Chemotherapy

SELECTIVITY: exploiting qualitative and quantitative differences between the host and pathogen, can be structural or biochemical.

Antibacterial chemotherapy

Several targets: cell wall synthesis, cell membrane, protein synthesis and nucleic acid synthesis/repair. Compounds may be bacteriostatic or, bactericidal – the extent to which removal is beneficial is important.

Due to differences in structure of Gram +/- walls, the repertoire of drugs that can be used against each type is sometimes different. Usually more difficult for drug to cross Gram – due to highly impermeable outer membrane, as well as range of hydrolytic enzymes in the periplasm

Cell Wall as a target

- peptidoglycan is a unique target not present in mammalian cells. The extent of crosslinking and number of chains is greater in Gram +.
- Peptidoglycan is alternating polymer of NAG and NAM.
- NAG generated and converted to NAM (*fosfomycin* – inhibits pyruvyl transferase)
- NAM is modified to a glycopeptide: addition of pentapeptide: ala-glu-lys-D-ala-D-ala. (*cycloserine* – inhibits L-lanine racemase, D-ala-D-ala synthetase and ligase enzymes)
- This binds to the 'lipid membrane carrier' – bactoprenol phosphate, that has been dephosphorylated (*bacitracin* – inhibits this dephosphorylation) then NAG binds to form the cell wall monomer
- The unit is flipped to extracytosolic surface on bactoprenol phoshate
- Monomer encorporated into chain via transglycosylation (*vancomycin*) and the pentapeptide chains form branched networks via transpeptidation (*vancomycin* – inhibits by binding to D-ala-D-ala and preventing access of enzymes)

- *β-lactams* – prevent cross-linking. Includes penicillins, cephalosporins, monobactams, carbapenems. Side chains determine specificity, stability and pharmacokinetics
    - drug acts as a structural analogue of D-ala-D-ala. Transpeptidase/transglycosylase enzyme creates acyl enzyme intermediate, with open B-lactam ring, which is slow to hydrolyse
    - The drugs promote lysis only in proliferating cells. In the presence of bacteriostatic agents, β-lactams don't cause lysis.
    - Bactericidal activity requires autolysin activity - enzymes that break down and remodel the cell wall, causing lytic cell death in proliferating cells

Protein synthesis disruption

- 30S subunit
    - Tetracycline: blocks rotation of aminoacyl-tRNA into A site, leading t premature release without peptide bond formation
    - Aminoglycosides - streptomycin: bind near A-site, disrupting decoding and translational accuracy – decreased fidelity
- 50S subunit
    - Chloramphenicol: blocks amino-acyl tRNA interaction with A site of peptidyl transferase centre
    - Macrolides, e.g. erythromycin: bind entrance of polypeptide export tunnel, blocking elongation beyond 6-8 oligopeptides.
- Fusidic acid: binds and inhibits elongation factor G, blocking translocation
- Most of the agents are bacteriostatic rather than bactericidal.

Nucleic acid synthesis and repair

- Fluoroquinolones, e.g. ciprofloxacin – Inhibition of DNA topoisomerases (supercoiling)
- Rifampin: inhibit DNA dependent RNA polymerase binding nn covalently to allosteric site.
- Daunomycin – planar polycyclic molecule intercalating non-covalently causing partial unwinding.
- Bleomycins – metal-chelating glycopeptide, interacting with Oxygen and Iron generating free radicals causing DNA strand breaks
- Mitomycin C – alkylates DNA at GC positions

Anti-metabolites (folate synthesis)

- Sulfamethoxazole – blocks dihydropteroate synthase (DHPS)
- Trimethoprim – blocks dihydrofolate reductase (DHFR)
- Combination = co-trimoxazole

Cytoplasmic membrane

- low selectivity as mammalian cells also have membranes with similar characteristics
- Gramicidin A – dimerises in membrane forming monovalent cation channels
- Polymixin – aggregates into micelle-like complexes affecting permeability of cytoplasmic membrane
- Valinomycin – diffuses across membrane as a potassium uniporter exerting ionophoretic cpacity.

- Membrane is often the target for antifungal drugs. Fungi have ergosterol based membranes rather than cholesterol. *Amphotericin B* - bind preferentially to ergosterol facilitating pore formation; *Fluconazole* – inhibition of enzymes for ergosterol synthesis.

Antimalarial drugs

- interfere with nucleic acid synthesis – pyrimenthamine, sulfadoxine
- Chloroquine: concentrated within infected RBC and localises to acidic food vacuole. Parasite breaks down Hb to heme and amino acids. Heme (damaging and toxic) then polymerised to hemozoin (non toxic). Chloroquine prevents the polymerisation.

Antiviral chemotherapy

Viruses subvert many host processes to execute its life cycle. But several possible targets: attachment, penetration into host cells, translation, nucleic acid synthesis and protease inhibitors.

