormonal therapy for metastases (cancer spread in other tissues)
    - Testoterone reduction
- Combination chemotherapy:
    - **Estramustine** (estrogen linked to nitrogen mustard)
- Taxol or Vinca alkaloid

Leukemias and Lymphomas

- Problem, we can't remove these cancer cells because they're spread all-over the body (no surgery possible). Totally depend on anti-cancer drugs.

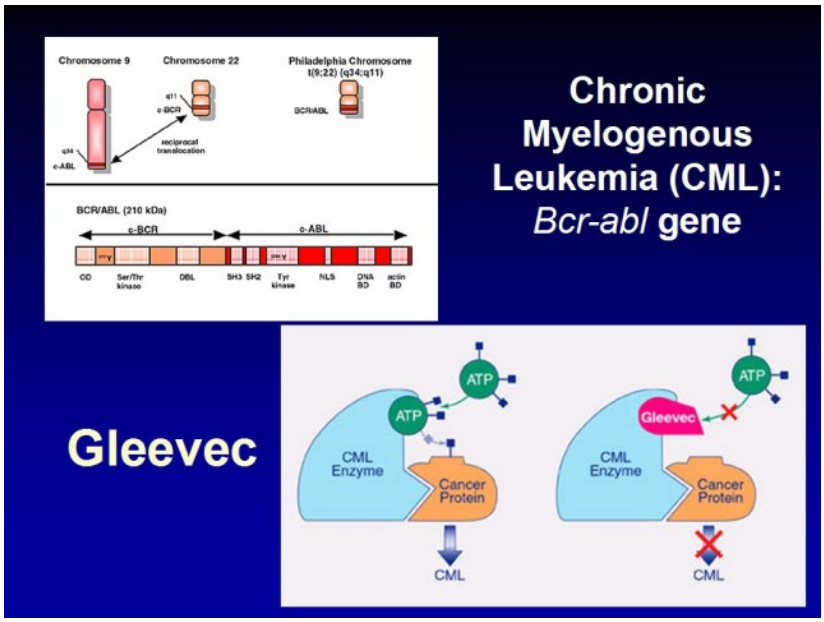
Combination chemotherapy

- Hodgkin's Lymphoma
  - MOPP – **Mechlorethamine**, **Vincristine**, **Procarbazine**, **Prednisone**
- Non-Hodgkin's Lymphoma
  - CHOP – **Cyclophosphamide**, **Doxorubicin**, **Vincristine**, **Prednisone**

These treatments are current "gold standards"; they have been found by trial and error.

Prednisone

- It interacts with glucocorticoid receptors (GR) within the cell (affect gene expression profile of these cells)
- Antilymphocytic effects in high doses (triggers apoptosis in some leukemia and lymphoma)



Chronic Myelogenous Leukemia (CML)

- CML is caused by genetic rearrangement (a new carcinogenic gene forms after chromosome stuck together: *Bcr-abl* gene)
- CML enzyme is turned on, kinase reaction never goes off, developing CML

Gleevec

- Specific inhibitor to that combination enzyme in leukemia cells (doesn't effect bone marrow, HIGHLY specific)
- This drug stops proliferation – targets kinase
- It took over 40 years to discover the disease and develop the drug.

EGFR Inhibitors

- Epithelial growth factor receptor inhibitors
- Antibody approach
- They inhibit proliferation, decrease survival, increase apoptosis, and decrease angiogenesis, metastasis and invasion.
- **Cetuximab** : drug that binds the interaction of the ligand (EGF) with the receptor
- **Gefitinib**: a drug that inhibits the tyrosine kinase domain of the EGF receptor

Immunomodulation - Interferon (IF) alpha or gamma

- When IF bind their receptor on cells, they trigger transduction cascade that leads to:
  - Decreased cell proliferation
  - Enhanced immune activities against potentially transformed cells

Block angiogenesis?

