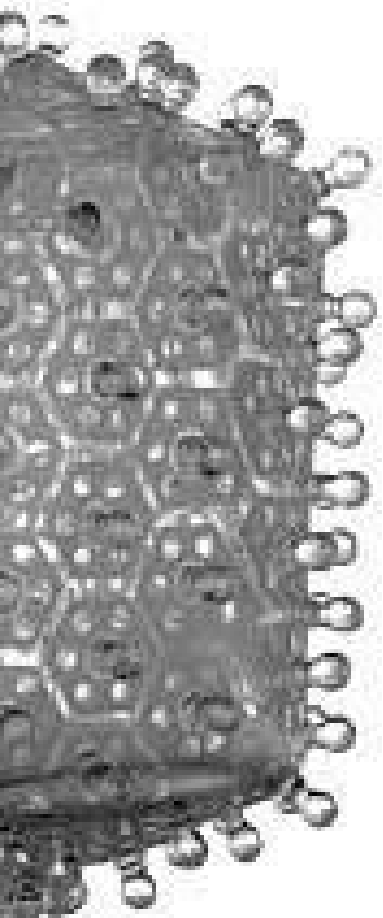

Microbiology

Lecture No: 30 (viro-10)

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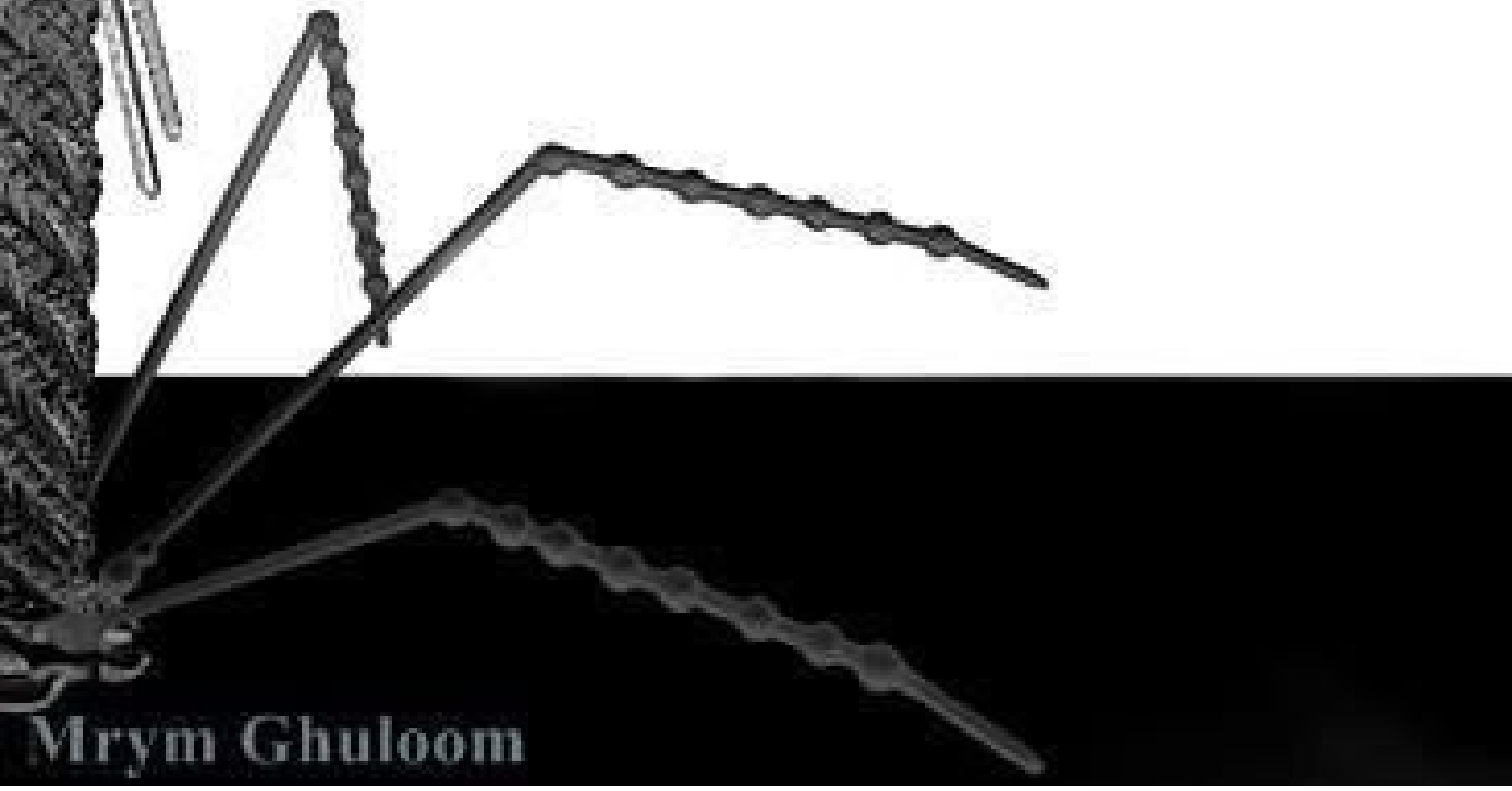
Sheet ■ Slide □



