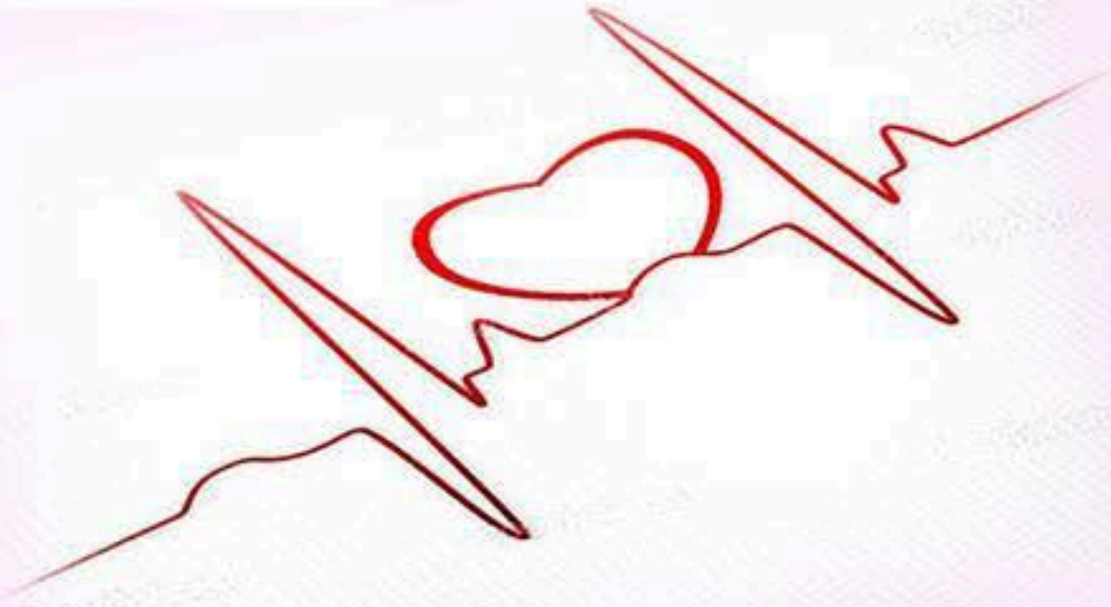
PATHOLOGY

Medical Committee
The University of Jordan

SHEET

SlIDE



Lecture Number: 3

Doctor: Mazen Al-Salhi

DONE BY: Asma Jisrawi

Designed By: Majida Al-foqaraa'

Cell Injury & Death

Causes and Mechanisms

In this lecture you will be able to understand the causes and mechanisms behind cell injury and death.

Causes of cell injury

1- **Hypoxia**

What is the difference between hypoxia and ischemia?

Hypoxia you are losing oxygen while ischemia you are losing blood supply

Ischemia is generally more severe because you are not only losing oxygen you are losing nutrients and everything else the cell requires.

Ischemia is not only from arterial damage or arterial closure, you can also get it from venous blockage, so this also blocks any solutes or toxins we need to get rid of, so they will accumulate and further injure the cell.

Examples of hypoxia → Pneumonia (and all respiratory diseases that affect oxygen transport to the blood), Anemia (less blood cells means less hemoglobin so you are not efficiently transporting oxygen from the lungs to the tissues), CO poisoning.

What gas can cause hypoxia? CO

How? CO binds hemoglobin more avidly (with more affinity) than oxygen and thus, it prevents oxygen delivery from your lungs to the tissues.

2- **Chemical agents:**

An example that we ingest every single day is glucose, too much glucose causes diabetes.

Other examples are water and salts (sodium) all these are important in the hemostatic balance in the cell, so if get too much or too little, that can cause chemical injuries to the cell.

Also, the more obvious examples are the chemicals that we get exposed to on our daily basis. They include: air pollution, asbestos (in the past), coal miners have their lungs affected.

3- **Physical agents :**
Trauma, extremes of temperature, acid exposure, radiation, electric shocks and sudden changes in atmospheric pressure.