- With angiogenesis feeding the tumor, the cancer continues to expand, eventually spreading to other organs
- Target Growth Factors – eg. VEGF (vascular endothelial growth factor, stimulates angiogenesis = blood vessel growth)
- Complementary approaches by using a VEGF receptor inhibitor

Promote Differentiation

- Can convert a tumor cell to a normal cell

Retinoic Acid

- Promotes terminal differentiation of leukemic promyelocytes
- Interacts with retinoid receptors (α, β, γ)
- Activates gene transcription and terminal differentiation in leukemia cells

DNA as a target

- try not to damage DNA, but rather alter its function
- using Antisense oligonucleotides, that target specific genes involved in the proliferation of cells or survival (Gene therapy approach)

One example is an Antisense Drug against Bcl-2 (pro-survival gene that blocks apoptosis)

Cancer Treatment

- Destroy neoplastic cells
- Removal via surgery
- Prevent metastases
- Convert tumor cells to normal cells
- Block angiogenesis
- Halt neoplastic cell division

But, prevention is the best. Avoid tobacco, eat a healthy diet, engage in physical activity, limit alcohol, limit exposure to ultra-violet light and maintain a healthy weight.

Tuesday, March 13, 2007
Dr. Clarke - Lecture #16

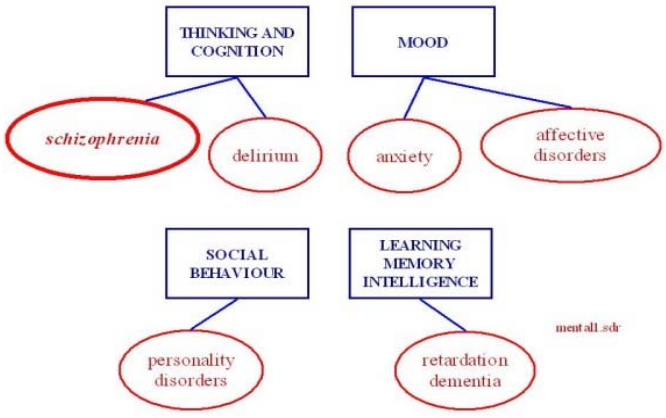
Drugs for Psychosis

Reading in Brody, Chapter 22: Dr. Clarke will not cover all the material in class, so it is important to go over it for the exam. Ignore Pharmacokinetics section and Table 22-1 and focus on the part about side effects.

Drugs to know by name:
- chlorpromazine (CPZ) – the first neuroleptic
- haloperidol (Haldol™)
- clozapine
- phencyclidine (i.e. PCP)

Terms:
- EPS (**ex**tra**p**yramidal **s**ymptoms i.e. Parkinson-like drug effects), movement problem that can be caused by first-generation antipsychotic drugs, especially when given in high doses
- 2 types of antipsychotic drugs:
  - *typical* antipsychotic = first generation = classical = traditional
  - *atypical* antipsychotic (better drugs i.e. little or no EPS and little or no ↑ prolactin)
- neuroleptic (any antipsychotic that produces EPS)
- depolarization block = depolarisation inactivation
- mesolimbic (a dopaminergic neuronal pathway that innervates forebrain)
- nucleus accumbens (main terminal area for mesolimbic DA pathway)
- Positron Emission Tomography (PET)

- Antipsychotic drugs typically do more than just block DA (dopamine) receptors as we will see through the lecture. An important question is whether these non-DA actions contribute to the therapeutic effects.



Schizophrenia (literally means *split-mind*)
- Classified as a disorder of thinking and cognition back in 1900
- was thought to be a thought disorder (out of touch with reality, affects thinking and cognition) but it is more than just this
- it is a split-mind, but not into two personalities → it's a splitting of cognition from emotion
- emotional withdrawal

Schizophrenia is defined by symptoms

Positive (things that you have that you shouldn't really have):
- delusions (e.g. Believe that you are the King.)
- hallucinations (usually auditory - voices might be coming from God, the CIA or others)
- thought disorders

- agitation
- grandiosity (might think you're Napoleon or Elvis!)
- suspiciousness (paranoia is common amongst schizophrenics, although not all are)
- hostility (if schizophrenic patient paranoids he/she might become hostile)

Negative (the symptoms reflect things that you lack and that you should have)
- blunted emotions (i.e. flattened affect or don't feel any emotions even when something terrible happens)
- emotional and social withdrawal
- poor rapport with people
- passive/apathetic (e.g. Don't want to get up in the morning)
- lack of spontaneity

Cognitive (some people, including Brody, consider thought disorders as a 3rd category)
- includes poor abstract thinking (e.g.. I am a man. Aristotle was a man; therefore I am Aristotle.)

