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Pathology

Doctor 2017 | Medicine | JU

◉ Sheet ○ Slides

DONE BY

shehab

CONTRIBUTED IN THE SCIENTIFIC CORRECTION

Moh Tarek

CONTRIBUTED IN THE GRAMMATICAL CORRECTION

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DOCTOR

Manar Hajeer

In the previous lecture we discussed the accumulation of oxygen- derived free radicals as a mechanism of cell injury, we covered their production and their pathologic effects.

As we know, Free radicals are very harmful molecules so cells have developed mechanisms to remove them and thereby minimizes their injurious effects:

1 – Decay spontaneously as they are unstable molecules

2 – By **SOD (superoxide dismutase**) that converts superoxide to hydrogen peroxide (H2O2). Hydrogen peroxide is also toxic so it must be eliminated. Elimination of H2O2 is done by **catalases** (present in peroxisomes) and **glutathione peroxidases** (found in the cytoplasm). Both of them convert H2O2 into **water**.

3 – Endogenous or Exogenous anti-oxidant (e.g., Vitamins E, A, C and B-carotene)

5-Defects in Membrane Permeability

(previous 4 mechanisms are in sheet 2)

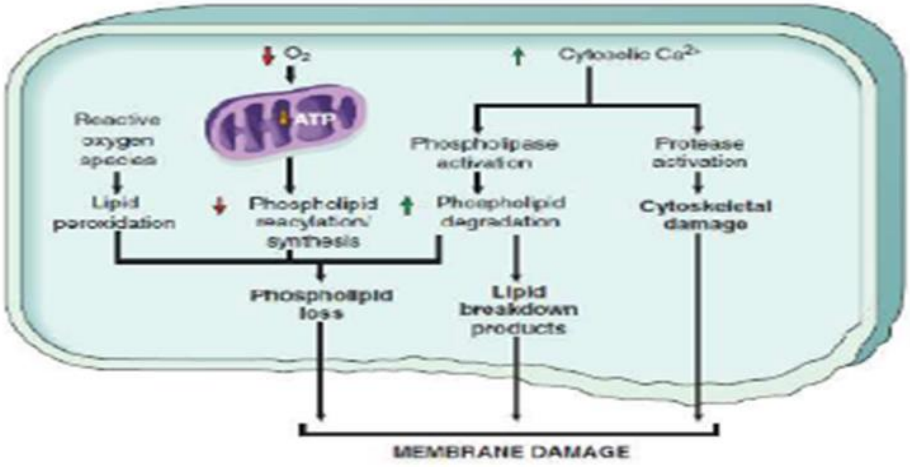
Membrane damage is a late event in cell injury caused by diverse mechanism. It leads to increased membrane permeability which causes a leakage of cell content and initiation of inflammation. The most important sites of membrane damage during cell injury are the mitochondrial membrane, plasma membrane, and membranes of lysosomes.

The membrane is composed of Proteins and phospholipids so we can say that any mechanism that affects both of these components may lead to Membrane damage. Mechanisms that leads to membrane damage:

Deficiency in oxygen supply to the cell leads to failure of many energy-dependent metabolic pathways such as decreased ATP production from the mitochondria which leads to

- decrease in the protein synthesis,
- sodium-potassium pump will fail that changes the membrane permeability
- ATP dependent calcium pump will fail leading to increased concentration of calcium in the cytosol and calcium causes activation to many enzymes such as phospholipases (degradation of phospholipid) and Proteases (degradation to protein , cytoskeletal damage).

Also**, Mitochondrial Damage** leads to production of ROS that causes lipid peroxidation.



6-DNA and Protein Damage

Exposure of cells to radiation or chemotherapeutic agents, intracellular generation of ROS may all induce DNA damage. DNA damage may be in the form Mutation, Mutated DNA leads to abnormal proteins that are unfunctional so a lot of processes in the cell will be disrupted leading to cell injury. If the damage is too severe it may trigger apoptotic death.