When we are at about one thousand meter above sea level, our bodies have adapted to a slightly less amount of oxygen than somebody at zero level. So if we are now on Himalayas (one of them of course) and we have gone there immediately without oxygen tanks, our body will not have enough time to adapt - that is called physical injury

Mechanical injury if a car accident happened → trauma → physical injury

4- **Infectious agents:**
From tiny viruses, to bacteria, to meters-long worms in the intestine – everything that causes infection will cause cell injury. Either directly, or indirectly when it leads to inflammation.

5- **Immunologic reaction:**
Examples are: autoimmune reactions against one's own tissues and antigens (as in Rheumatoid arthritis – Careful! Not all rheumatism is autoimmune-related) and allergic reactions against environmental substances that normally do not react with our bodies (like strawberries and dust) but the immune system here is inappropriately activated, inflammation can result and cause cell damage.

A chronic infection that constantly activates the immune system, is also going to cause cell damage. An example is the infection that causes caseous necrosis in the lungs – which is Tuberculosis.

6- **Genetic defects:**
From obvious genetic defects as the Down syndrome, and chromosomal translocation, to the small point mutations all can cause cell injury.

7- **Nutritional defects:**
It can be divided to two major groups: deficiency and excess.

Examples → Vitamin deficiency: Scurvy is due to deficiency of vitamin C

Excess of fats (cholesterol) which causes atherosclerosis causing ischemia and cell damage.

Excess of glucose causes diabetes.

Drinking water excessively also causes cell damage.

*This seems to be only mentioned in section 2*In the case of gout, excess of proteins can lead to the accumulation of uric acid crystals from red meat or mushrooms which deposits in blood when it is super-saturated with it (usually this occurs when the temp. decreases). So it precipitates in the coldest parts of the body as in the big toe and joints of the feet because these are cold and cold temperatures reduce solubility.

8- **Aging**: Cellular senescence leads to alterations in replicative and repair abilities of individual cells and tissues. All of these changes result in a diminished ability to respond to damage and, eventually, the death of cells and of the organism.

Mechanisms of cell injury:

The cellular response to injurious stimuli depends on the type of injury, its duration, and its severity (dose of injury).

But it also depends on the cell itself (cell type) and genetic makeup and capability to adapt (adaptability) of the cell.

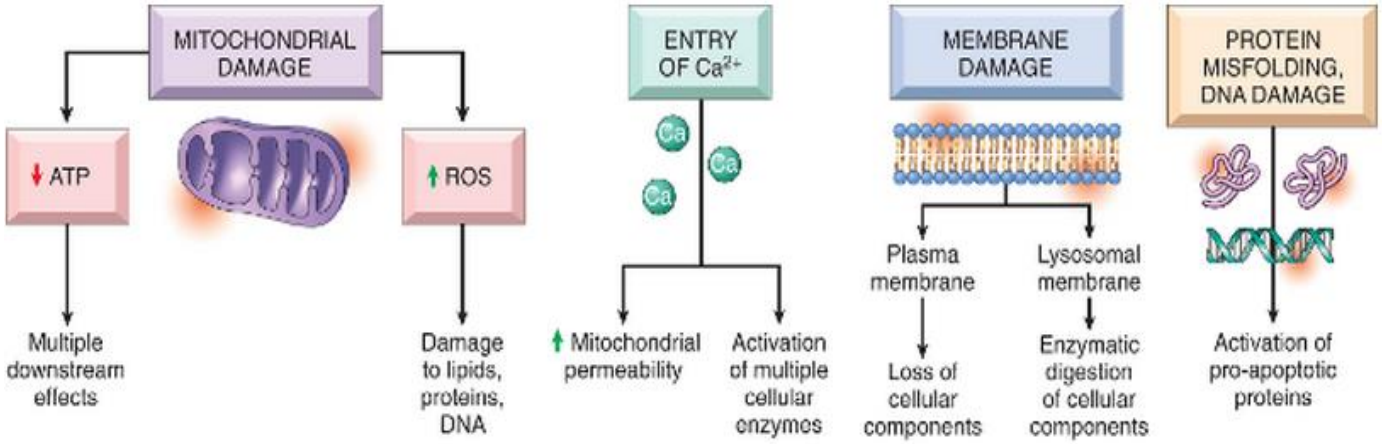
Skeletal muscles can resist ischemia longer than the heart does