Mrym Ghuloom

بسم الله الرحمن الرحيم

Antivirals

We will take antivirals in two lectures, this is the first one.

General introduction about how they approach antivirus drugs:

First of all, you will face a patient, you have to determine whether it is an infection or not, then decide if it is: bacterial , viral, fungal or parasitic infection? How can we determine that?

From **history**, **symptoms**, **examination and diagnosis**. Once you figured out that this is a viral infection, you have to decide whether to manage it with specific antiviral or just supportive treatment. **Most** of the cases of the viral infections the treatment is supportive.

The common viral infections are **the respiratory tract infections** and we treat symptoms mainly, i.e. If there is fever we give antipyretic, if there is loss of fluids we compensate that. If it is a rhinovirus for example (which causes common cold), we don't have to specifically diagnose that this is a rhinovirus, just treat supportively and there is no need for antiviral. Now, in some serious and life threatening conditions you have to use specific antiviral to save the life of the patients in many cases .

There are about 30 types of antivirals and as you notice that the number of antivirals isn't like antibiotics, they aren't available as much as antibiotics. We have limited options and the spectrum is usually narrow.

Written by Nadira Turk

You might use broad spectrum antibiotics that cover gram negative bacteria e.g.: *E.coli*, *kelbsiella*, at the same time they cover gram positive, e.g. *staph.aureus and streptococcous*

On the other hand in viruses, you don't have antivirals that work on many types of viruses. They are specific and have narrow spectrum. Also, the options are limited for many reasons we will talk about them later.

From the options we have:

1) Anti-herpes: drugs work on Herpes viruses
2) Anti-CMV: specific category
3) Anti-influenza.
4) Ribavirin : pediatrics use them in children
5) Anti-HIV,HBV (hepatitis B) and HCV
6) Interferons

Note:

Cytomegalovirus is from the herpes family but Anti-herpes drugs don't work on it

This lecture should cover the first 4 types.

- Vaccines help a lot; they kept viral infections under control. However, we don't have vaccines for all viruses, e.g.: Rhinovirus, is a virus that causes common cold, we have more than 100 serotypes of rhinoviruses , so it is difficult to make a vaccine for each of them, especially making vaccines is very costy, but usually this virus causes mild infection.((remember that viruses can be mutated also, producing new serotypes))

- On the other side we have a vaccine against Hepatitis B, but not all people take it, that's why we need antivirals .Usually it is given to risky group of people who deal with bloody things (like syringes for example) like: doctors, nurses and the paramedical staff . However, you might find some people in community who are not vaccinated and they are infected with HBV , so they need the antiviral

How did they discover antiviral drugs?

A) Random biological screening: Chemical substrates. They use a cell culture and they found that this substrate will stop the replication of the virus.

B) X-ray crystallography: it's like the x-ray used in hospitals to examine the body, but here for viruses. It will show pharmacists some viral proteins in the three dimensional (3D) form. So once they decide the structure then they can make a certain substance to block this structure. **Example**: Neuraminidase inhibitors.

- **Selective toxicity**: there is an ultimate association between viral life cycle and the human cell; sometimes it is difficult to find a target in the virus without harming the human cell, so the antiviral must work on something that is specific to the virus.

