Pathology

Modified slides no. 3 V3

Writer : Karim Shawagfeh & Hebah Khraisat
corrector : Karim Shawagfeh & Hebah Khraisat
Doctor : Manar Hajeer

MECHANISMS OF CELL INJURY

cell injury and adaptations

Manar Hajeer, MD, FRCPath
University of Jordan , school of medicine

MECHANISMS OF CELL INJURY

Principles

- The cellular response to injury depends on:
  - type of injury
  - duration
  - severity
- The consequences of injury also depend on:
  - type,
  - status,
  - adaptability, and genetic makeup of the injured cell (precision medicine concept)
- Cell injury results from functional and biochemical abnormalities in one or more of several essential cellular components.
- Same injury may trigger more than one mechanism.

Check the next slide for examples on each point specified with their respective highlighter color.

- ischemic injury (cutting the blood supply of an organ which leads to lack of oxygen/nutrients reaching it) is more severe than an insect bite (causes artery carrier rxn) since it leads to immediate cell injury

- Cardiac ischemia for 2 minutes leads to reversible cell injury (since it's in need of continuous O2 supply) however if the ischemia goes on for 30 minutes it may lead to myocardial infarction (necrosis)

- atherosclerosis by 70% reduction of the blood flow leading to chronic ischemia vs total occlusion by thrombosis /90% atherosclerosis (or extremely high atherosclerosis)is more severe

- ischemia to the cardiac muscle would lead to myocardial infarction within 30 mins (very sensitive to lack of o2) whereas it would take 3 hours of ischemia at the lower limb muscles to lead to infarction

- ischemia on a previously healthy tissue would face less consequences than the already compromised tissue (previous ischemia to the tissue aka chronic ischemia). "Remember that things aren't 2 =1 +1 there are exceptions so in the case of an elderly with chronic ischemia, although her or his cardiac muscle is compromised, his body adapted and for a collateral circulation and for new blood vessels to form so he can withstand ischemia more than a young man that doesn't have collateral circulation so, it depends on many things.

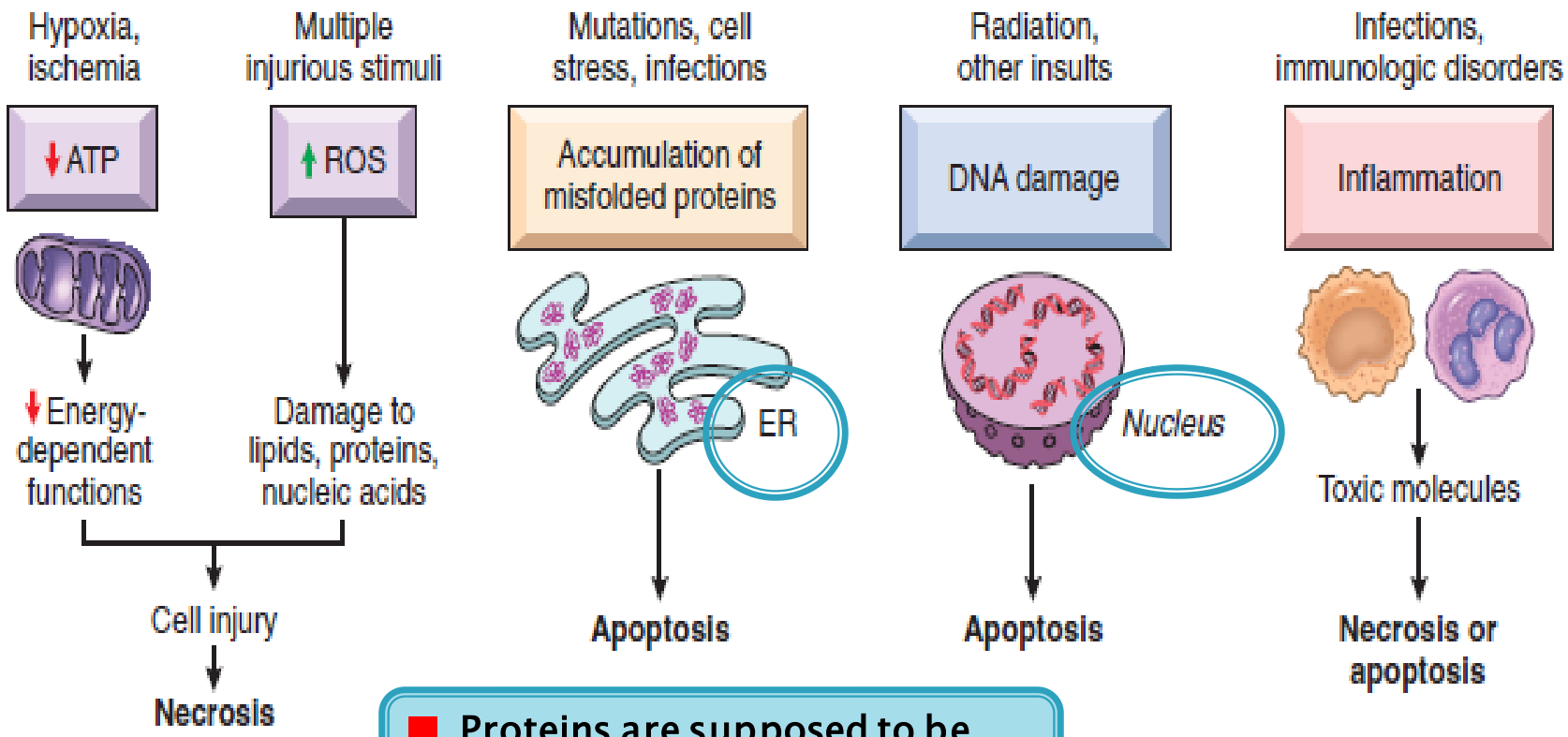
- cardiac muscles/ brain/epithelium of organ (eg: GI tract) they can adapt way less than for example skeletal muscle to hypoxia or ischemia; because when you are sitting you are not using your legs, but your brain is continuously working meaning that it depends on O2 all the time.