Liver can resist nutritional defect longer than heart does because it stores glycogen while heart has just burned its last glucose (heart doesn't store glycogen)

Useful mutations → polymorphisms produce same protein and same function with a slight differences in function so some people have polymorphisms in their liver (cytochrome P450 system) which makes it more active, these people can get rid of their chemical injuries faster or metabolize drugs faster. However, some chemical injurious agents require to be activated by the P450 system to cause damage. So these patient have advantages and disadvantages regarding their polymorphism.

Hypertrophic heart is less capable of withstanding ischemia than a normal heart because hypertrophic heart is working much harder and is taking more oxygen and nutrients.

Mechanisms of cell injury can be divide into 4 major groups:



(1) Damage to cellular (plasma and lysosomal) membranes

(2) Damage to DNA and misfolding of proteins:

They can specifically induce apoptosis.

(3) Mitochondria and their ability to generate ATP and ROS under pathologic conditions

They produce ATP, and sometimes during their production of ATP they produce some reactive oxygen species ROS.

Mitochondria require hydrogen ions, they use the difference in concentration of hydrogen in and out to drive ATP production.

ATP reduction (depletion):

If there is a reduction in O_2 supply, or there are certain toxins or radiation that can affect electron transport chain or damage the mitochondria itself, then what will happen? There is going to be reduction in oxidative phosphorylation and so less ATP available for the cell.

In our body we use 50-75 kg of ATP a day so when ATP is reduced a lot of things will be affected.

Significant reduction (depletion) of ATP has widespread effects on many critical cellular systems:

The activity of plasma membrane ATP-dependent sodium pumps is reduced, resulting in intracellular accumulation of sodium (influx) and efflux of potassium. The net gain of solute is accompanied by iso-osmotic gain of water, causing cell swelling and dilation of the ER.

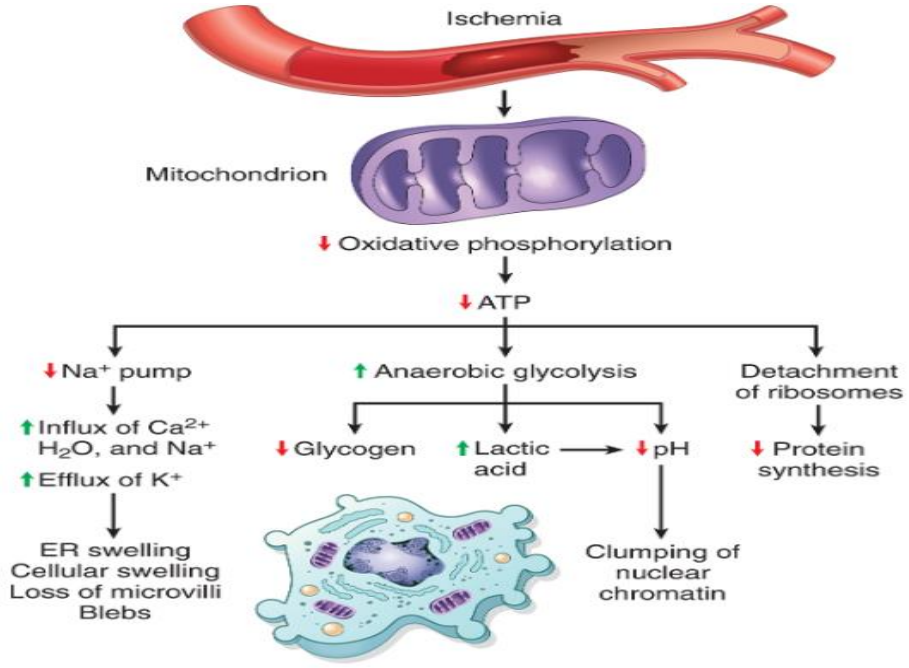
There is a compensatory increase in anaerobic glycolysis in an attempt to maintain the cell's energy sources. As a consequence, intracellular glycogen stores are rapidly reduced, and lactic acid accumulates, leading to decreased intracellular pH and decreased activity of many cellular enzymes along with clumping of nuclear chromatin.