Misfolded proteins have similar effects, the accumulation of misfolded protein will cause a stress on the ER leading to cell injury.

Physiologically, cells have mechanisms to detect and repair damaged DNA. Any cell contain Damaged DNA will undergo apoptosis (if necrosis doesn't take place) to avoid cancer development.

Clinicopathologic correlation

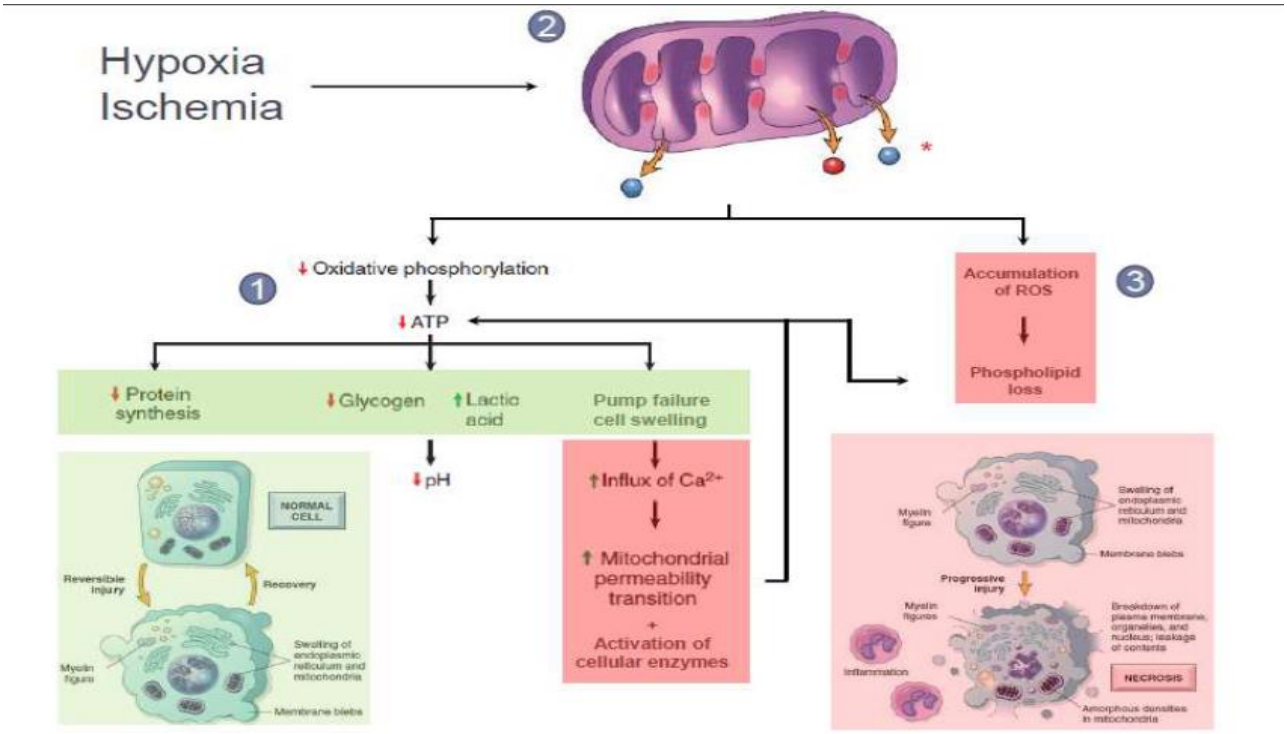
All of these mechanisms of cell injury can explain certain pathologic cases e.g:

1**- Ischemic and hypoxic injury**. Both of these cases cause deficiency of oxygen supply to the cell. Three mechanisms are involved in both:

A – Reduction of ATP generation

B – Mitochondrial damage and formation of mitochondrial permeability transition pore.