- Additional information: The effect of ischemia isn't correlated to the regeneration ability of the cell (the GI tract is able to regenerate but it is effected more than the skeletal muscles that aren't regenerative, the GIT dies immediately), It is more related to the O2 supply need so, any epithilial tissue is highly sensitive to ischemia.

The principal biochemical mechanisms and sites of damage in cell injury

- All different mechanism can act at the same time

- We need O2 in the mitochondria for oxidation reduction reactions in order to produce energy which is needed for Na/K pumps, protein and lipid synthesis, and transport so that is why hypoxia and ischemia is the most important mechanism



- Proteins are supposed to be folded well to function

Hypoxia and Ischemia

- One of the most frequent causes of injury.
- Defective oxidative phosphorylation >>Failure of ATP generation>>>depletion of ATP in cells
- Failure of energy dependent pathways (membrane transport, protein synthesis, lipogenesis and phospholipid turnover)
- Anaerobic glycolysis.
- Liver cells and skeletal muscle cells Vs brain and heart.

- The cell becomes nonfunctional (even if it's still alive)
- One of the most important transport is Na/K pump and a non-functional pump will lead to accumulation of Na inside the cell and attracting water causing swelling.
- The anaerobic glycolysis happens in most cells (eg. Skeletal muscles and liver cells) but not all (such as heart or brain). That is NOTED through the difference in time that leads the ischemic cell to necrosis
- Also anaerobic glycolysis leads to acidosis

Hypoxia effects:

- Reduced activity of membrane ATP dependent sodium pumps>> cell swelling
- Lactic acid accumulation >> decreased PH>> failure of enzymes.
- Disruption of the ribosomes>> decreased protein synthesis.
- Accumulation of ROS
- Damage to mitochondrial and lysosomal membranes.
- Necrosis is the end result.
- Apoptosis can contribute.

protein denaturation

- Because of detachment of ribosomes from ER

- Main
- Sometimes

Ischemia-Reperfusion Injury

- Mostly happens in brain and heart tissue (O2 demanding)

- Paradoxical cell injury after restoration of blood flow to ischemic but viable tissues.
- After myocardial and cerebral ischemia.

- Restoration of blood supply through medications (like: Tissue plasminogen activator or thrombolytic drugs) /endogenous lysis, we expect the body to react well BUT the body might end up with an ischemic reperfusion injury

- Main mechanism is:
- **Increased generation of ROS from:**
- Injured cells with damaged mitochondria & defective antioxidant mechanisms.
- Infiltrating new leukocytes.

