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كلية العلوم

قسم الكيمياء الحيوية

المضادات الحيوية (BCH 476)

Antibiotics

Lecture 17-18: Mechanism of action of antibiotics

Antibiotics affecting membrane structure & function

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Lecture No.	Topics
17-18	-Antibiotics affecting membrane structure & function. (e.g.Valinomycin, gramicidinA , polymyxins, polyene antibiotics). Ionophosphorous antibiotics.

The cytoplasm of all living cells is bounded by the cytoplasmic membrane, which serves as a selective permeability barrier, carries out active transport functions, and thus controls the internal composition of the cell.

If the functional integrity of the cytoplasmic membrane is disrupted, macromolecules and ions escape from the cell, and cell damage or death ensures.

The cytoplasmic membrane of bacteria and fungi has a structure different from that of human and animal cells and can be more readily disrupted by certain agents. Consequently, selective chemotherapy is possible

- **Example:**
  - Polyenes acting on fungi.
  - Polymyxins acting on gram-negative bacteria
- Polyenes require binding to a sterol which is present in the fungal cell membrane but lacking in the bacterial cell membrane.

- Conversely, polymyxins are inactive against fungi and polyenes are inactive against bacteria a striking example of selective toxicity.

Polymyxins

The polymyxins are a family of compounds produced by *Bacillus polymyxa* and related bacteria.
Only polymyxins B and E are used therapeutically.
Polymyxin E is called colistin.
Structurally, the polymyxins are cyclic polypeptides with a long hydrophobic tail.
Mode of action: They act like cationic detergents by binding to the cell membrane and causing the leakage of essential cytoplasmic contents.
The effect is not entirely selective, and both polymyxin B and colistin exhibit considerable toxicity.

The Cytoplasmic Membrane as the Site of Antibiotic Action

- The cytoplasmic membrane of bacteria is only affected by two clinically-used antibiotics.
- These are polymyxinB and polymyxinE (colistin).
- They act by competitively replacing Mg^{2+} and Ca^{2+} from negatively charged phosphate groups on membrane lipids.
- The result is disruption of the membrane.

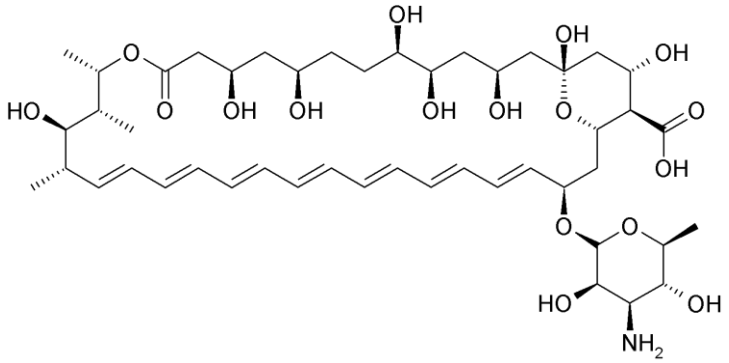
Gramicidin (anti G +ve bacterial)

- Antibiotics of the tyrothricin complex (gramicidin and tyrocidine), which are used in some topical preparations, are cyclic peptides that bind to the cell membrane and interfere with its function.
- These agents possess good activity against Gram +Ve organisms, but they also bind to mammalian cell membranes and are toxic to be used in humans.
- Toxicity also precludes the systemic use of the many disinfectants, including phenols, quaternary ammonium compounds, biguanides, and others, that achieve their antibacterial effect wholly or in part by interfering with the integrity of the cell membrane.