Debate: Does schizophrenia progress from positive symptoms to negative symptoms?

It was thought that you get the positive symptoms early in life, and later, the negative symptoms become more apparent.

- Other people think that positive and negative are two separate disorders.
- Most people think that schizophrenia is a basket of different disorders or different traits.
  - It is a complex disorder and maybe, in the future, we will be able to subtype it and say that it is not just one disorder, but rather ten different disorders and some people can have more than one type of disorder at a time.

There is a lot of uncertainty!

Schizophrenia is quite common:
- 1% lifetime prevalence (1% of population will become schizophrenic)
- life-long disorder, there's no cure, tend to need constant medication

What causes schizophrenia?
- Genes (lifetime risk of schizophrenia if a relative has the disease):
  - high incidence of schizophrenia among family
  - high risk of developing schizophrenia (48%) in identical twins, then fraternal twins (17%), children (13%), etc
  - Results show there's clearly a genetic component involved in the transmission (the more related you are to someone with schizophrenia, the higher the risk of you becoming schizophrenic)
- environmental factors
- neurodevelopmental problems
  - subtle symptoms start early in life (poor social rapport, the if you worthwhile suggested by videotapes of children who later manifests symptoms of schizophrenia).
  - Even in fetal development, the cells in the brain do not proliferate and migrate properly.
- Virus
  - Higher risk of developing the disease if the mother contracted influenza during the 2nd trimester of pregnancy.
  - No particular virus has been implicated definitely in contributing to schizophrenia but likelihood that there is some viral component at least in some people.
- Stress (is a triggering factor)

- Structural changes in the brain (pretty obvious now with imaging techniques)
    - MRI scans of identical twins – only one has schizophrenia (the one with the larger cerebral ventricles)

- Disordered (DA) neurotransmission
    - drugs that tend to reduce the symptoms of schizophrenia block DA transmission
    - Henri Laborit (surgeon in France, observations led to discovery of antipsychotic drugs)
        - He wanted drugs that could calm agitated patients before surgery (anti-histamine tend to produce sedation). Drug company sent him chlorpromazine.
        - Chlorpromazine (produces sedation, but is not a very good anti-histamine)
        - described it as *beatific quietude* – 'saint-like quietude' in his patients
    - Delay and Deniker (psychiatrists)
        - Found out that chlorpromazine worked well for schizophrenia, not just tranquilizing (takes away the "craziness" without sedating the patient too much)
        - "neuroleptic" (these early antipsychotic drugs caused many EPS)
            - this makes sense to us today because we know that these drugs block DA transmission in the nigrostriatal system as well as other systems
        - were able to separate the antipsychotic effect from the sedative effect.
            - Psychiatrists were able to dissociate the antipsychotic action from the sedative and Parkinson actions, because drugs are differed in the relative amounts of these effects

Figure on the right:

- People started realizing that drugs, even though not perfect, had an important effect - reduced the psychotic symptoms. However, the patient was never completely normal even on the medication.
- The therapeutic effect is delayed (maximal effects of drug seen only after 6 weeks).
- There are adverse effects (Brody).
- Negative symptoms vary largely with the drug.

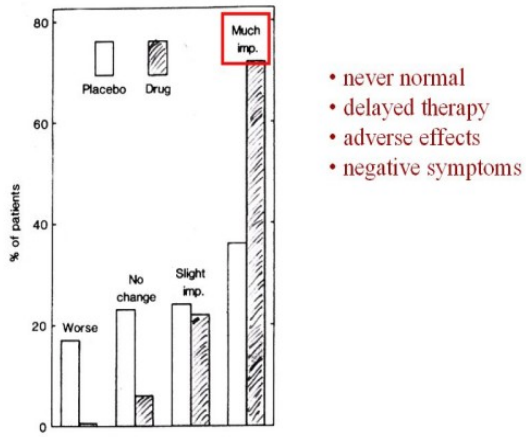


Fig. 28.7 Clinical trial of phenothiazines in acute schizophrenia. (Results of NIMH Collaborative Study 1964 Arch Gen Psychiat 10: 246)

