Antiviral therapy - General Principles

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Effect of chemical & physical agents on viruses

- Heat is the most reliable method of virus disinfection.
- Most human pathogenic viruses inactivated by exposure to 60°C for 30 mins.
- Viruses are stable at low temperatures and are routinely stored at -40 to -80°C.
- Some viruses are rapidly inactivated by drying, others survive well in a desiccated state.
- UV light inactivates viruses.
- Enveloped viruses (lipid in envelope) are inactivated by organic solvents.
- Phenols, alcohols, QACs active against viruses.
- Most active agents are chlorine, hypochlorites, iodine, aldehydes, ethylene oxide.
Antiviral Therapy – important notes

1. Virus replication is dependent to the host cells
   Antiviral must be selective for viral enzyme or protein, or inhibit virus-specific process

2. Usually antivirals inhibit replication, don’t kill virus
   Reliance on host immune response for ultimate virus elimination

3. High error rate of viral replication
   Rapid development of drug resistance
   Need to suppress virus replication rapidly and efficiently
VIRAL LIFE CYCLE

ATTACHMENT

Click after each step to view process

PENETRATION

UNCOATING

HOST FUNCTIONS

Transcription

Translation

REPICATION

ASSEMBLY (MATURATION)

RELEASE

MULTIPLICATION
Potential sites of action for antiviral agents

- **Site of action**
  - attachment to the host cell
  - uncoating of the viral genome
  - nucleic acid synthesis
  - assembly of progeny virions
  - release of virus particles from host cell

- **Drugs inhibit ongoing replication at host cell level** and
  - replication will resume on removal of drug
  - agents are not effective in elimination of non-replicating or latent virus
Attachment

1. Using agents which mimic the virus attachment protein (VAP) and bind to the cellular receptor
Attachment

2. Agents which mimic the receptor and bind to the V.A.P:
Attachment

Example: Fuzeon – anti-retroviral drug (Inhibition of gp41 mediated infusion)
1. gp120 binds to CD4 molecule
2. gp120 changes shape, peels back and binds to chemokine receptor
3. gp41 darts out and pierces cell membrane and anchors virus
4. Fusion of membranes begins
Penetration/Uncoating

- Difficult to specifically target these stages of the life cycle as relatively little is known about them
  - Pleconaril – blocks attachment & uncoating of picornaviruses (eg rhinovirus)
  - Amantadine & rimantadine - ? Block cellular membrane ion channels
- Drug treated cells unable to lower pH of the endosomal compartment – needed to allow influenza virus HA protein to fuse to the cell membrane
Pleconaril Mechanism of Action

Blocks uncoating and attachment by binding into a hydrophobic pocket within the capsid.
The entry of influenza virus into cells and amantadine

M2, this viral protein forms a channel in the membrane that actively pumps protons from the endosome into the interior of the virion. These protons lower the pH in the interior of the virion, releasing the viral RNAs from M1. In this way the RNAs can enter the nucleus.
Amantadine Hydrochloride

Pleconaril
Genome replication

- Many viruses have evolved their own specific enzymatic mechanisms to preferentially replicate virus nucleic acids at the expense of cellular molecules.
- There is often sufficient specificity in virus polymerases to provide a target for a specific antiviral agent,
- The majority of these antiviral drugs function as polymerase substrate (i.e. nucleoside/nucleotide) analogues.
- The toxicity of these drugs varies considerably from some which are well tolerated (e.g. acyclovir) to others which are highly toxic (e.g. AZT (azidothymidine)).
Antiviral Drugs
Nucleoside and Nucleotide Analogs

Guanine

Deoxyguanosine

Acyclovir

Acyclovir

Viral thymidine kinase

Acyclovir monophosphate

Cell thymidine kinase

Acyclovir diphosphate

Cell thymidine kinase

Acyclovirus triphosphate
Antiviral Drugs
Nucleoside and Nucleotide Analogs

(b) Synthesis of normal viral DNA guanine nucleotide

(c) Synthesis of false viral DNA nucleotide with acyclovir
Nucleoside analogue drugs include:

- **deoxyadenosine analogues**
  - Didanosine (ddI)(HIV)
  - Vidarabine (chemotherapy)

- **deoxycytidine analogues**
  - Cytarabine (chemotherapy)
  - Emtricitabine (FTC)(HIV)
  - Lamivudine (3TC)(HIV, hepatitis B)
  - Zalcitabine (ddC)(HIV)

- **deoxyguanosine analogues**
  - Abacavir (HIV)
  - Entecavir (hepatitis B)

- **(deoxy-)thymidine analogues**
  - Stavudine (d4T)
  - Telbivudine (hepatitis B)
  - Zidovudine (azidothymidine, or AZT)(HIV)

- **deoxyuridine analogues**
  - Idoxuridine
  - Trifluridine
Assembly/Maturation/Release

- Processes poorly understood

Prevention of assembly
- HIV protease inhibitors – see later

Prevention of release:
- Relenza and Tamiflu - neuraminidase inhibitors (active against influenza viruses)
  - prevent release of budded virus from the cell
  - drugs target the enzyme active site - a very conserved region
- Both influenza A and B are blocked
- Need to be taken very early in course of infection
- No effect on non-influenza neuraminidases
  - (different substrate specificity
- Tamiflu effective as a prophylactic agent
Neuraminidase release the assembled new influenza virus particles from cells

Virus hemagglutinin sticks new virus particle to sialic acid on cell surface
Virus cannot escape from infected cell

Neuraminidase of virus removes sialic acid from cell surface thereby releasing virus

Neuraminidase removed sialic acid
Influenza virus neuraminidase complexed with Relenza

Left: The enzyme is shown as strands. Relenza is space-filled. The single N-acetyl glucosamine residue on each chain of the dimer is shown as ball and stick. Right: The enzyme is space-filled showing the inhibitor at the active site in a cleft in the surface of the molecule.
Механизм действия Тамифлю

1. Вирус инфицирует клетку организма
2. Появляются новые частицы вируса
3. Частицы покидают клетку и она умирает. Вирус распространяется на другие клетки

За размножение вируса и проникновение в клетки “отвечает” один из 8 его белков – нейраминидаза. Этот белок одинаковый во всех штаммах гриппа

1. Вирус попадает в клетку организма
2. Составляющая Тамифлю – озльтамивира фосфат – блокирует действие белка нейраминидазы в клетке и вирус не может размножаться
3. Другая составляющая – озльтамивира карбоксилат – действует вне клетки – не дает вирусу распространяться

Действие препарата

1. Тамифлю блокирует нейраминидазу
2. Вторая составляющая препарата проникает в клетку
3. Оказывает противовирусное действие

Прием препарата

Профилактика: 1 капсула 1 раз в сутки, 7 дней
Лечение: 1 капсула 2 раза в сутки, 5 дней

Tamiflu-
neuramidase inhibitor
Zanamivir
neuraminidase inhibitor
Interferons

- Low molecular weight proteins produced by mammalian cells in response to viral infections
- 3 classes of interferon
  - interferon-\(\alpha\) (produced by lymphocytes)
  - interferon-\(\beta\) (produced by fibroblasts)
  - interferon-\(\gamma\)
- Interferons are host-species specific
- Can be produced by recombinant genetic techniques
Interferon signaling
INTERFERON + IFNR

2-5(A) synthetase expression

Active RNaseL

mRNA degradation
Inhibition of protein synthesis
Viral replication blocked

PKR expression

Active PKR

eIF-2α-P
Inhibition of protein synthesis
Viral replication blocked

Protein phosphatase 1α
ICP34.5

HSV-1

Protein synthesis
Viral replication
Interferon-α

- Renders cells resistant to wide range of viruses
- It takes several hours to develop and persists for days thereafter
- Used in treatment of
  - Chronic Hepatitis B and C infection
  - Hairy cell leukaemia
  - Kaposi’s sarcoma
- Route of administration: parenteral
- Dosage depends on the condition being treated
- Adverse effects
  - flu-like symptoms
  - depression
  - hypotension
Examples of treatment of viral infections