Failure of ATP-dependent Ca2+ pumps leads to influx of Ca2+, with damaging effects on numerous cellular components, described later.

Prolonged or worsening depletion of ATP causes structural disruption of the protein synthetic apparatus, manifested as detachment of ribosomes from the rough ER (RER) and dissociation of polysomes into monosomes, with a consequent reduction in protein synthesis.

Remember: one molecule of glucose give us two molecules of ATP when only glycolysis takes place (anaerobic respiration).

Ultimately, there is irreversible damage to mitochondrial and lysosomal membranes, and the cell undergoes necrosis.



Mitochondrial damage and dysfunction

Mitochondrial damage may result in several biochemical abnormalities:

Failure of oxidative phosphorylation leads to progressive reduction of ATP, culminating in necrosis of the cell, as described earlier.

Abnormal oxidative phosphorylation also leads to the formation of reactive oxygen species, which have many deleterious effects.

Damage to mitochondria is often associated with the formation of a high-conductance channel in the mitochondrial membrane, called the mitochondrial permeability transition pore. The opening of this channel leads to the loss of mitochondrial membrane potential and pH changes, further compromising oxidative phosphorylation.

The mitochondria also contain several proteins that, when released into the cytoplasm, tell the cell there is internal injury and activate a pathway of apoptosis.

Note: necrosis and apoptosis are distinct but they can happen together in the same tissue.

Free radical and reactive oxygen species:

Free radical is missing one electron from the outer electron pairs so this means they are very unstable; they react with other molecules to gain electron pairs.

The molecule which they will react with will become a free radical and so on until two radicals react together and complement each other.

Reactive oxygen species (ROS) are a type of oxygen-derived free radical whose role in cell injury is well established. Cell injury in many circumstances involves damage by free radicals; these situations include ischemia-reperfusion, chemical and radiation injury, toxicity from oxygen and other gases, cellular aging, microbial killing by phagocytic cells, and tissue injury caused by inflammatory cells.

There are different types of ROS, and they are produced by two major pathways:

- ROS are produced normally in small amounts in all cells during the reduction-oxidation (redox) reactions that occur during mitochondrial respiration and energy generation (electron transport chain). In this process, molecular oxygen is sequentially reduced in mitochondria by the addition of four electrons to generate water. This reaction is imperfect, however, and small amounts of highly reactive but short-lived toxic intermediates are generated when oxygen is only partially reduced. These intermediates include superoxide, which is converted to hydrogen peroxide (H_2O_2) spontaneously (Hydrogen peroxide is not a free radical itself, but it is highly reactive

and highly damaging) it can transverse membranes, and it is either spontaneously degraded to water and oxygen or, in the presence of metals, such as Fe^{2+}, H_2O_2 is converted to the highly reactive hydroxy radical •OH by the Fenton reaction. Nitric oxide (NO) is another reactive free radical produced in leukocytes and other cells. It can react with H_2O_2 to form a highly reactive compound (but not a free radical) peroxynitrite, which also participates in cell injury.

- ROS are produced in phagocytic leukocytes, mainly neutrophils and macrophages, as a weapon for destroying ingested microbes and other substances during inflammation and host defense. Using NADPH and phagocyte oxidase the take two 2 oxygen species and strip away their electrons producing superoxide that is further converted into hydrogen peroxide or peroxynitrite. Or the ROS are generated in the phagosomes and phagolysosomes of leukocytes by a process that is similar to mitochondrial respiration and is called the respiratory burst (or oxidative burst). In this process, a phagosome membrane enzyme catalyzes the generation of superoxide, which is converted to H_2O_2. H_2O_2 is in turn converted to a highly reactive (but not free radical) compound hypochlorite HOCl (the major component of household bleach) by the enzyme myeloperoxidase, which is present in leukocytes.