Ergosterol inhibitors (antifungal)

- These drugs are largely **anti-fungal** agents. Their selective target is ergosterol (characteristically fungal) which replaces cholesterol as the major sterol component in eukaryotic cell membranes.
- Two major classes of inhibitors exist.
  1. Polyenes
  2. Azoles

Polyenes (include Amphotericin B, Nystatin)



- Bind to cell membrane ergosterol causing membrane leakage & altered membrane transport
- Amphotericin is usually given by intravenous injection (i.v.) to treat systemic fungal infections.
- i.v. injection causes many unpleasant side effects
- Nystatin is used in the topical treatment of Candida vaginal & oral thrush infections

- A polyene is a molecule with multiple conjugated double bonds. A polyene antifungal is a macrocyclic polyene with a heavily hydroxylated region.
- The polyene antimycotics bind with sterols in the fungal cell membrane, principally ergosterol.
- This changes the transition temperature (Tg) of the cell membrane, thereby placing the membrane in a less fluid, more crystalline state.
- As a result, the cell's contents leak and the cell dies.
- Animal cells contain cholesterol instead of ergosterol and so they are much less susceptible.

Azoles (miconazole, clotrimazole, ketakonazole)

- They exhibit broad antifungal activity by disrupting ergosterol synthesis.
- They are used as topical agents to treat fungal skin infections and *Candida* yeast infections
- Drug resistance is being reported with increasing use.

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What are *Ionophores?*

Ionophores are agents that enter the cell membrane and change its permeability.

They make the ions move inward and outward of the cell membrane, so the ion concentrations will become equal in both sides.

How does the structure of ionophores favor its function?

- A characteristic property of all the ionophores is their ability to adopt a cyclic ring formation by concentrating all the oxygen functions at the centre of the structure where they are available for complexing mono-or divalent cations.
- Following complexing the branched alkyl groups are spread over the molecular surface, rendering the compound highly lipid soluble.
- As a result the complex can facilitate the transport of cations through cell membranes of the target organisms (protozoa and gram-positive bacteria).

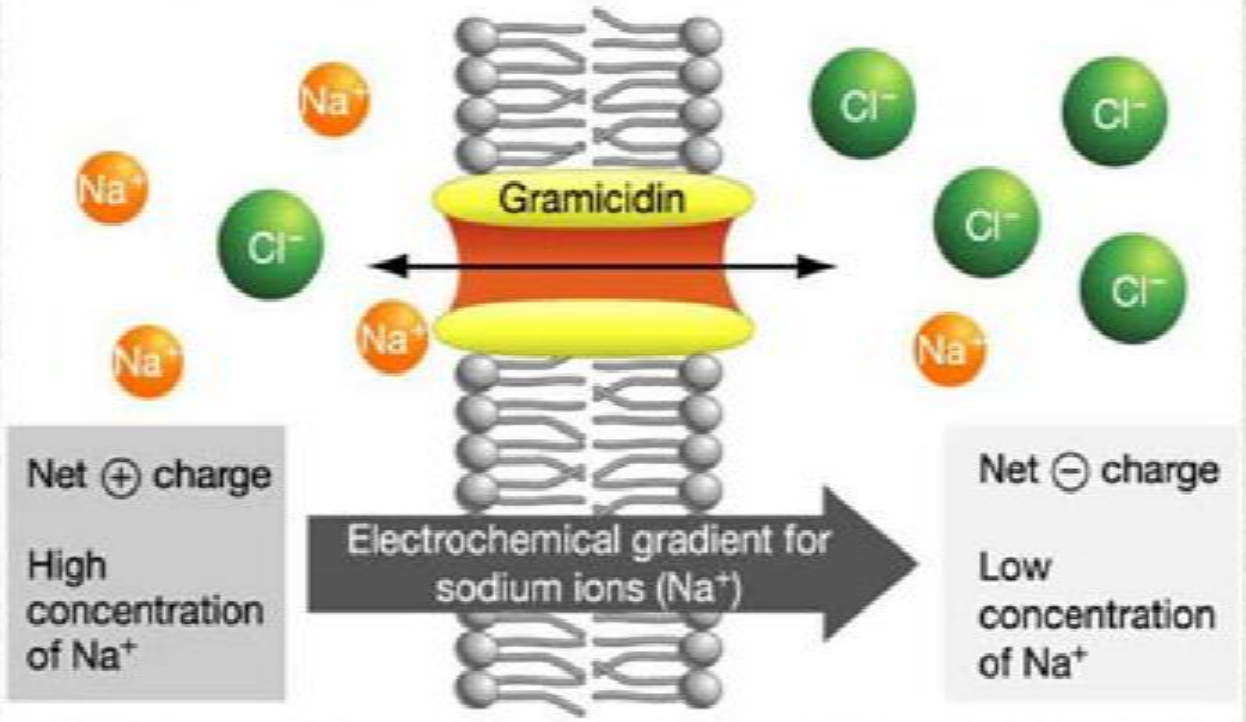
- This facilitated transport of K^+, Na^+, Ca^{2+} or Mg^{2+} across the cell membrane results in an increase in intracellular calcium to toxic levels.
- In coccidia, which do not have osmoregulatory organelles, the osmotic balance of the cell is disrupted resulting in ingress of water and vacuolisation of the cell.
- Cell death is the result of the increased intracellular pressure destroying cellular structures and eventually bursting the cell.
- In bacteria the ion transfer induced by ionophores causes a collapse in the trans-membrane electrochemical potentials.