  "Selective toxicity: is the ability of a chemical or drug to kill a microorganism without harming its host."

Sites of action for antivirals:

1) Binding to the free virus particles:

Stabilizing the virus by cross linking its structural proteins and inhibiting release of the viral genome

2) Interference with the virus attachment:

- You can imagine that the attachment is mediated by receptors, so if you block the receptor, then there will be no attachment.

✓ **Example:**

They tried to synthesize synthetic CD4"receptor for HIV" so such soluble receptors might bind HIV before attaching to the cell "actual receptors".

-Also, the fusion of this virus (HIV)to the cell has been blocked using some inhibitors called **"enfuvirtide"**, which will bind to a glycoprotein(gp41)on the surface of the virus, this glycoprotein is very

helpful for fusion of the virus to the cell , so such antiviral will inhibit the fusionto the cell.

3) Inhibition of viral uncoating :

-as you know uncoating is an important event in the virus's life cycle , as by uncoating the genetic material will be exposed to the replication mechanism within the cell, so we can make an antiviral that inhibits uncoating .

- ✓ **Example:** Amantadine blocks the (M2) channels of influenza virus and these channels have a role in uncoating (will be known later).

4) **Inhibition of viral nucleic acid replication** :

- If you have something that blocks or inhibits the replication enzymes, then there will be no replication.

✓ **Examples:**

A) Influenza RNA polymerase, if you stop this enzyme then you'll stop the life cycle of the virus.

B) Herpes thymidine kinase (TK) & DNA polymerase.

C) HIV reverse transcriptase (RT) , protease and integrase

So all of these enzymes are **specific** for viruses and are not found in human cells, therefore the antiviral will target them only.

- ❖ ***Note:*** human cells have some enzymes that can be found in viruses, but they are different in structure and parameters (like Km for example), so specificity is still there.

5) **Interference with cellular processing of viral protein** :

We talked previously that viral proteins after translation undergo processing steps like: acylation and glycosylation .This is the least good approach since human cell and viral proteins are processed in similar way. So the toxicity will be higher if we design a drug that act in this way.

6) **Interference with budding and virus maturation:**
If you prevent the virus from budding out then it will not be able to infect the neighboring cells.

✓ **Examples**: 1- inhibitors of HIV proteases: which inhibit maturation and post-maturation that are mediated by proteases.
2- Inhibitor of NA that mediates budding of influenza virus.

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Usually you use antivirals for:

1- **Treatment** "the commonest"
You should use them as earlier as possible, usually within couple of days to be effective.
Acyclovir: used to treat Herpes (cold sores) and many other infections, as early as possible
e.g.: when we have a patient with recurrent herpes around the mouth (cold sores) and varicella zoster virus ' that causes shingle الحزام الناري', we can use acyclovir .
If the symptoms passed 48 hours (dermatologists say 72 hours) > antiviral will not be effective.
So the early use is very important

2- Prophylaxis:
*We can use them for prophylaxis : Acyclovir and Penciclovir (which is the same as Acyclovir, it has the same spectrum but has longer half life and you can give it with less frequency than acyclovir (3 times a day rather than 5 times a day as in case of acyclovir) .
***Anti-Influenza drugs**: as prophylaxis in case of pandemics.
Advantage of using it despite we have a vaccine: actually prophylaxis using antiviral will provide you a very rapid protection, vaccine takes time to act as vaccine depend on the stimulation of the immune system to produce antibodies , which may need 3 months whereas antiviral will provide you protection within hours.

Disadvantage of these drugs: Once stopped the patient becomes susceptible to infection.

- Antiviral is contraindicated in pregnancy, however a pregnant woman with HIV positive infection should be given Anti-HIV (like zidovudine and lamivudine) to protect the baby from infections, as the HIV mother is immunocompromized and very prone to infections.

Prodrugs:"not active"

Chemical side chains are added to enhance the bioavailability as these side chains will be protective to the active moiety of the antiviral, so there will be better adsorption and penetration to the cells and the active drug will be released at the site of the infection in concentrations Higher than the concentrations that could be achieved when we give the active drug directly. These side chains will be cleaved by the *host* enzymes to be active.