- The cell already has ROS and the restored blood has O2

- **Inflammation induced by influx of leukocytes, plasma proteins and complement**

- Also has ROS so the damage is tripled

Oxidative Stress

- Cellular abnormalities induced by ROS (free radicals)
- Chemical species with single unpaired electron (extremely unstable)

So it has high energy

- **ROS generated in:**
- Chemical injury (CCL4)
- Radiation injury (UV, Xray)
- Hypoxia
- Cellular aging
- Inflammation
- Ischemia-reperfusion injury.

Every ROS catalyzes the activation of another ROS in a chain rxn that leads to severe damage

Generation and Removal of Reactive Oxygen Species

Normally, in redox rxns O2 becomes water, and we get ATP, during this process small amounts of ROS are produced that are short lived, so the damage isn't obvious.

- **1-Normally produced in small amounts in all cells during the redox reactions.**
- Oxygen is reduced to produce water.
- Small amounts of highly reactive but short-lived toxic intermediates are generated.
- Superoxide ($O_2^{\bullet}$), hydrogen peroxide (H_2O_2), hydroxyl radical $^{\bullet}OH$.

highly damaging

- **2-Produced in phagocytic leukocytes (neutrophils and macrophages) during inflammation.**
- In phagosomes and phagolysosomes to kill microbes.
- O2 >> superoxide >> H2O2 >> hypochlorite.
- Myeloperoxidase (H2O2 into hypochlorite).

It is a damaging substant that's why it was used in bleaching, and it has a killing effect on bacteria.

The reaction is catalyzed by myeloperoxidase enzyme that is found in macrophages and neutrophils

Removal of free radicals

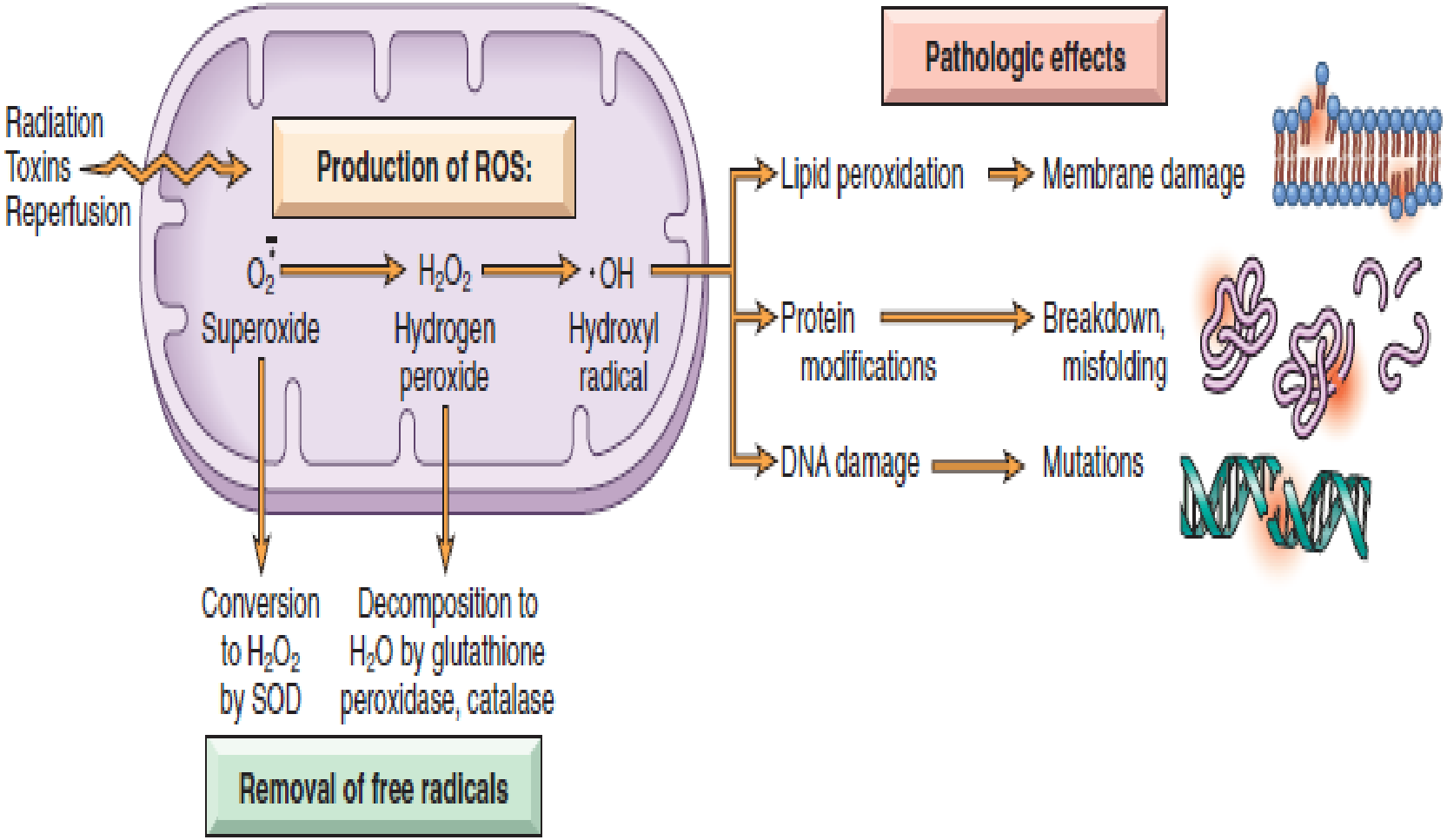
- Decay spontaneously
- Superoxide dismutase (SOD).
- Glutathione (GSH) peroxidases.
- Catalase (one of most active enzymes known)

