ANTICANCER AGENTS

(CYTOTOXIC AGENTS)

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Overview

- Cancer is a disease in which there is uncontrolled multiplication and spread within the body of abnormal forms of the body's own cells. Such cells are characterized by 5 things:

1. Abnormal genetic content (mutations, deletions or translocations)
2. Altered chromosomal structures
3. Uncontrolled growth (proliferation)
4. Loss of differentiation & loss of function
5. Invasiveness and or metastasis

- The terms cancer, neoplasm & tumor are synonymous (can be used interchangeably)
- It is ***not*** generally true that cancer cells have a faster rate of multiplication than normal cells, the only difference concerning multiplication is their loss of processes that normally regulate cell division (e.g. contact inhibition)
- Carcinogenesis appears to be caused by the activation of specific dominant growth genes (*oncogenes*) or loss of negative feedback (*tumor suppressor genes*)

- Cancers should be seen as diseases of faulty cell signaling pathways- be it growth factors or cell death signals
- Cancer is largely a disease of later age groups, as people live longer lives (due to advances in public health & medicine), cancer is poised to be a major health issue.
- Solid tumors arising from epithelial cells are termed *carcinomas* while those arising from connective tissue are called *sarcomas*
- Cancers arising from heamopoetic tissue are called *leukemias* or *lymphomas*

- Cancers may either be benign or malignant
- Malignant cancers are characterized by 1) *loss of differentiation, 2) invasiveness & 3) ability to spread (metastasis).* Benign cancers show uncontrolled growth without above features.
- The 3 main approaches to treating established cancer include: surgical excision, irradiation & chemotherapy. The role of each depends on the type of cancer & its stage of development

- Clinically, the major problem in successful control and treatment of malignant neoplasms is that by the time malignant cells are detected, the neoplasm is already large (>10^9 cells) & often, it has metastasized
- Chemotherapy is the main treatment for only a few cancers, but is used as an adjunct to surgery or irradiation for many cancers.
- Overall approach to anticancer chemotherapy is destruction of neoplastic cells while minimizing toxicity to normal (non-neoplastic) cells

- Cancer chemotherapy compared 2 antimicrobial chemotherapy presents a difficult problem
- In biochemical terms, microorganisms are both quantitatively and qualitatively different from human cells yet cancer cells & normal cells are so similar, that it has proved difficult to find good exploitable differences between them
- Different drugs work best for different cancers and in different locations (sites) in the body
- Effectiveness of anticancer agents is greater on cells that are going through the cell cycle compared to those that are resting (G_0 phase)

- Most anticancer drugs act in the S-phase but some act in the M-phase, while others have complex actions in the cell cycle
- Of the 4 major cancer types, the faster growing hematological types are more responsive to chemotherapy due to rapid doubling times and the ease of drug distribution and access
- Solid tumors are usually well vascularized on the outside but poorly vascularized inside
- Normal cells of the hair follicle, bone marrow, intestinal epithelium are also rapidly dividing & become inhibited by cytotoxic drugs (S/effect)

Pharmacodynamics

- Basic mechanisms of anticancer drugs include:

1. Inhibition (or less effective) DNA synthesis- antimetabolites
2. Damage or disruption DNA/RNA, interfering with Topoisomerases- Alkylating agents, Intercalators and Antibiotics)
3. Interfering with transcription –steroids
4. Disruption of mitosis- alkaloids
5. Disruption of translation- Asparaginase

- Of the above mechanisms, those that directly affect the cell cycle have prominent cytotoxic side effects. Other groups (like hormones and enzymes) may be free of these adverse effects
- Cytotoxic agents include alkylating agents, antimetabolites, antibiotics & the alkaloids
- Other approaches to cancer treatment include; immunotherapy, angiogenesis inhibitors, gene therapy & the use of biological response modifiers (e.g. Interferons, heamopoetic growth factors)

ALKYLATING AGENTS

- Alkylating agents & related compounds contain chemical groups which have a property of forming covalent bonds with nucleophilic substances in the cell like DNA/RNA/Proteins
- The main step is formation of a carbonium ion which reacts instantaneously with electron donor (nucleophilic) groups like -NH_2, -OH, -SH
- Most alkylating agents are bifunctional, having two alkylating groups

- The 7 nitrogen of guanine, being strongly nucleophilic is the main molecular target, N1 & N3 of adenine, N3 of cytosine also targeted
- A bifunctional agent being able to interact with 2 groups ,can cause intrachain or interchain cross-linking, interfering with transcription & replication of nucleic acids
- Their main action occurs during replication when some parts of DNA are unpaired and more susceptible to alkylation
- The final outcome is apoptotic cell death.