For example:

1) Famciclovir : it's a prodrug that will be later on converted into penciclovir.
2) Valaciclovir: is a prodrug for Aciclovir. When it is given orally (Valaciclovir) ,60% of the active Aciclovir will be in the desired tissues , while if you give direct active Aciclovir then the concentration will be around 20%.

- Famciclovir is the prodrug of penciclovir , it has *two acetyl groups* and these two acetyl groups will be removed and hydrolyzed in the intestinal wall and then the molecule will be oxidized in the liver , and the active moiety will be released which is penciclovir

- Aciclovir and penciclovir (Anti-herpes drugs) are very common; they are very specific to Herpes virus

Mode of action of Aciclovir:

There are three phosphorylation steps for the Aciclovir to become effective and bind to its target. (Be careful, it is not the idea of prodrugs)

First step: is carried out by the Herpes thymidine kinase (TK) .Without this step, the other two steps which are carried out *by cellular host kinases* will not take place, this means that *selective toxicity* works here and if the cell isn't infected, the drug will not work in it . Thymidine kinase will phosphorylate Aciclovir as it is not so *accurate enzyme* .Once acyclovir becomes phosphorylated, it will not be able to come out from the cell as it is now negatively charged. So the drug accumulates only in virus-infected cells

Second & Third steps: *cellular kinases* carry out these steps, then it will become active Aciclovir **tri-phosphate** (the phosphorylated form of acyclovir), this acyclovir tri-phosphate will bind Herpes DNA polymerase and inhibits it .It has little or no effect on cellular DNA polymerase, mainly no effect as there are *structure differences* between the polymerase of us and the polymerase of the virus.

***How acyclovir tricks Herpes DNA polymerase and terminate DNA synthesis?**

It is guanosine analogue that has 5' phosphate group but doesn't have 3' OH group , it is like guanosine but with deficient 3' OH group . so *DNA polymerase* will take it to add it as usual on the 3' end of the last nucleotide in the DNA chain , now the acyclovir is connected to the DNA chain but with no 3' hydroxyl group to add further nucleotide on it , and that's how we terminate the DNA elongation , and this is indirect method for blockage.

This drug has no side effect (but sometimes it could affect the GIT and could cause nausea), it is not Toxic and considered clinically safe. For example, patients with recurrent herpes simplex have been effectively treated with daily doses for many years without side-effects.

- **Latency**: as we remember when the virus hides in another place; e.g.: Herpes simplex virus type1 which affects the area around the mouth and the dermatomes ,it will go back to the retrospectively and hid in a ganglion, it will keep its copy number to the lowest possible number and hide as integrated form or episomal form.

THESE antivirals don't detect and treat the latent status of the virus.

Penciclovir (Anti-Herpes) has similar mode of action for Aciclovir (ACV), same chemical effect and the differences are: the potency is 100 less than the Aciclovir, but has a longer half life, so it will be kept for a longer time inside the cell and by that you can give Penciclovir less frequently, i.e: instead of 5 times/day we can give it 3 times/day.

Usage:

Why do we use these anti-herpetics?

1) Treatment of herpes simplex encephalitis (acute inflammation in the brain), it's important to diagnose encephalitis as soon as possible because we may lose the patient. It might be bacterial or viral (commonly) or fungal, if the patient is immunocompormised, expect anything to be the cause.

Encephalitis: inflammation in the brain.

Meningitis : inflammation in the brain coverings.

Meningoencephalitis = both of them .

One of the viruses that cause encephalitis and you may save the life of the patient if you treat it early with Acyclovir is Herpes .

****Nice story to read , not required**☺

You diagnose it firstly as brain infection from *symptoms* like: headache, photophobia , vomiting, confusion and fatigue .so you examine the patient and clinically diagnose that it is an infection in the brain .It could be viral or bacterial, if you are 100 %sure that it is bacterial then start with antibiotics, if you are not sure give antibiotics and antivirals until you get the lab results. How to be 100 % that it is bacterial although the culture results need 2-3 days to appear ?