The neuroleptic revolution

- The patient population in public mental hospitals in the US increased largely since 1900s (up to 550,000 patients in 1950). But then, with the introduction of antipsychotic drugs, the population in these mental hospitals decreased rapidly.
- Emptying of hospitals, many of the patients can go back in the society (don't need continuous care in hospitals), but others did not integrate and became street people

Antipsychotic drugs – diverse chemical structures

- Typical (traditional) antipsychotics:
    - chlorpromazine (remember)
    - haloperidol
- Atypical (Novel) antipsychotics:
    - clozapine (remember)
    - risperidone

Is DA just a precursor for NA (noradrenaline)?

- Tyrosine → DA → NA
- It is also a neurotransmitter, discovered because of reserpine (drug extracted from the snakeroot plant – given to babies to calm them down; produces a depressive state).

How do neuroleptics work?

- Reserpine (RES) had the same effects as chlorpromazine, reducing schizophrenia (antipsychotic) and produced EPS.
- *Question:* What might mechanism(s) of action chlorpromazine and RES have in common?
- In 1955, sensitive techniques were developed to measure small amounts of monoamine transmitters in the brain. (Carlsson)
- Was found that RES ↓↓↓ brain levels of DA, NA, 5-HT and induces PD (Parkinson disease) -like symptoms. It is the loss of DA that causes Parkinson symptoms.
- Both RES and CPZ reduce schizophrenia, but does CPZ reduce DA?
- Found out that CPZ causes no change in DA content in brain tissue in rats.
- Carlsson tested many antipsychotic drugs by measuring the ratio DA metabolite: DA in the brain. This ratio gives a good idea of the DA cell activity. CPZ (and many antipsychotics) *increased* this ratio, therefore suggesting an *increase* in DA cell activity.

How come RES decreases DA, CPZ increases DA cell activity *and both suppress EPS?*

Carlsson´s explanation: some DA receptors are **autoreceptors**.

- Antipsychotics block DA receptors on post-synaptic cells (reducing the psychotic symptoms).
- Autoreceptors are found on somatodendritic (firing) and terminal (release) membranes of DA neurons (when stimulated by agonist, autoreceptors work to reduce DA transmission).
- DA autoreceptors are on the DA cells and function to reduce DA cell firing and to reduce DA release when an action potential comes.
- DA is released from terminals and dendrites (DA acts on autoreceptors – negative feedback).

In 1972, Paul Greengard discovered that DA increased cAMP (using molecular assays on rats striatum in vitro).

- DA stimulates receptors → stimulates adenylyl cyclase activation → increasing levels of cAMP
- Greengard found out that CPZ strongly inhibits DA action (therefore maybe CPZ blocks DA receptors).
- Haloperidol (another widely used antipsychotic) was needed in **huge** concentrations to block DA receptor (very weakly inhibits DA activity), meaning that not all antipsychotics block this adenylyl cyclase-stimulating effect of DA.
- In 1975, an experiment was performed using radioactive DA binding in rat striatum. CPZ is very potent compared to haloperidol. CPZ blocks these DA receptors in striatum better than haloperidol.
- Experiment: Radiolabel Haloperidol and use it as a radioligand to figure out what it is binding to – it was found that it actually binds to DA receptors!
    - Haloperidol binds to DA receptors but only inhibits ^{3}H-DA binding at very high concentrations
- Conclusion both CPZ and haloperidol bind DA receptors, but not the *same* DA receptors.