transforms superoxide to H_2O_2

Both glutathione and catalase transform H_2O_2 into water but remember that H_2O_2 is sometimes transformed to hypochlorite by myeloperoxidase in macrophages and neutrophils.

- Endogenous or exogenous anti-oxidants (e.g., vitamins E, A, and C and β-carotene)

The food that contains these substances is important to protect our bodies (against cancer for example).



- Whether the lipids are in Plasma membranes or Organelle membranes so ROS can lead to membrane damage
- Misfolding of proteins could cause, as we said before, the cell to go into Apoptosis
- Mutations in the DNA include its degradation
- These are the three main, vital pathological effects of ROS.

Effects or ROS:

- Damage to Lysosomal & Mitochondrial membranes leads to their enzymes leaking from their respective organelles, causing cell injury especially in Lysosomes.

- **1-Lipid peroxidation of membranes.**
- (plasma, lysosomal & mitochondrial membranes)
- **2-Crosslinking and other changes in proteins.**
- (degradation, fragmentation, loss of enzymatic activity & misfolding).
- "Inactivation of enzymes"
- **3-DNA damage.**
- Single strand breaks, mediate: apoptosis, aging, malignant transformation
- **4-Killing of microbes.**

Cell Injury Caused by Toxins

- Environmental chemicals & substances produced by infectious pathogens.

- **Direct-acting toxins**
- **Latent toxins.**

- Any environmental chemical/substance that could damage the tissue is called a "Toxin"

- Direct-acting: the substance by itself goes to the cell and causes damage directly to it
- Indirect/Latent: The substance is converted into an active substance (within the liver mostly, by Cyt P450), which goes on to cause damage to cells

Direct-acting toxins

- Act directly by combining with a critical molecular component or cellular organelle.

- **Mercuric chloride poisoning**
- Contaminated seafood
- Mercury binds to sulfhydryl groups of membrane proteins>>inhibit ATP-dependent transport and increase permeability.
  - Cell swelling
- **Chemotherapeutic agents**
- **Toxins from microorganisms.**

Latent toxins

- Not intrinsically active
- Must be converted to reactive metabolites, then act on target cells.
- Via cytochrome P-450 in SER of the liver.
- Damage mainly by formation of free radicals>>membrane phospholipid peroxidation.

- **CCl4 and acetaminophen**.
- Membrane peroxidation>>>>damage
- ER membranes >> detachment of ribosomes>>decline in synthesis of enzymes and proteins +decreased synthesis of apoproteins >> fatty liver
- Mitochondrial membranes>> decreased ATP >> cell swelling >> cell death.

And other effects (protein misfolding, DNA damage), but mainly membrane peroxidation

Acetaminophin is the old name for Paracetamol. It has Hepato-toxic effects, but then it'd convert in the liver into it's more reactive form.