By CSF test: in one hour the result will be known , it will tell you that there is gram +ve or –ve as the microbiologist will see it under the microscope. If there is no bacteria , the lab needs 48 hours to make sure that there is herpes or not . so you should have a clinical sense to suspect Herpes , especially if the patient is very confused and there are personality changes and loss of orientation , and CT scan help U also. so if the cause is bacterial stop the antiviral and continue with the antibiotic , but if it is herpes ,continue with the antiviral .**Aciclovir is given IV usually 10-14 days for the patient with herpes simplex encephalitis.**

- Treatment of varciella zoster virus (VZV) for elderly and immunocompromised ((VZV) from Herpes family), you need a higher dose of Acyclovir than for HSV.

2) Prophylaxis:

*HSV-1infects the mouth and causes cold sores.

*HSV-2 infects genital areas.

- Aciclovir is given for prophylaxis for both Herpes simplex and zoster infections in bone marrow and heart transplant patients. Also, it is given to prevent the spread of virus and you can use it for a long time (years) like in cases when we want to prevent recurrent HSV1&2 ,but especially genital herpes(HSV-2)which is common in Western countries.

Anti-CMV: "remember that acyclovir doesn't work on CMV"

- We use Ganciclovir which is Acyclovir derivatives & Cidavir which is nucleoside analogue.

Foscarnet:

- It blocks the pyrophosphate binding site of Herpes DNA polymerase *directly* (not like the indirect blockage by acyclovir), however it is toxic to the kidney and to the bones, and it is used in life-threatening infections that are resistant to Aciclovir.

Anti-Influenza: we have two of them:

1) Amantadine and Rimantadine: prevent genome release:

- If you remember there are M2 channels in influenza virus, the function of this proteins actually is to pump the hydrogen (the doctor said to pump but it is more accurate to say to allow the flow of hydrogen as it is a channel) inside the virus to change the acidity, especially when the virus is inside the endosomal vesicle .If the acidity is changed using this ion channels , two proteins that are closely associated with viral RNA (structural nucleoproteins NP and the M proteins) will dissociate from the viral RNA and the RNA will move to the nucleus through pores and start to replicate .

If you stop the acidity change then the proteins will stay associated with the genome. So **Amantadine and Rimantadine** are blockers for M2 channels. They will inhibit the acidity change ,so the sub-sequence progressive steps of the infection will stop. Clinically, you can use them to treat Influenza A .

Also , they can be used to prevent the outbreak in the community (80% of population taking it). Prophylactically , you can use them when there is an Influenza infection in the community , and they are taken for at least 5 weeks or until outbreak is finished and it is highly recommended prophylaxis for some special group like elderly people who have problems in lung and heart or immunocompromised or diabetic patients.

2)Neuraminidase inhibitors :

- We have two :**oseltomivir** and **zanamivir** .

- NA normally acts at the stage of virus release from the cell (if you remember , it breaks the connection between heamagglutinin and sialic acid to allow the release of the virus) , so they will inhibit the release of the virus and the virus will aggregate at the surface of the cell .

-They are common more than Amantadine and Rimantadine ,especially when you face a new pandemic and there is no special known treatment or vaccine then you use them. (zanamivir ,tamiflu & oseltomivir)

- These antivirals are effective against influenza A and B Viruses

3) Ribavirin:

- Nucleoside analogue , you can use it in lab as it has an antiviral effect on cell culture actually.

-it has a *slightly* broad spectrum.

-used to treat Influenza ,hantavirus infection and Lassa fever "hemorrhagic fever in Africa and kills people there ".

-It is given to children as aerosolized when they have a serious infection with respiratory syncytial virus(RSV)

- Therapy of Hepatitis C involves a combination of : INF (interferons) and Ribavirin .

Side Effects:

1)Acyclovir (anti-herpes):

Some gastrointestinal side effects . however it is commonly use and generally safe with no significant side effects.

- Be careful if your patient has abnormal kidney functions or renal failure as 70% of acyclovir is excreted unchanged in urine .

2)Amantadine: Has neuro effects like :insomnia and jitteriness الأرق والعصبية, they will disappear once you stop taking it

3)Ganciclovir (anti CMV): "the doctor didn't mention them"

Neutropenia in 1/3 of patients :it means decreased number of neutrophils.

Less common; thrombocytopenia (decreased number of thrombocytes), rash and nausea.

"Thank you all ☺ I am sorry if it wasn't very nice sheet but I tried to be good one"
"Special thanks for correction team and our Lajneh :D I really appreciate their efforts"

GOOD LUCK ;)