ANTIVIRAL DRUGS

Looking back

• 1950 Wellcome discovers that methisazone could be used as prophylaxis against smallpox (used at the epidemic in 1963). Later the vaccine was used.

• Amanadine against influenza was licensed in 1966. Mechanism of action unknown, but was later shown to inhibit the influenza virus ion channel protein M2.

• 1960ies: nucleoside analogs previously tested on cancer cells, were tested on DNA viruses. Resulted in acyclovir for treatment of herpesvirus infections in the 1970ies. The first selective and effective an antiviral drug. Mechanism of action was worked out later.

Viruses are parasites

• Using the cellular machinery for replication.

How are antiviral drugs identified?

• Virus infected cell cultures.

• Inhibition of virus enzyme function in vitro.

• Structure based analysis of viral proteins (enzymes).

Antiviral drugs - success

- Successful development of antiviral drugs against:
  - HIV
  - herpesvirus (VZV, CMV, HSV-1, HSV-2)
  - Hepatitis B virus (HBV)
  - Hepatitis C virus (HCV)
  - influenza virus

Relatively unspecific antiviral drugs

- Interferon

- Ribavirin
Interferon

- Interferon was discovered by Isaacs and Lindenmann in 1957.
- It “interfers” with viral replication.

Interferon inhibits virus replication

IFN-alpha

- **HCV**:  
  - pegylated IFN-alpha (stable) + ribavirin.  
  - Chronic HCV infection can be cleared.
- **HPV (condylomas, warts)**:  
  - IFN-alpha  
  - “Imiquimod” a Toll-like receptor ligand that activates innate immune system.

Interferon

- **Side effects**: fatigue, myalgia, chills, fever, suppression of bone marrow, neuropsychiatric problems.
- **Not all viruses are affected** by interferon – RNA viruses are often sensitive while low or no effect is seen with DNA viruses.

Ribavirin

- Resembles riboguanisine.
- Inhibits many processes required for viral replication: nucleoside biosynthesis and mRNA capping and is hypermuthagenic.

Ribavirin

- Inhibits primarily RNA viruses.
- Has been used to treat HCV infections.
- Oral and intravenous treatment of hemorrhagic fever caused by Lassa virus.
Inhibition of specific processes during the virus replication cycle.

- Virus binding to the cell and uptake.
- Replication of viral genome.
- Processing of viral proteins.
- Often enzymes.

ANTIVIRAL DRUGS TO HERPESVIRUSES

HERPESVIRUS FAMILY

- DNA viruses.
- Use own DNA polymerases.
- Persistent infections.

HERPESVIRUS FAMILY

- Herpes simplex types 1 & 2 (HSV).
- Varicella-zoster (VZV) – chicken pox.
- Cytomegalovirus (CMV).
- Epstein-Barr virus (EBV) – “Kissing disease”.
- Human herpesvirus-6 (HHV-6). “Roseola”.

Deoxynucleoside

DNA synthesis
Acyclovir is a nucleoside analog and chain terminator

Acyclovir must be phosphorylated in the cell to become active

1) Herpesvirus thymidine kinase (tk).
2) Cellular kinases.

Acyclovir –“prodrug.” Acyclovir –active substance

Road of acyclovir to target

ACYCLOVIR summary

• Herpesvirus thymidine kinase (tk) phosphorylates acyclovir more effectively than cellular thymidine kinases.
• Chain terminator at DNA synthesis.
• Herpesvirus DNA polymerase is more effectively inhibited than cellular DNA polymerases.
• Acyclovir –prototype for polymerase inhibitors.
**ACYCLOVIR FUNCTIONS BEST ON HSV-1 AND HSV-2**

- Functions best on HSV-1 & 2, then EBV and VZV and lastly CMV, in vitro. In vivo, acyclovir is ineffective to CMV.
- Effectivity in vivo correlates with ability of these viruses tk-enzyme to phosphorylate acyclovir.

**ACYCLOVIR IMPROVEMENTS**

- Chemical modifications of acyclovir have: improved pharmacological properties, including
  - improved half-life.
  - improved gastrointestinal uptake.

**GANCICLOVIR**

- Ganciclovir is a modified version of acyclovir with activity to CMV.
- CMV particularly problematic at organ transplantations and HIV infections.
- CMV may cause pneumonia in immunosuppressed, and retinitis and gastroenteritis in AIDS patients.
- Treatment required as long as immunosuppression remains, often throughout life. Reactivation is common if treatment ceases.

**VALACYCLOVIR**

- Valacyclovir has a valine-group that causes improved uptake.
- Prodrug converted to acyclovir in liver.
- Higher levels in blood (may therefore be given orally).

**ANTIVIRAL DRUGS TO HIV**
**HIV RT inhibitors**

**Nucleoside analogs**
- Nucleoside analogs were tested on HIV and AZT was licensed as first antiviral drug to AIDS.
- Chain terminator that lacks 3'-OH.
- Oral administration. Active at 0.1-0.5ug/ml.
- A series of nucleoside analogs against HIV has been developed, e.g. ABC, ddi, 3TC, d4T, TDF, ddC.