C – Accumulation of reactive oxygen species (ROS)



Ischemia injures tissue faster than hypoxia. Cellular Function will be lost long before cell death occurs (e.g., myocardial cells become noncontractile after 1 to 2 minutes of ischemia, but may not die until 30 minutes of ischemia elapsed. Striated skeletal muscle in the leg tolerates complete ischemia for 2 to 3 hours without death). During the period of reversible injury if oxygen is restored, disturbances are reversible. **10 minutes**

2- Ischemic-Reperfusion Injury

Reperfusion= restoration of blood flow. Under certain circumstances, the restoration of blood flow to ischemic tissue in reversable injury state results in increased cell injury and cell death.

This is the reverse of the expected outcome of the restoration of blood flow, which results normally in the recovery of reversible injured cells.

Several mechanisms may account for this injury including:

1 – Generation of ROS from parenchymal, endothelial cells and by infiltrating leukocytes that are carried with the blood.

2 – the influx of leukocytes and plasma proteins that may increase the inflammation. Complement proteins may bind to the injured tissues and increase inflammation.

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3 – chemical (Toxic) injury

Different types of toxins induce cell injury by two general mechanism:

A – **Direct acting toxins**: some toxins act directly by combining with a critical molecular component or cellular organelle. Cells that use, absorb, excrete, concentrate that chemical encounter the greatest damage among other cells.

Examples: mercuric chloride poisoning (occur from ingestion of contaminated seafood), mercury binds to sulfhydryl groups of various cell membrane proteins causing inhibition of ATP-dependent transport and increased membrane permeability.

Chemotherapeutic agents also induce cell damage (a feature that is being used to kill cancer cells).

B – **Indirect toxins**: Many toxins are **not active** until they are converted to another reactive metabolites, which then act on the target cells so these toxins affect cells in which they are activated. The activation usually accomplished by **cytochrome P-450** in the smooth ER of liver and other organs. The most important mechanism of cell injury involves the formation of **free radicals**.

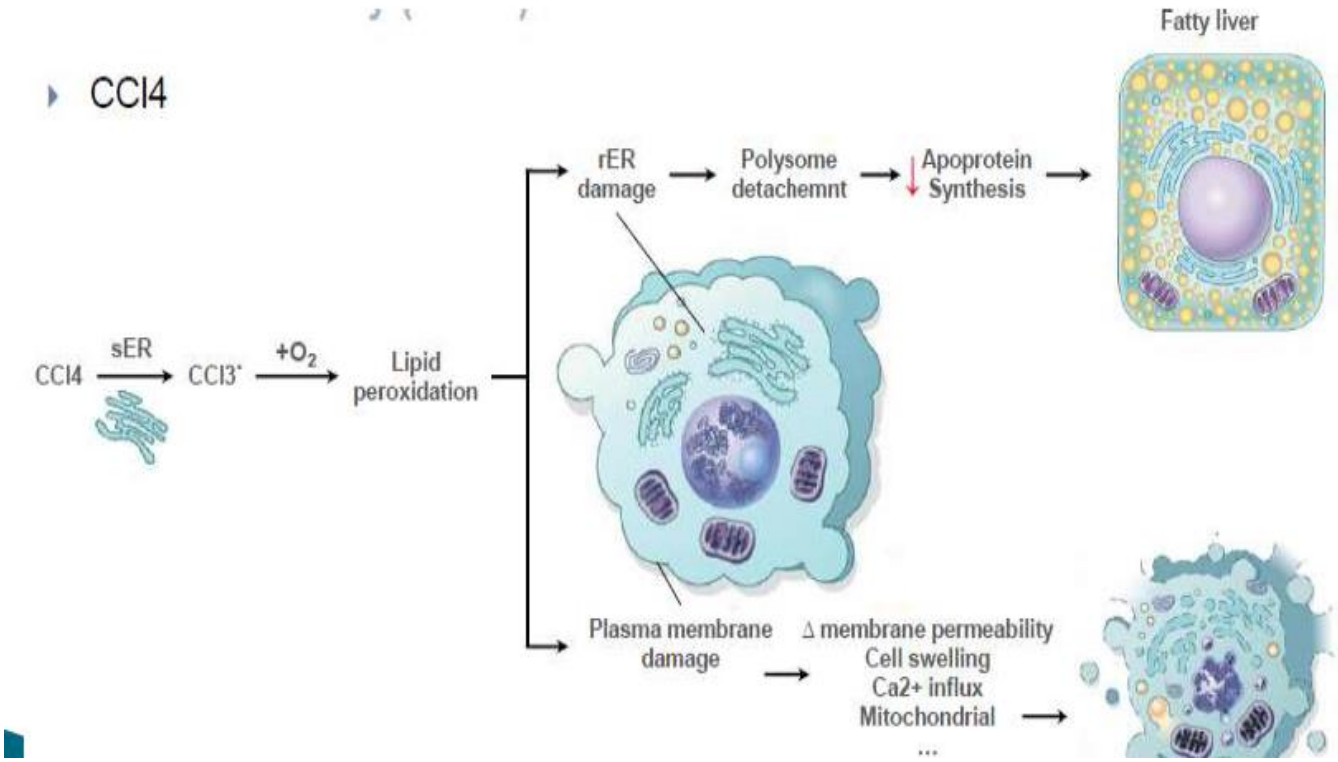
Example: **Carbon tetrachloride** (CCl_4, it was used in dry cleaning but now is banned) and **acetaminophen** (paracetamol, if it is taken in large amounts it will be toxic). Both works by the same mechanism (formation of free radicals).