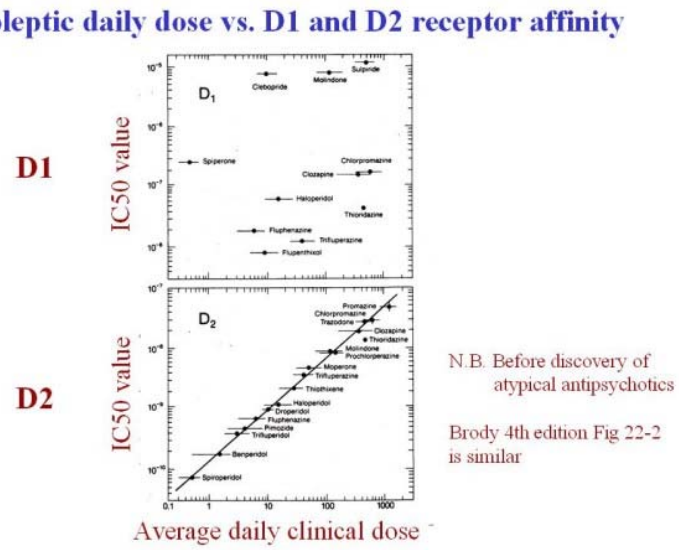
<u>The initial DA receptor classification</u>

- D1 subtype → stimulates adenylyl cyclase → ↑ cAMP
- D2 subtype → inhibits adenylyl cyclase → ↓ cAMP
    - D1 subtype is predominant in Greengard's cAMP assay

- So CPZ blocks D1 receptors potently (could be seen with radioactive DA binding in striatum) – haloperidol only blocks at very high concentrations
- In the ^{3}H-DA binding assay, under the conditions used (buffer, temperature, etc.) ^{3}H-DA actually binds **preferentially** to D1 receptors (presumably the D1 receptors have higher affinity for DA and/or are more abundant than D2 receptors)
- **MAIN POINT:** the DA receptor binding assay was mostly an assay of D1 receptors, that's why CPZ worked well and haloperidol did not

o Haloperidol acts potently at D2 receptors.

<u>Neuroleptic daily dose vs. D1 and D2 receptor affinity</u>



- Graph of average daily clinical dose of many different antipsychotic drugs (x-axis) plotted against their respective affinities for D1 and D2 receptors (IC50 value).
- the more potent the drug is as an antipsychotic, the more potently it interacts with the D2 receptors (D2R) – very good correlation.
- This correlation is NOT found with D1 receptors
- This is strong evidence that antipsychotic drugs (at least those discovered up until this point) produce their antipsychotic affect by blocking D2R.
- CPZ, even though it acts on D1 receptors, actually produces (it is thought) its therapeutic effect by blocking D2 receptors; it is not very potent clinically nor does it interact very potently with D2R (see graph above).

Can drugs cause temporary schizophrenia?

Some amphetamines cause temporary schizophrenia-like symptoms in normal people, and do so more potently in people who are schizophrenic or predisposed to schizophrenia.

Amphetamine and cocaine psychosis

- ideas of reference (e.g. A landslide is announced on news, you think it has something to do with you.)
- paranoid delusions
- neuroleptics (block positive symptoms)
- schizophrenic patients
  - o more sensitive to drugs (positive symptoms recur)

<u>Other drugs that reproduce some schizophrenia-like symptoms</u>

- **PCP** (phencyclidine)
  - o A drug of abuse also known as "angel dust"
  - o Produces negative symptoms of schizophrenia
  - o Used as animal model to model negative symptoms of schizophrenia
- **LSD**
  - o Typical psychedelic experience is not schizophrenia-like

- Creates more visual hallucinations whereas schizophrenia is typically associated with auditory hallucinations, therefore not a close model

DA hypothesis of schizophrenia

- Evidence
  - Stimulant psychosis
  - Neuroleptics
  - Brain DA content?
    - Schizophrenic do not necessarily have an abnormal DA concentration.
    - However, DA tissue content is not a good guide to levels of DA *transmission*.
    - The amount of DA released in the synapse might be higher, but not necessarily higher in the overall brain.
  - DA receptor abnormalities?
    - D2 receptors may be changed; but this is not very consistent.
- Problems:
  - How can we explain the therapeutic delay (4-8 weeks before maximal effect reached)
  - Neuroleptic non-responders
    - 25% of patient don't respond to antipsychotic drugs
  - **Clozapine**
    - atypical drug
    - doesn't block D2R very much at clinically relevant doses

Several DA pathways are thought important to neuroleptic action:

SYSTEM	PROJECTS TO	EFFECT OF NEUROLEPTIC
I. Mesolimbic (bottom left arrow)	Nucleus accumbens, etc.	↓ positive symptoms?
II. Mesocortical (upper left arrow)	Parts of cortex	↓ negative symptoms?
III. Nigrostriatal (right arrow)	Striatum	EPS

The arrows in the figure below show the main terminal areas for these pathways.