**Resistance**
- Some mutations affect binding of RT till nucleoside analog.
- Other mutations increase the ability of RT to replace “erroneous” nucleoside analog.

**HIV RT inhibitors**

**Non-nucleoside analogs**
- Screening in infected cell cultures resulted in TIBO.
- Screening in enzymatic RT-assay resulted in NEVIRAPIN.
- These substances do NOT bind the active site in RT-enzyme.
HIV RT and non-nucleoside analogs

HIV RT inhibitor

HIV RT inhibitors

- Non-nucleoside analogs
- Resistance develops faster than to nucleoside analogs that bind the active site in RT.
- Crystallization of resistant RT facilitates “rational drug design”.

HIV protease (PR) cleaves gag/gag-pol proteins within the virion.

HIV protease inhibitors

- Peptidomimetic inhibitors of the protease.
- Pseudosubstrate consisting of a peptide that binds HIV PR but can’t be cleaved.
Protease inhibitors bind HIV protease.

HIV protease and inhibitor

HIV-1 binds CD4 receptor and CCR5 co-receptor

CCR5-antagonist

Inhibitors of HIV coreceptor binding. (CCR5 antagonists)

- Bind directly to CCR5 receptor and blocks HIV binding.
- Inhibits HIV-1 infection.
- Resistant HIV-1 virus has already been discovered.

HIV Antiviral drugs to HIV – September 2013.
- Nucleoside analogs (7).
- Nucleotide analogs (1).
- Non-nucleosidanalogs (5).
- Protease inhibitors (10).
- Fusion inhibitors (1).
- Integrase inhibitors (1).
- Coreceptor antagonists (1).

Totally 26 inhibitors.

On top of this, there are licensed combinations of these substances, so called HAART.
**HAART**

- Since the end of the 1990ies.
- Three antiviral drugs simultaneously to HIV:
  - Two nucleoside analogs.
  - One protease inhibitor.
- Some drugs cannot be combined due to toxicity.

**Nucleoside analogs to HIV RT are being used against HBV**

- In addition to retroviruses, Hepatitis B virus (HBV) also has an RT-enzyme.
- Some HIV RT inhibitors are active on HBV.
- Adefovir and lamivudine (3TC) are active towards HBV at lower doses than against HIV.
- New nucleoside analogs specific for HBV RT are under development.

**ANTIVIRAL DRUGS TO INFLUENZA**

**“Flu Five Times Deadlier Than World War”**

**Influenzavirus**

M2 – ion channel pumping in H⁺ into the virion.
Hemagglutin – binds virus receptor (sialic acid).
Neuraminidase – cuts off sialic acid from cell surface.

**How to Make Mask for Prevention of Influenza**
Influenzavirus replication cykel

H protein binds sialic acid.
N protein cleaves off sialic acid.

Influenzavirus M2 is an ion channel that pumps H⁺ into the virion

M2 protein pumps H⁺ into the virion.

Influenzavirus M2 with amantadine

Amantadine/rimantadine bind influenza A virus M2.

• Probably sterical inhibition of H⁺ import into the virion (H⁺ import is required to release RNP's from the virion).
• Effective to influenza A, but not influenza B.
• Used prophylactically.
• Single amino acid substitutions can cause resistance.

Influenzavirus neuraminidase

• Influenzavirus hemagglutinin (H) binds sialic acid on the cell surface.
• Influenzavirus neuraminidase cleaves off terminal sialic acid groups when virus is being produced.
• Cleavage of sialic acid by neuraminidase is required to prevent newly synthesized virus from sticking to the cell surface.
• Neuramindase is essential for influenza virus virulence.
**Neuraminidase inhibitors resemble sialic acid**

- Neuraminidase-inhibitor
- N-acetylneuraminic acid = sialic acid

**Neuraminidase inhibitors**
- Oseltamivir and zanamivir specifically inhibit influenza virus neuraminidase.
- Prophylactic use.
- Effective on amantadine/rimantadine resistant virus.

**ANTIVIRAL DRUGS TO HEPATITIS C VIRUS**

**Hepatitis C virus is a flavivirus**

RNA viruses

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<th>ssRNA(-)</th>
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Hepatitis C virus is a flavivirus

- 80% of primary infections are symptom free.
- 75–85% develop a chronic infection.
- 5–20% develop liver cirrhosis within 20–30 years.
- 1–5% mortality due to liver cancer or cirrhosis.

**Sustained virological response (=cure) of HCV of various interferon and ribavirin drugs**

- Cure rate: 15–45%
Hepatitis C virus mRNA is translated to a polyprotein cleaved by viral proteases

Hepatitis C virus replication cycle

Novel substances to hepatitis C virus - advanced stage in development

Antiviral treatment of HCV

Novel substances to hepatitis C virus in clinical trials.