- All alkylating agents depress bone marrow and gastrointestinal health. With prolonged use, depression of gametogenesis & increased risk of non-lymphocytic leukemia may occur
- Major subgroups of alkylating agents include:

1. Nitrogen mustards (cychlophosphamide, mechlorethamine, chlorambucil & melphalan)

- Cyclophosphamide is the most used agent. It is metabolized by P450 enzymes to its active form it has a pronounced effect on lymphocytes and can be used as an immunosuppressant.
- A unique side effect is hemorrhagic cystitis, this

is due to a metabolite *acrolein*. It can be improved by increased fluid intake & administration of ***mensa*** or N-acetylcysteine. These compounds interact with acrolein forming a non-toxic cmpd.

2. Nitrosoureas, include carmustine & lomustine. They readily cross the BBB and are used in brain & meningeal tumors

3. Others related agents include: dacarbazine, procarbazine cisplatin, carboplatin & busulfan

- Cisplatin has low myelotoxicity but may cause severe nephrotoxicity, nausea & vomiting. Good hydration & the use of *Ondansetron* are advised

ANTIMETABOLITES

- These are compounds that mimic structures of normal metabolic constituents like folic acid, pyrimidines or purines
- Such analogs inhibit the relevant metabolic enzymes. They are active in the S-phase
- Methotrexate, 5-fluorouracil, 6-thioguanine, 6-mercaptopurine & cytarabine are examples
- Methotrexate is selectively polyglatamated in cancer cells due to higher *polymerase activity* hence greater antimetabolite effect on cancer cells

- Methotrexate competitively inhibits the enzyme dihydrofolate reductase (just like Trimethoprim)
- This blocks regeneration of FH_4,a necessary cofactor in synthesis of purines & pyrimidines
- 5-fluorouracil is converted to 5-fluorouridylate, which in turn inhibits *thymidylate synthase* by covalent coupling to the enzyme
- Folinic acid enhances activity of 5-Fluorouracil by stabilizing its complex with enzyme, less enzyme is available to convert dUMP to dTMP
- DNA synthesis is then reduced

- Cytarabine also inhibits pyrimidine synthesis. It is converted to a *cytosine triphosphate derivative*, which is the active form incorporated into DNA. At high doses, cytarabine also inhibits *DNA polymerase* competitively
- 6-mercaptopurine & 6-thioguanine are purine analogs that must first undergo activation to form nucleotides which then act as competitive inhibitors of several enzymes in purine biosynthetic pathways

ANTIBIOTICS

- These include: Doxorubicin, Actinomycin D, Mitomycin C, Plicamycin & Bleomycin
- Doxorubicin acts by: 1) intercalation between bases in double stranded DNA, 2) inhibition of topoisomerase II, 3) generation of free radicals
- The major anticancer effect is thought to be thru inhibition of DNA topoisomerase II
- Topoisomerase II is important DNA replication by catalyzing the uncoiling & breakage of both strands of double stranded DNA

- The drug stabilizes a covalent complex of an *enzyme-DNA intermediate*, preventing the DNA strands breaks from re-joining
- Doxorubicin is the single most active agent against breast cancer
- Bleomycin forms tertiary complex oxygen & Fe (II) competent to cause nucleotide-sequence specific single & double DNA strand scission
- Plicamycin and Actinomycin D both intercalate DNA, with preference for *guanosine-cytosine base pairs*. This results in double strand DNA cleavage or inhibition of transcription

PLANT ALKALOIDS

- Vincristine & Vinblastine bind strongly to tubulin and block *microtubule polymerization* disrupting mitotic spindle formation at metaphase.
- Cell death results from inability to segregate chromosomes properly
- Etoposide is semi-synthetic, prepared from the mandrake plant (mayapple). It is used in first line regimens against lung cancer & testicular carcinoma
- Teniposide is a close analog of Etoposide used in acute leukemias in children

HORMONES

- Steroids act by passing thru' the cell membrane & binding to cytoplasmic receptors, which then enter the nucleus & interact with specific *hormone-responsive chromatin,* thus inducing synthesis of special mRNAs.
- Translation of these mRNAs leads to proteins that alter physiological or biochemical reactions in a beneficial way.
- Pathogenesis of breast cancer has a hormonal element. Treatments have included attempts to eliminate estrogen production by the adrenals,

- & blockade of estrogen receptors using anti-estrogen drugs
- *Tamoxifen* is the main anti-estrogen clinically used against breast cancer. It acts by binding to estrogen receptors and blocking estrogen dependent translation in cells in the G_1 phase.
- In prostate cancer, orchiectomy is employed along with the estrogen *diethylstilbestrol* which reduces testosterone concentration
- *Leuprolide & Goserilin*, analogs of Luteinizing hormone-releasing hormone (LHRH) act on the hypothalamus. They are both agonists as well as antagonists of this hormone

- They produce an initial rise in gonadotropins (LH & FSH), followed by decline in 2 or 3 weeks
- This results in castrate level testosterone concentrations (pharmacological castration)
- *Flutamide* is an anti-androgen that inhibits androgen binding to its nuclear receptors. This leads to a rise in testosterone levels which is ineffective as Flutamide blocks the action of testosterone at its receptors.
- Recent attempts to achieve total androgen blockade (testis & adrenal) involves concurrent use of Flutamide & LHRH analogs