Note: reactive oxygen species is example on free radicals

The damage caused by free radicals is determined by their rates of production and removal. When the production of ROS increases or the scavenging systems are ineffective, the result is an excess of these free radicals attacking lipids, DNA, proteins, leading to a condition called oxidative stress.

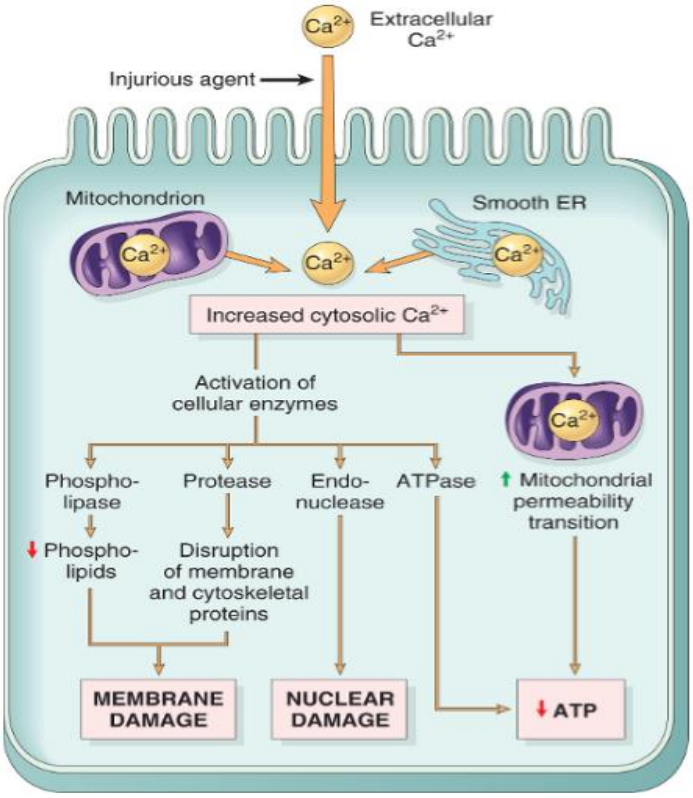
(4) Disturbance in Calcium homeostasis

Calcium concentration outside the cell is 10 thousand times higher than the inside of the cell so when Calcium enters the cell a lot of things will happen. There will be activation for certain enzymes, increasing mitochondrial permeability so it's related with ATP.

Remember: when (ase) is at the end of a word it means it is a name of an enzyme that destroys whatever the rest of word is.

They found that if we depleted extra cellular Calcium from a cell that has been injured, that cell is able to resist death longer than a cell that is flooded with Calcium. This will be important when we talk about reperfusion injury
So why maintaining the Calcium outside the cell, what will happen when Calcium enter the cell?

Increase in cytosolic Calcium concentration, initially because of release of Ca2+ from the intracellular stores, and later resulting from increased influx across the plasma membrane. Increased cytosolic Ca2+ activates a number of enzymes, with potentially deleterious cellular effects. These enzymes include phospholipases (which cause membrane damage), proteases (which break down both membrane and cytoskeletal proteins along with mitochondrial damage, this will further reduce ATP production further increasing Ca2+ influx and so on – a vicious cycle), endonucleases (which are responsible for DNA and chromatin fragmentation) which cause nuclear damage and then apoptosis, and adenosine triphosphatases (ATPases) which also reduce ATP. Increased intracellular Ca2+ levels may also induce apoptosis, by direct activation of caspases and by increasing mitochondrial permeability.



Free radicals push cell into necrosis or apoptosis. In clinical pathological state, we'll see how this leads to cell's death.