Influenza

- Amantadine: block function of M2 channel (ion channel triggering uncoating when exposed to low pH) and interfere with haemagglutinin processing.
- *Zanamavir*: neuraminidase inhibitor, enhancing viral aggregation and inhibit release from cells.

Herpes viruses

- *Aciclovir*: purine analogue – monophosphorylated by herpes thymidine kinase, then cellular enzymes convert to tri-phosphate, which competitively inhibits viral DNA polymerase. Lacks 3 prime OH and so is chain terminating
- *Foscarnet*: analogue of pyrophosphate, blocking cleavage of nucleoside triphosphates during polymerisation.

Human immunodeficiency virus

- *Reverse transcriptase inhibitors*: nucleoside (zidovudine), non-nucleoside (nevirapine) inhibitors of reverse transcriptase as chain terminators with no 3 prime OH.
- *Protease inhibitors*: saquinavir

Much resistance has occurred due to the high mutation rate in viral replication and so now multidrug combinations are used e.g. HAART

Anticancer chemotherapy

Cancer cells are fundamentally 'self' which makes finding unique targets difficult. Proliferation is itself one target that is used.

Drugs binding to DNA

- NITROGEN MUSTARDS: bifunctional alkylators – target nucleophilic areas of DNA and form intrastrand branches. Chlorethyl group undergoes cyclization and loses Cl, reactive intermediate binds to N7 of a guanine covalently. Second side chain also cyclize and attacks another nucleophile. E.g cyclophosphamide; melphalan – accumulates in melanomas
- NITROSUREAS: Lomustine – alkylating and carbamoylating, producing interstrand cross-links in duplex DNA at N7 and O6 sites in guanine
- Mitomycin C – DNA alkylation and cross-linking, inhibiting DNA synthesis.
- Cis-platin – intrastrand cross-link, binding two neighbouring guanines causing a major bend, inhibiting DNA polymerases.
- ANTHRACYCLINES: linear planar 3 aromatic ring structure. Intercalate non-covalently between bases of DNA causing local unwinding. No base specificity, e.g. doxorubicin. Leads to changes in sedimentation of the DNA.
- Many of the drugs that interact with DNA cause lesions, requiring that there are various DNA repair pathways e.g. mismatch repair or alkylation repair etc.

Antimetabolites

- targeting quantitative differences between cancerous and normal cells rather than qualitative
- prevent formation of normal precursors to DNA/RNA synthesis.
- Methotrexate: inhibits DHFR (+ leucovorin to rescue normal tissue from folate depletion)
- Pyrimidine/purine antagonists – taken up by nucleoside uptake systems and converted to nucleotides. Converted to 'fraudulent nucleotide' that cannot become encorporated

Topoisomerase inhibitors

- important for unwinding DNA which allows for transcription, replication and separation at division (Topo I = transcription; Topo II = replication)
- Type I inhibitor e.g. topotecan
- Type II inhibitors e.g. etoposide, daunorubicin – the inhibitors stabilise the cleavage complex and inhibit the religation step

Antimitotic drugs

- interfere with microtubular function. Microtubule dynamics are particularly important during mitosis. Disruption of network will affect progression through metaphase, by preventing formation of the spindle.
- E.g. vinblastine and paclitaxel.
- Other roles of microtubules are also disrupted e.g. phagocytosis etc.

Hormone therapy

- steroid hormones can affect proliferation of certain tumour types, due to the effects of transcription their receptors have.

- Some tumours, hormones inhibit growth, e.g. glucocorticoids against certain leukaemias
- Some tumours depend on hormones for growth, so removal of hormone producing organs, preventing synthesis, or blockade of the action of the hormone.
    - Tamoxifen – antioestrogen – but although antagonises the proliferative aspects of oestrogen signalling it actually acts as an agonist for the anti-osteoporotic functions!

The aim of chemotherapy is to target selectively rapidly dividing cells. But there are different effects on different stages of the cell cycle, and the tumour is a heterogenous population

The drugs attempt to cause death by inducing apoptosis

RESISTANCE

Effectiveness of nearly all chemotherapy is limited by resistance. Now found that resistance elements pass not only down generations (vertical transfer), but also can be transferred between bacteria via horizontal transfer which has lead to the widespread resistance there is today.

Multiple drug, vs multidrug resistance
Multidrug resistance is where a single feature renders the cell resistant to a wide range of antibiotics. The most usual example of this, are the non-specific efflux pumps which can pump out a range of compounds. In contrast multiple drug resistance is due to a series of genes, that each confer resistance to a single drug, but these elements have become concatenated together on genetic elements such as plasmids. Use of one drug will therefore select for the element, and hence resistance against other unrelated compounds.