OTHER AGENTS

- *L- Asparaginse* is administered to hydrolyze asparagine required for growth in tumor cells in higher concentrations than in normal cells
- This shuts down protein synthesis & eventually, nucleic acid synthesis.
- This approach is selective for those neoplastic cells devoid of *asparagine synthetase* and thus unable to synthesize essential asparagine
- *Hydroxyurea* inhibits *ribonucleotide reductase* which reduces ribonuleoside diphosphates to deoxyribonucleotides required 4 DNA synthesis

Targeting anti-tumor drugs

- Extensive side effects produced by most anti-cancer drugs on normal cells have resulted in efforts to develop procedures for targeting these drugs to tumor cells
- This is to maximize exposure of tumor cells to the drugs while minimizing exposure to normals
- Regional infusions thru' intra-arterial catheters are used where circulatory system of the tumor can be localized. Intra-cavity infusions are employed as well (e.g. peritoneal cavity)

- Monoclonal antibody-drug conjugates are being investigated for targeting drugs to tumor cells surfaces, but the heterogeneity of tumor cell surface antigens makes this approach difficult
- Encapsulation of drug in liposomes is also under investigation
- Recent approaches using magnetic cells as carriers of drug are also under investigation

Resistance

- May manifest as patients who initially respond to treatment but later the tumor may return and the same drugs are ineffective
- In others, a drug combination may show little positive result even though the same protocol has proved successful in many other patients
- Resistance may be present initially or develop after exposure to drugs
- It may be due to 1) reduced intracellular drug concentrations, 2) to repair of drug induced damage & 3) to modification of drug targets

- Methotrexate & Actinomycin D require carrier proteins for transmembrane transport- cellular uptake may become decreased
- Methotrexate resistance may also be due to increased intracellular enzyme concentrations *(DHFR)*, this is a result of gene amplification
- An altered enzyme, still biochemically active with a lower binding affinity to Methotrexate is yet another mechanism
- In another example, Methotrexate is not conjugated with polyglutamate & is therefore not retained within the tumor cell. (Remember *Methotrexate-polyglutamate* is more active)

- Cyclophosphamide requires cellular metabolic activation and in the absence of this metabolic pathway, tumor cells are resistant
- Increased activity of *aldehyde dehydrogenase* also leads to enhanced metabolism of Cyclophosphamide (due to gene amplification)
- Bleomycin resistance shows a mechanism in which cells *rapidly repair the DNA breaks* caused by the drug. Repair of covalent cross-links in DNA strands is also thought a possible mechanism of resistance to DNA directed drugs

P-glycoprotein

- Mammalian cells possess a 170,000 Dalton *phosphoglycoprotein* called P-glycoprotein
- It acts like an ATP-driven membrane associated transport protein pump that transports complex ring systems containing: hydrophobic, positively charged compounds out of the cell.
- Doxorubicin, Daunorubicin, Actinomycin D, Etoposide, Teniposide, Vincristine & Vinblastine all show resistance in cells expressing a high activity of multidrug-resistance P-glycoprotein

- Current effort to develop molecules that block activity of this pump are underway
- Verapamil & other calcium channel blockers block this pump, though at unacceptably high concentrations
- Sulfahydryl compounds including glutathione & metallothioneins act as cell protective groups *particularly against alkylating agents*. Increased concentrations of such protective compounds scavenge highly reactive compounds and are yet another mechanism of resistance
- A *decease in topoisomerase II activity* leads to resistance to Etoposide & Teniposide.

Drug interactions

- 6-mercaptopurine undergoes enzyme catalyzed metabolism with *Xanthine oxidase* as principal enzyme, Allopurinol (gout) is also metabolized by the same enzyme
- Co-administration of these drugs leads to decreased metabolism of both
- Allopurinol also lengthen Cyclophosphamide $t_{1/2}$ increasing its myelotoxicity by decreased renal elimination of its metabolites
- Methotrexate & NSAIDs compete for plasma binding as well as renal tubular excretion

Side effects & adverse effects

Tissue	Undesirable effects
Bone marrow	Leukopenia, Immunosuppression & resulting infections
	Thrombocytopenia & anemia
Gastrointestinal tract	Oral & intestinal ulcerations, diarrhea, nausea & vomiting
Hair follicles	alopecia
Gonads	Menstrual irregularities, impaired spermatogenesis
Wounds	Impaired wound healing
Fetus	Teratogenesis (especially in first trimester)

- Bleomycin pulmonary fibrosis
- Busulfan pulmonary fibrosis
- Doxorubicin cardiotoxicity
- Cisplatin nephrotoxicity & neuropathy
- Cyclophosphamide hemorrhagic cystitis
- Vincristine neurotoxicity/neuropathy
- Cytarabine cerebral damage
- Methotrexate, Vinblastine, Etoposide & 5-FU the major side effect is myelosuppression.
- Nearly all anticancer drugs have side effects which are considered by the patient as very unacceptable!

Relation of pharmacodynamics to clinical response