To tie everything together, we said that one of the things that happens from the ROS is lipid peroxidation, we're talking about membranes. We said that if we have hypoxia or ischemia and you have reduction in ATP production where ATP is required for building the cell, we'll also get reduction in phospholipids synthesis. Because the cell is the outer periphery, it is also going to see wear-and-tear, it will exchange phospholipids (to renew) in the membrane. Also, cytosolic Calcium, we said that when it enters the cell, it is going to activate certain enzymes like phospholipase 1 so a combination of increased phospholipid degradation, reduction of phospholipid synthesis and damage by ROS, All leads to phospholipid loss that can affect the cell's membrane and the mitochondria.

So cell's membrane that is damaged which will eventually burst, changing the solutes across the membrane, further Calcium entering the cell, since the mitochondria is already producing less ATP it will decrease the production even more which is a vicious cycle leading to further membrane and mitochondrial damage.

This is an important point to differ between reversible and irreversible injury. When you see irreversible membrane damage and mitochondrial dysfunction, then that cell is usually tipped towards necrosis by that vicious cycle. Once those two have occurred, they perpetuate themselves, when one happens, it comes back to the original injury. Cytosolic Calcium also activates proteases, which are proteins that damage proteins, either proteins in the membrane and these proteins for example are the pumps that maintain sodium and potassium and Calcium or they could be cytoskeleton elements which hold the cell in shape. All this leads to membrane damage.

Now that we've seen the underlying mechanisms, what does this mean in practice?

Take for example hypoxia ischemia, early on, the first thing that's going to stop is oxidative phosphorylation, reduction of ATP will lead to less protein synthesis and a decrease in glycogen storage with an increase in lactic fatty acids affecting the pH and heart failure occurs as well, there may be some but not massive influx of Calcium. These happen at an early stage (green=early, as shown in the book) which is *still* a reversible injury.

When there's a massive influx of Calcium, cellular proteins will be activated therefore increasing the mitochondrial permeability for transport, further reducing ATP, this is

late. It's going to perpetuate that damage that we talked about. If that damage is perpetuated because of the increase in mitochondrial permeability, you're damaging the mitochondria itself causing accumulation of reactive oxygen species and this can lead to causing phospholipids loss. So a combination of mitochondrial effects, cellular enzymes as well as phospholipids loss all feedback into reduction of ATP, that's when it becomes a vicious cycle leading towards necrosis.

The figure in the slides is from outside the book, only to show the early and late events.

All that happens in hypoxia ischemia once the blood supply was cut off. What will happen if we resupply the blood flow?

If its early on (the green phase), it's reversible.

If it got to the red, that means it's going to exasperate (irritate) the injury because oxygen and Calcium is being reintroduced, this is especially important in the cardiac muscle and in brain tissues like in strokes (perfusion injury), where reintroducing oxygen, the mitochondria is already damaged and you'll get excessive production of ROS.

These cells have a reduction in ATP and for these cells to get rid of free radicals, glutathione peroxidase, catalase or superoxide dismutase, these are ATP-dependent though they don't have ATP so their homeostatic balance is towards ROS accumulation. So you'll not only get accumulated ROS, you will also get producing more ROS, all this will lead to the damage that we talked about before.

Normally what you'd expect when you reperfuse (resupply it with blood) a tissue is this tissue regaining its function but if it's gone beyond a certain point, we're causing more damage by reperfusion. So there are certain situations where you need to stop that from happening.

Unfortunately, in ischemic tissues, complement systems are activated and so antibodies also get attached to ischemic tissues. And when reprefusing, you're bringing all these white blood cells to the tissue and when they see the accumulation of antigen they'll see this tissue as a sign of something wrong and that it needs to be fixed, leading to inflammatory cell infiltrating in the tissue and these produce HOCL, ROS thinking they're combating whatever injury/stimulus/infectious agent etc. it is eventually producing more damage.

Lastly but not least, you're reperfusion the cell with more Calcium, these cells while lacking ATP, they can't maintain their Calcium homeostasis so Calcium will come into the cell an cause all the damage that we mentioned. That's reperfusion injury.