Principles of Therapy of HIV infection

- HIV infection is always harmful
- Ongoing HIV replication leads to progressive immune system damage and ultimately to AIDS
- True long-term disease-free survival is unusual
- The major factor limiting the ability of antiretroviral drugs to control HIV replication and delay disease progression is the development of resistance
- The selection of resistant HIV variants is limited by potent combination therapy that suppresses HIV replication below the limit of detection
- The goal of therapy is therefore the maximum achievable suppression of HIV replication
- Combination treatment is required
  - HAART – highly active anti-retroviral therapy
Actual and potential anti-retroviral targets

- Integrase inhibitors
- Reverse transcriptase inhibitors
- Protease inhibitors
- Fusion/entry inhibitors
Current antiretroviral therapy

- Nucleoside (analogue) reverse transcriptase inhibitors (NRTI)
  - competitive inhibitors of reverse transcriptase by competing with natural pyrimidines (AZT, d4T [stavudine]) or purines (ddl [didanosine]) and inhibiting chain elongation
- Non-nucleoside reverse transcriptase inhibitors (NNRTI)
  - noncompetitive inhibitors of RT by binding to a pocket near the reverse transcriptase active site, changing its conformation and so inactivating it
- Protease inhibitors
  - Competitive inhibitors - rationally designed, based on the 3D structure of HIV protease.
    - prevent proteolytic cleavage of final translation product necessary for assembly and release of virus
- Attachment prevention
  - Prevents fusion of the virus to the cell membrane
HAART (highly active anti-retroviral therapy)

Examples of approaches:

1. Protease Inhibitor with 1-3 nucleoside reverse transcriptase inhibitor (NRTI) drugs - (PI + NRTI + NRTI)

2. Double Protease Inhibitor combinations with 2 NRTI or a an NRTI + nNRTI - (PI + PI) + 2NRTI or nNRTI

3. Protease Inhibitor with 2 NRTI drugs and a non-nucleoside reverse transcriptase inhibitor (nNRTI) (PI + nNRTI + NRTI + NRTI)
Resistance to antiviral drugs

- This is often a big problem – especially with RNA viruses.
- Resistant mutants arise spontaneously (even in the absence of drug) and are selected,
  - e.g., acyclovir-resistant mutants are unable to phosphorylate the drug (TK mutants) or,
    - do not incorporate the phosphorylated drug into DNA (pol mutants).
  - Amantadine-resistant mutant influenza A viruses emerge rapidly in treated patients.
- To overcome resistance it is crucial to use drugs at sufficient concentration to completely block replication.
- The use of more than one drug, with more than one target, reduces significantly the emergence of resistant mutants.
New antiviral drugs

- Entry inhibitors -
  - Receptor interactions are very specific (- but often alternative routes for entry exist)
  - Fusion inhibitors
- Protease inhibitors -
  - Very specific if virus-encoded
    - Herpesvirus,
    - Hepatitis C virus
    - not influenza
- Nucleic acid synthesis and processing
  - RNA-dependant RNA polymerase,
  - Virus-specific helicases,
  - Herpesvirus cleavage/packaging
- Small interfering RNA (si RNA), antisense RNA, and ribozymes
- Regulatory proteins
- Virus Release / Spread
Computer-generated antiviral drugs
Conclusions

- Field of antiviral therapy has matured dramatically in past 30+ years
- Greatest progress made for
  - Herpesviruses
  - HIV
  - Respiratory viruses
  - Hepatitis viruses
- Preventive vaccination remains the key to global control of viral infections
Questions

What antiviral drugs could be used to treat patients with HIV infection? Please explain it, and put forward your idea how to design new drugs for anti-HIV infection.