Mechanism of action of CCl_4: CCl_4 is converted to a toxic free radical (CCl_3-), principally in the smooth ER in liver, and this free radical is the cause of cell injury, mainly by membrane phospholipid peroxidation in the presence of oxygen. phospholipid peroxidation causes two things:

1 - Damage to the ER membranes of hepatocytes and dissociation of ribosomes from the rER (rough endoplasmic reticulum) to cause a decline in the synthesis of enzymes and plasma proteins (detachment of ribosomes from rER will make them unfunctional)

Therefore, results in the decreased synthesis of **apoproteins** that carry lipids out side the cell, this defect results in the accumulation of lipids in hepatocytes and other cells and the **(fatty liver)** of CCl_4 poisoning.

2 – Plasma membrane damage and all related consequences



20 minutes

Apoptosis, Programmed cell death, suicide

Apoptosis is a pathway of cell death in which cells activate enzymes that degrade the cells' own nuclear DNA and nuclear and cytoplasmic proteins.

It is genetically determined and highly organized process. It ends in fragmentation of the cell into apoptotic bodies giving the name (apoptosis; Falling off). The apoptotic bodies are cleared by phagocytes. Fragments are cleared with **little** leakage of cellular contents, so apoptotic cell death doesn't elicit an inflammatory reaction.

Apoptosis VS Necrosis

Feature	Necrosis	Apoptosis
Cell size	Enlarged (swelling)	Reduced (shrinkage)
Nucleus	Pyknosis → karyorrhexis → karyolysis	Fragmentation into nucleosome-sized fragments
Plasma membrane	Disrupted	Intact; altered structure, especially orientation of lipids
Cellular contents	Enzymatic digestion; may leak out of cell	Intact; may be released in apoptotic bodies
Adjacent inflammation	Frequent	No
Physiologic or pathologic role	Invariably pathologic (culmination of irreversible cell injury)	Often physiologic means of eliminating unwanted cells; may be pathologic after some forms of cell injury, especially DNA and protein damage

However, Apoptosis and necrosis may coexist. For example: in ischemia cells undergo apoptosis at first but if ischemia is severe apoptosis may progress to necrosis. Also, in viral hepatitis necrosis and apoptosis coexist.

Causes of Apoptosis:

Apoptosis occurs in many normal situations and serves to eliminate potentially harmful cells and cells that have outlived their usefulness. It also occurs as a pathologic event when cells are damaged, especially when the damage affects the cell's DNA or proteins.