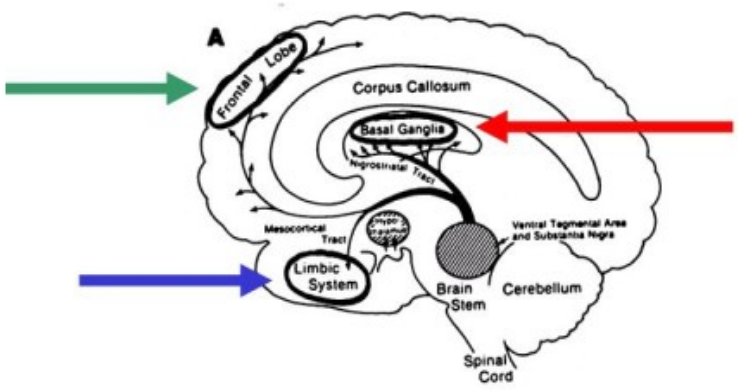
DA receptors – further subtypes (discovered through molecular genetic screening approaches; homology, cloning) :

D1-like

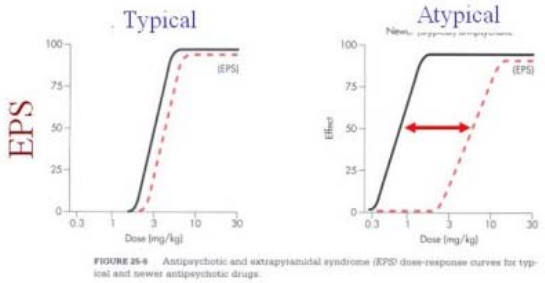
- D1 (most numerous)
- D5

D2-like

- D2 (most numerous)
- D3 (potential target)
- D4 (potential target)



Atypical antipsychotics – definition



Above figure:
Black – antipsychotic effect
Red - EPS

Prototype – **Clozapine**

- Have more favourable profile
- little or no EPS
    - Typical drugs (1st diagram): dose-response curve for producing an antipsychotic effect (black line) is very close to that which produces Parkinsonian-like side effects, so you tend to get both (not good!)
    - Atypical drugs (2nd diagram): A dose that produces the antipsychotic effect produces little or no EPS – have to give huge dose to produce Parkinsonian side effects
- little or no prolactin surge
    - prolactin stimulates milk production
    - DA inhibits that effect via D2 receptors (in pituitary)
    - Classical but not atypical antipsychotics block D2 receptors (therefore blocking effect of DA); therefore antipsychotic drugs increase milk secretion (even in men!)
- more efficacious?
    - Atypicals are more effective than classical drugs overall
    - more effective at treating negative symptoms (older drugs had little or no effect on negative symptoms)
    - also effective in patients that were unresponsive to typical drugs
    - atypical such as clozapine (see figure) produce fewer EPS)
- no tardive *dyskinesia* (major motor side effect of the older drugs)
    - tardive – takes months or years to develop
    - involuntary movements, particularly in mouth, face, and neck
    - dyskinesia – face and mouth
    - 20-40% of patients affected
    - If withdraw from the drug, it gets worse, not better

Clozapine in treatment-resistant schizophrenics:
fewer extrapyramidal side-effects

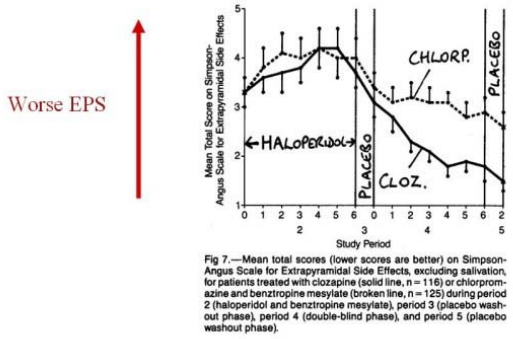


Fig 7.—Mean total scores (lower scores are better) on Simpson-Angus Scale for Extrapyramidal Side Effects, excluding salivation, for patients treated with clozapine (solid line, n = 116) or chlorpromazine and benztropine mesylate (broken line, n = 125) during period 2 (haloperidol and benztropine mesylate), period 3 (placebo washout phase), period 4 (double-blind phase), and period 5 (placebo washout phase).