- *inactivation of antibiotics*
    - β-lactamases. Many different enzymes with different substrate preferences, chromosomal or plasmid encoded etc. Hydrolysis of beta-lactams. 4 groups: A,C,D serine hydrolases; B zinc dependent.
    - aminoglycosydes: N-acetylation; O-phosphoryl transferase; O-adenyl-transferase

- *modifications to the target sites*
    - acquisition of new genes encoding a target with low affinity
    - increased amounts produced
    - acquisition of genes producing modification of targets
    - mutational events lowering affinity of target
    - E.g. MRSA: MecA (transpeptidase), FemA,B,D (generate appropriate substrate as standard NAM-NAG not optimal substrates for Mec A enzyme)
    - E.g. Vancomycin resistance – D-ala-D-Lac! Much reduced affinity for vancomycin active site. Expressed on 5 genes: VanH VanA VanX VanS VanR.

- *decreased accumulation of antibiotic: increased efflux, or decreased uptake*
    - decrease in overall permeability due to mutational loss of porins (low level resistance only)
    - changes in existing uptake systems
    - increased active efflux in conjunction with repression of porin synthesis

Strategies to circumvent resistance include:
- design derivatives of existing drugs not metabolised
- specific inhibitors of drug resistance mechanisms, e.g. clavulanic acid a beta lactamase inhibitor – combined with amoxicillin in Augmentin
- reducing usage of drugs, decreasing selection pressure
- identification of new drug targets
- using combinations of medications to reduce the selection pressure on each
- Drug companies are reluctant to invest in antibiotics

Resistance to anticancer drugs
- intrinsic: tumour, even on first exposure is relatively insensitive
- acquired: appears following a course of chemotherapy
- resistance can be due to poor access, altered targets, enhanced drug inactivation, increased repair, changes in drug targets
- p-glycoprotein transporter conferring multidrug resistance. High expression of P-gp in many tumours.
- 'resistance modifiers' are drugs that can disrupt the efflux action of P-gp.
- To minimise unwanted side effects, and minimise risk of resistance, combinations of drugs are often used.

MCQ – True/False – Negatively marked

Antiviral chemotherapy

a) Blocking attachment with antibodies is one strategy employed
b) HIV can be targeted using cytokine receptor antagonists
c) Protease activators can be used to disrupt the final processing
d) Resistance has arisen due to the great mutation rate that is demonstrated by many viruses

Antibacterial chemotherapy

a) Gram + organisms have a cell wall that is highly impermeable to most drugs
b) Cytoplasmic membrane of bacteria is a useful target due to the significant differences in sterol content compared to mammalian cells
c) Peptidoglycan synthesis involves 'penicillin-binding proteins' which catalyse the transpeptidation and transglycosylation stages
d) β-lactam antibiotics act by enhancing the transpeptidation stage
e) β-lactam antibiotics work best in conjunction with a bacteriostatic agent
f) tetrahydrofolic acid biosynthetic pathways in mammals and bacteria are identical

Antiplasmodial chemotherapy

a) drugs are used which target the breakdown of haemoglobin to Heme and amino acids

Resistance to antibacterial chemotherapy

a) Inactivation of drugs is a common mechanism of resistance, such as the β-lactamases
b) MRSA resistance is an example of resistance due to efflux of drugs
c) Decreased expression of porins is a means of achieving high-level resistance
d) Drug companies are becoming more willing to invest into drug research to attempt to overcome the resistance that is now becoming prevalent

Anticancer chemotherapy

a) Targeting qualitative rather than quantitative differences between tumour and normal cells is commonly employed
b) Topoisomerase inhibitors are used to disrupt the DNA
c) Drugs are used to both covalently and non-covalently interact with DNA to disrupt its function
d) Base specificity is important in drugs that are found to intercalate with DNA
e) Anticancer chemotherapy aims to prevent apoptosis of cancer cells

MCQ – True/False – Negatively marked

Antiviral chemotherapy

e) Blocking attachment with antibodies is one strategy employed **T**
f) HIV can be targeted using cytokine receptor antagonists **F CHEMOKINE**
g) Protease activators can be used to disrupt the final processing **F INHIBITORS**
h) Resistance has arisen due to the great mutation rate that is demonstrated by many viruses **T**

Antibacterial chemotherapy

g) Gram + organisms have a cell wall that is highly impermeable to most drugs **F CELL WALL IS NOT IMPERMEABLE**
h) Cytoplasmic membrane of bacteria is a useful target due to the significant differences in sterol content compared to mammalian cells **F (FUNGI TRUE)**
i) Peptidoglycan synthesis involves 'penicillin-binding proteins' which catalyse the transpeptidation and transglycosylation stages **T**
j) β-lactam antibiotics act by enhancing the transpeptidation stage **F DISRUPT**
k) β-lactam antibiotics work best in conjunction with a bacteriostatic agent **F DON'T WORK IN THIS ENVIRONMENT**
l) tetrahydrofolic acid biosynthetic pathways in mammals and bacteria are identical **F**