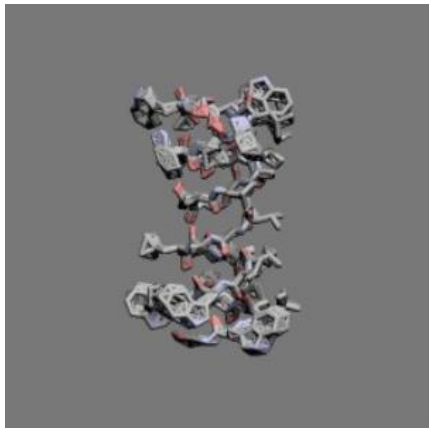
Types of Ionophores

- There are two types of ionophores:
  1)Those which make a hole in the cell membrane which permits the ions to move upon gradient of concentration: Gramicidin and Tyrotricin.
  2)Those which carry cations from one side to the other side of the cell membrane: Valinomycin and synthetic molecules such as crown ethers and cryptates.

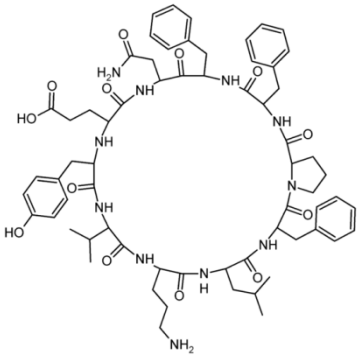
Mechanism of action of Gramicidin

- It acts as an ionophore in the bacterial cell wall and makes a hole in it
  Bactericidal.





Gramicidin as an ionophore



- Two molecules of gramicidin make a channel in the bacterial cell membrane, N-terminals meet in the middle of the membrane and C-terminals are outside it.
- Each gramicidin molecule is in the form of a left-handed helix, which results in the polar groups lining the interior of the channel. This facilitates the transfer of polar ions through the channel.
- A single gramicidin channel can allow the transport of up to 107 K^+ ions per second.

- In general, gramicidin channels are ideally selective for monovalent cations.
- Divalent cations like Ca^{2+} block the channel by binding near the mouth of the channel. So it is basically impermeable to divalent cations.
- The channel is permeable to most monovalent cations, which move through the channel in single file.
- The channel is filled with about six water molecules, almost all of which must be displaced when an ion is transported. Thus, ions moving through the gramicidin pore carry along a single file of water molecules

Carrier Ionophores

- Carrier ionophores are specific for particular ions.
- Valinomycin will transport K^+ but not Na^+ or Li^+.

- It forms an octahedral complex with six carbonyl-group oxygen atoms acting as ligands.
- The resulting complex has a hydrophobic exterior, which allows the complex to diffuse through the membrane.

- The rigid nature of the molecule coupled with its size makes the binding site of it too large to form complexes with Na^+ or Li^+.