How do atypical antipsychotics work at the anatomical level? (several hypotheses)

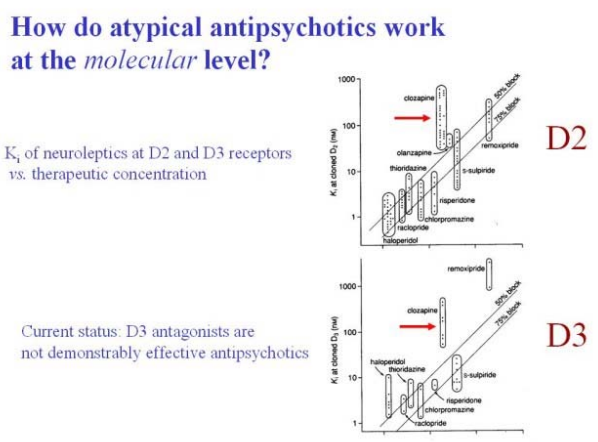
- Rat behavioural studies suggest that clozapine acts selectively on the mesolimbic DA system (not in the nigrostriatal system).
- Experiment: give amphetamine to rats to increase DA transmission
    - High doses of **Amphetamine** causes *stereotypy* (the rat runs in a fix circle in its cage, or stop in one corner and scratch its face) by increasing *nigrostriatal* DA release.
    - In this nigrostriatal assay, clozapine, given acutely, somewhat enhances amphetamine effect (definitely not blocking nigrostriatal DA transmission then).
    - Lower doses of **Amphetamine** causes *locomotor stimulation* (only makes the rat run more) by increasing *mesolimbic* DA release.

- In mesolimbic, clozapine has a depressant effect, it inhibits amphetamine (reduces DA transmission).
- With a typical antipsychotic drug, DA transmission is blocked in both regions (nigrostriatal and mesolimbic).

Other evidence of possible relevance to the previous experiment:

- D3 DA receptors are abundant (preferentially found) in mesolimbic DA terminal areas (nucleus accumbens).
- Few D3 DA receptors in nigrostriatal

- Clozapine blocks mesolimbic but not nigrostriatal DA, whereas typical drugs block both mesolimbic and nigrostriatal DA receptors



- Hypothesis: clozapine acts on D3 DA receptor

An experiment shows that clozapine doesn't really bind D3 receptors a whole lot; therefore, not a good hypothesis. At clinical doses, clozapine does not have a big effect on D3 receptors.
Current status: D3 antagonists are not demonstrably effective antipsychotics.

<u>Three hypotheses to determine how atypical antipsychotic drugs produce antipsychotic effects:</u>

1. D4 receptor blockade
   - D4 DA receptors have unusually high affinity for clozapine
   - At clinical doses, clozapine blocks a lot of D4 DAR
   - But selective D4 antagonists are not antipsychotic

   Therefore not a good explanation

2. DA + 5-HT2 receptor block
   - Maybe the antipsychotic effect also requires blockade of 5-HTR
   - Risperidone (an atypical drug) occupies 5-HT2 receptors as well as D2 receptors in human brain at a clinical dose.
   - Relative affinities at D2 and 5-HT2 Receptors:
     - Compared to classical drugs, atypicals:
       - Block fewer D2 receptors (>70% for classical and 40-60% for atypical)
         - Produce less EPS (we have good evidence that EPS is caused by blocking D2 receptors so this makes sense!)
       - Block more 5-HT2A receptors (~70-90%)