1 – Physiologic Apoptosis:

a – **Steady state population** (to maintain constant number of cells). Example; old cells in the skin die and are replaced by new ones, cells in the gut are being replaced continuously.

b – **End in function/Life** (cells that have outlived their usefulness). Example; polymorphic neutrophils (PMNs) are being eliminated at the end of immune responses by apoptosis.

c – **During embryogenesis**

d – **involution of hormone dependent tissues**. Example, Endometrium after menopause, as Estrogen level decreases cells undergo atrophy and apoptosis, the same happen to lactating breast.

e – **Self reacting lymphocytes**, these lymphocytes recognize self-antigens and could cause autoimmune disease if they were not killed by apoptosis.

30 minutes

2 – Pathologic Apoptosis: Apoptosis will eliminate cells that are damaged beyond repair

a – **Severe DNA damage**, for example after exposure to radiation (UV light) or treatment with chemotherapeutic drugs, high temperature or hypoxia.

b – The accumulation of **misfolded proteins** that causes a stress to ER triggers apoptotic death.

c – **Some infections** (adenoviruses, HIV, hepatitis viruses).

d – **Duct obstruction** causes pathologic atrophy and apoptosis. Ex: pancreas, parotid gland, kidney (all of these organs have a duct).

Mechanism of Apoptosis:

Apoptosis is highly regulated and controlled process by biochemical pathways that control the balance of death and survival signals and ultimately the activation of enzymes called **caspases**. Two distinct pathways;

- **Mitochondrial pathway** (intrinsic, as the mitochondria is found inside the cell)
- **Death receptor pathway** (extrinsic, as receptors are found on the cell surface). Both of these pathways converge to activate **caspases**.

1 – Mitochondrial pathway:

Its is responsible for apoptosis in most physiologic and pathologic conditions.

In order to understand the mechanism of action, you must know a family of proteins known as **Bcl-2** that is composed of 20 proteins and divided into 3 groups:
Antiapoptotic proteins that inhibit apoptosis such as Bcl-2 and Bcl-xl

Proapoptotic proteins that promote apoptosis such as Bak and Bax

Sensor proteins that are sensitive to stimuli such as BH3.

When cells are deprived of growth factors and survival signals, or are exposed to agents that damage DNA, or accumulate unacceptable amounts of misfolded proteins(any cause of apoptosis), BH3(sensor) is activated. The activation of BH3 leads to the activation of proapoptotic Bak and Bax. As a result, Bak and Bax dimerize, insert into the mitochondrial membrane and form channels through which **cytochrome c** and other mitochondrial proteins escape into the cytosol.

After **cytochrome c** enters the cytosol, it, together with certain cofactors, activates caspase-9. Activation of Caspase-9 leads to activation of additional caspases (Executioner caspases) which in turn activate endonucleases (Nuclear fragmentation) and breakdown of cytoskeleton then formation of apoptotic bodies.

As a summary :-

Stimuli → BH3 activation → Bak and Bax activation → Bak and Bax dimerize forming a channel → cytochrome c and other proapoptotic proteins leak to the cytoplasm → caspase-9 activation → activation of downstream caspases → apoptosis.

2 – The Death Receptor pathway:

As the name indicates, this mechanism involves activation of a receptor (found on the surface of the cell that will undergo apoptosis) by a certain ligand

The receptor → type **1 TNF receptor and Fas** . TNF (tumor necrosis factor) is a family of receptors which contain in their cytoplasmic region a death domain, when activated it mediates interactions with other proteins involved in cell death.

The ligand → Fas ligand (FasL) is a membrane protein expressed mainly on activated T lymphocytes.

When the ligand is attached to the receptor → death domain is activated → activation of caspase-8 → activation of downstream caspases → apoptosis.

The death receptor pathway is involved in the elimination of self-reactive lymphocytes and in the killing of target cells by some cytotoxic T lymphocytes.

The end result of both pathways are cellular fragments called the Apoptotic bodies that contain ligands for phagocytic cell receptors. Phagocytes will recognize these ligands and engulf the fragment and destroy it without inducing any inflammatory reactions.