Don't forget that in both reperfusion and ischemic injury, because we're affecting mitochondrial permeability, we can also induce apoptosis because certain molecules can come out of the cell. So depending on how severe the injury is and how directed it is to how mitochondria is affected it can be either necrosis or apoptosis or a mixture of both.

Chemical toxic injury. .

There's two types of toxic injury, direct and indirect.

Direct means that the chemical agent itself can affect a specific process in a specific cell. For example, cyanide attaches to Complex-IV Cytochrome C and prevents it from passing on the electron to the next part of the chain. What do you do in that case? You've got plenty of oxygen, you got nutrients, but you've shut down specifically the electron transport chain. So all the resulting loss of oxidative phosphorylation that we saw, all the effects from the reduction of ATP happened because of cyanide so depending on how much cyanide you have ingested or been given, many if not all of your cells will die.

Mercury chloride poisonous injury, this specifically binds the sulfhydryl group of some membrane proteins. These membrane proteins are important in maintaining membrane permeability, solutes bounded etc. So this injury will lead to a change in membrane permeability.

The difference from cyanide is that cyanide is general, affecting all tissues since all tissues produce ATP. Mercury chloride (you may get it from fesh) poisoning will only affect cells that absorb, use, excrete or concentrate that particular chemical. In this case it's specifically the kidney and the GI. So certain chemicals affect certain organs because they directly injure these organs but those organs are responsible for detoxification, concentration or getting rid of these chemicals.

For example, CCL4 (Carbon tetrachloride) was used extensively in the past in dry-cleaning as it turns out that that chemical is specifically transformed to its free radical in the sER of the liver. So this free radical in addition to oxygen can lead to microscopic lipid peroxidation and lipid peroxidation can lead to membrane damage, change in membrane permeability, cell swelling, Calcium influx, mitochondrial damage etc.

But what's specific about the damage of CCL4 to the liver is that because it's causing lipid peroxidation, it can also damage the RER (rough endoplasmic reticulum that has the ribosomes attached to it) along with the mitochondrial damage and the reduction of ATP, the polysomes and ribosomes detach and they will start producing less protein. The liver produces apoproteins, these proteins are required for exporting fat from the liver to other parts of the body that require fat for metabolism and building. These are the chylomicrons, VLDL's, LDL's and HDL's, which are the good and bad cholesterol. So the liver, either because of reduction of ATP or because of membrane damage and the detachment of ribosomes and polysomes, couldn't produce these apoproteins that will lead to accumulation of fat in the liver and we'll end up with fatty liver cells. A specific damage because of a specific chemical.

The last example, again from cytochrome p-450, acetaminophen (paracetamol), the common drug in suicide where people end up with prominent liver damage because this toxic metabolite is produced in the liver and it damages the liver specifically. This can be converted to a non-toxic metabolite which is diglutathionone which is normally found in body but we only have a certain amount of glutathione that is available in body. That is why there's only a certain amount in grams per day that a person is allowed to take. This is the maximum amount that the least of people (people with least active p-450's) can get rid of without damaging themselves and their liver.

If you find a person that has taken too much paracetamol, you can give then n-acetylcysteine that gets rid of the toxin and hopefully there won't be damage the liver so the damage won't become permanent.

SUMMARY

Mechanisms of Cell Injury

ATP depletion: failure of energy-dependent functions → reversible injury → necrosis

Mitochondrial damage: ATP depletion → failure of energy-dependent cellular functions → ultimately, necrosis; under some conditions, leakage of mitochondrial proteins that cause apoptosis.

Influx of Calcium: activation of enzymes that damage cellular components and may also trigger apoptosis

Accumulation of reactive oxygen species: covalent modification of cellular proteins, lipids, nucleic acids

Increased permeability of cellular membranes: may affect plasma membrane, lysosomal membranes, mitochondrial membranes; typically culminates in necrosis

Accumulation of damaged DNA and misfolded proteins: triggers apoptosis

The End

(((Special thanks to the magnificent Leen Younis)))