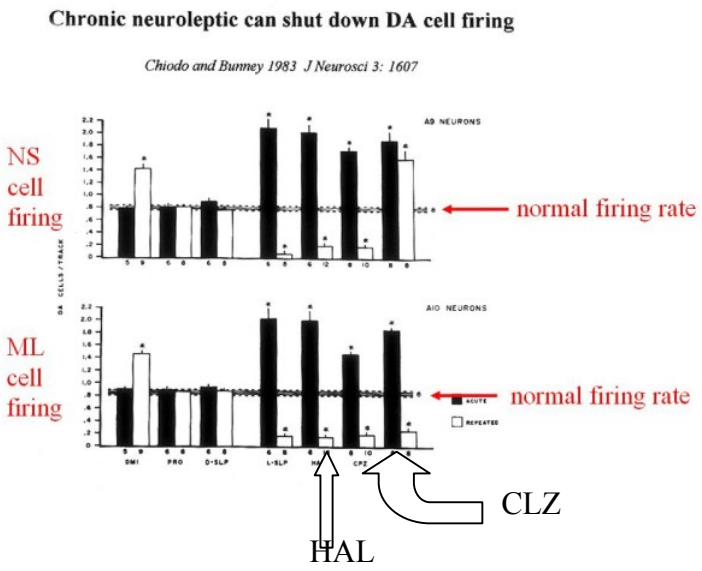
   - Classical drugs have higher affinity for D2 than 5-HT2.
   - Atypical drugs tend to have higher affinity for 5-HT2 than D2.
     - Exceptions for **clozapine** and **risperidone** that occupy a little more D2 than 5-HT2.

<u>Caveat: Many antipsychotic drugs have little or no selectivity for DA receptors</u>
All antipsychotic drugs have side actions on other receptors
- other common targets include:

- muscarinic receptors – if block these, get urine retention and dry mouth
- adrenergic receptors – if block these, get hypostatic hypotension

- See Brody textbook 4th edition
  - Section about side effects (IMPORTANT to read!!!)

3. Depolarization inactivation in mesolimbic system
   - Chronic action is what counts
   - Atypical drugs select DA neurons in mesolimbic system and blocks them (leaving nigrostriatal (NS) pathway not blocked, therefore you get no EPS)
   - Whereas classic drugs, given chronically, turn off DA transmission in nigrostriatal **and** mesolimbic (ML) DA neurons.
   - DA cells have pacemaker activity in the absence of drug (fire action potential when reach threshold).
   - If you give a DA agonist, it *hyperpolarizes* DA cells by *stimulating* autoreceptors, lowering or stopping cell firing (membrane potential becomes even more negative); it takes longer each time for the pacemaker to ramp up to the point where an action potential can be produced - the cell fires slower or stops firing.
   - DA antagonist *depolarized* DA cells by *blocking* autoreceptors; the cell fires faster, but… if you shift the membrane potential too much (too depolarized), the ion channels cannot function (cell can't fire anymore = *DEPOLARIZATION INACTIVATION* or BLOCK if antagonist given chronically)
   - Typicals do this to mesolimbic and nigrostriatal cells
   - Atypicals do this to mesolimbic but ***not*** nigrostriatal cells (maybe this is how they take away craziness and don't produce EPS)
   - Figure on the right; black=acute and white=chronic (given to rats)



   - Anesthetize rats, put electrode in head, measure firing rate of individual DA cells
   - All drugs given acutely ↑ firing rate through blocking DA autoreceptors (no negative feedback)
   - Chronic typical drug shuts down NS and ML DA cells (like haloperidol)
   - Chronic CLZ (clozapine) shuts down only ML cells but **increases** firing in NS (no EPS)

<u>Smoking and Schizophrenia</u>
- among normal population, about 25% are smokers
- among schizophrenics, 90% are smokers!
  - Possibly to try to get over the side effects of their medications (nicotine can stimulate DA release)
  - Selective attention & cognition: schizophrenics have trouble processing incoming info
  - Schizophrenic relatives also have some of the symptoms (can't tune out some sensory stimuli like the sound of the fridge) and yet they are not taking medications.
  - But when the relatives take nicotine, they normalize (increase their ability to concentrate). Relatives are useful to study here because typically they are not on antipsychotic medication, which would otherwise complicate the interpretation of results.