Antiplasmodial chemotherapy

b) drugs are used which target the breakdown of haemoglobin to Heme and amino acids **F HEME TO HEMOZOIN**

Resistance to antibacterial chemotherapy

e) Inactivation of drugs is a common mechanism of resistance, such as the β-lactamases **T**
f) MRSA resistance is an example of resistance due to efflux of drugs **F ALTERED TARGET**
g) Decreased expression of porins is a means of achieving high-level resistance **F LOW LEVEL BECAUSE NUTRITION**
h) Drug companies are becoming more willing to invest into drug research to attempt to overcome the resistance that is now becoming prevalent **F!**

Anticancer chemotherapy

f) Targeting qualitative rather than quantitative differences between tumour and normal cells is commonly employed **F QUANTITATIVE**
g) Topoisomerase inhibitors are used to disrupt the DNA **T**
h) Drugs are used to both covalently and non-covalently interact with DNA to disrupt its function **T**
i) Base specificity is important in drugs that are found to intercalate with DNA **F HOWEVER NOW MORE SPECIFIC DRUGS BEING DESIGNED**
j) Anticancer chemotherapy aims to prevent apoptosis of cancer cells **F PROMOTE**

MCQ – True/False – Negatively marked

Antiviral chemotherapy

a) Acyclovir is a nucleoside analogue that results in chain termination
b) Saquinavir is used to disrupt the nucleic acid of the virus

Antibacterial chemotherapy

a) Bacitracin is involved with disrupting the function of the lipid membrane carrier
b) Vancomycin binds to D-glu-D-glu and disrupts transglycosylation
c) Monobactams are part of the β-lactam family
d) Tetracyclines disrupt nucleic acid synthesis
e) Streptomycin is able to act in anaerobic bacteria
f) Chloramphenicol acts to prevent the peptidyl transfer reaction
g) Macrolides act to disrupt completion of peptide by stimulating dissociation of peptidyl-tRNA
h) Sulphonamides inhibit DHFR
i) Quinolones are used to inhibit RNA polymerase

Antiplasmodial chemotherapy

a) Chloroquine concentrated in the food vacuole and inhibits the polymerisation of Heme to hemozoin
b) Artemisinin is a slow acting antimalarial

Anticancer chemotherapy

a) Tamoxifen is a complete antagonist of oestrogen function
b) Methotrexate is used to inhibit the DHFR enzyme in the folic acid synthetic pathway
c) Nitrogen mustards are monofunctional alkylators
d) Cyclophosphamide is a drug that non-covalently intercalates into DNA
e) Both the *cis* and *trans* isomers of cisplatin have antitumour activity
f) Doxorubicin activity leads to a change in the sedimentation of the DNA
g) Vincristine has a similar function to taxol

MCQ – True/False – Negatively marked

Antiviral chemotherapy

c) Acyclovir is a nucleoside analogue that results in chain termination **T**
d) Saquinavir is used to disrupt the nucleic acid of the virus **F PROTEASE INHIBITOR**

Antibacterial chemotherapy

j) Bacitracin is involved with disrupting the function of the lipid membrane carrier **T**
k) Vancomycin binds to D-glu-D-glu and disrupts transglycosylation **F D-ALA-D-ALA**
l) Monobactams are part of the β-lactam family **T**
m) Tetracyclines disrupt nucleic acid synthesis **F PROTEIN SYNTHESIS**
n) Streptomycin is able to act in anaerobic bacteria **F REQUIRES O_2 DEPENDENT UPTAKE**
o) Chloramphenicol acts to prevent the peptidyl transfer reaction **T**
p) Macrolides act to disrupt completion of peptide by stimulating dissociation of peptidyl-tRNA T
q) Sulphonamides inhibit DHFR **F PABA ANALOGUE**
r) Quinolones are used to inhibit RNA polymerase **F INACTIVATE DNA GYRASE**

Antiplasmodial chemotherapy

c) Chloroquine concentrated in the food vacuole and inhibits the polymerisation of Heme to hemozoin **T**
d) Artemisinin is a slow acting antimalarial **F FAST ACTING**

Anticancer chemotherapy

h) Tamoxifen is a complete antagonist of oestrogen function **F**
i) Methotrexate is used to inhibit the DHFR enzyme in the folic acid synthetic pathway **T**
j) Nitrogen mustards are monofunctional alkylators **F BIFUNCTIONAL**
k) Cyclophosphamide is a drug that non-covalently intercalates into DNA **F NITROGEN MUSTARD**
l) Both the *cis* and *trans* isomers of cisplatin have antitumour activity **F ONLY CIS**
m) Doxorubicin activity leads to a change in the sedimentation of the DNA **T**
n) Vincristine has a similar function to taxol **T ANTIMITOTIC DRUGS**