CCL4 and Acetaminophen are converted into ROS which exert damage in their respective methods.

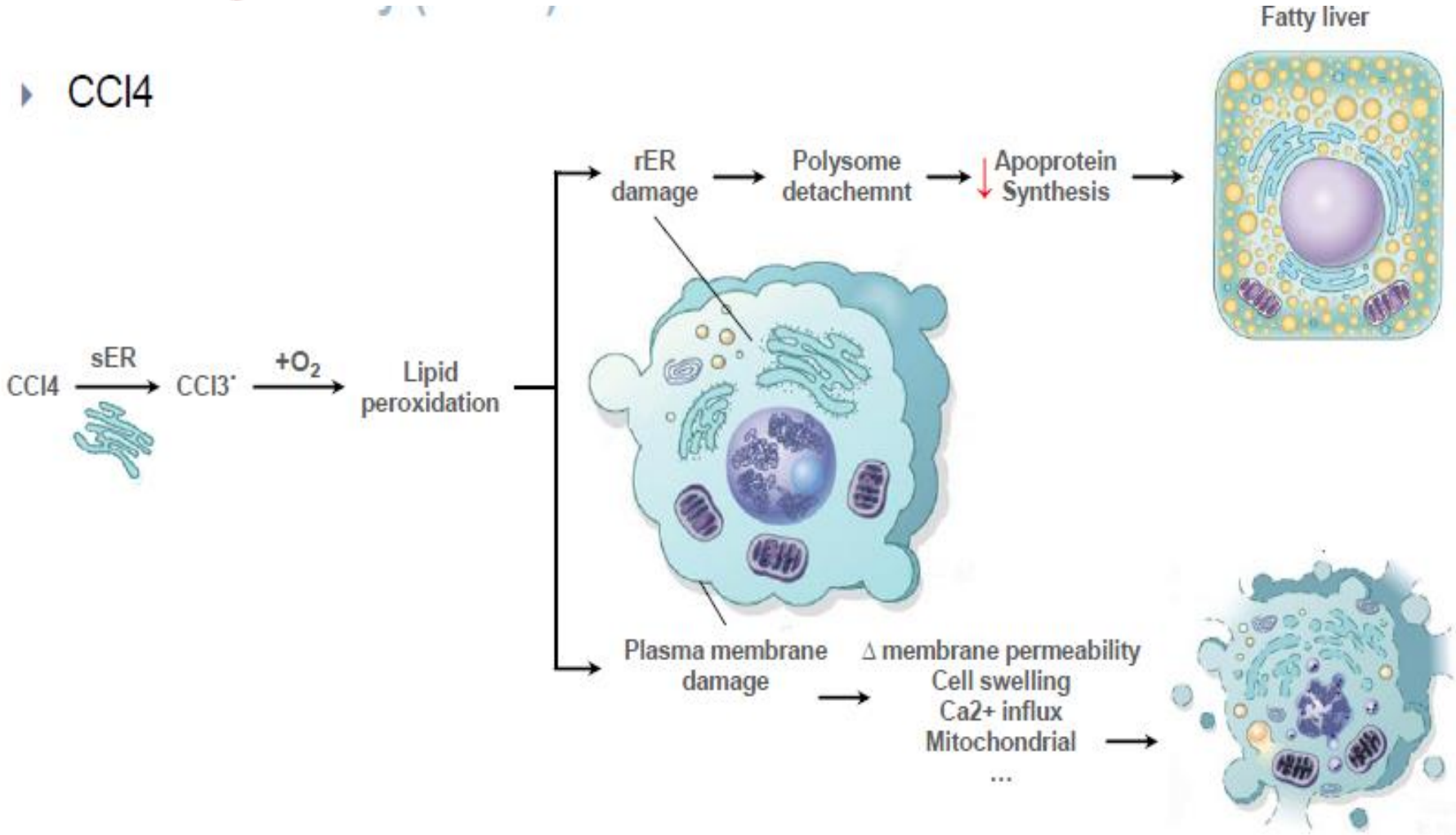
- The liver is a key organ in regards to fat synthesis and distribution. For the fat to be excreted from the liver to the circulation, it needs to bind apoprotein to form lipoproteins, but when there is decreased synthesis of those proteins due to lipid peroxidation of ER (which causes detachment of ribosomes), fat accumulates within the liver, causing the manifestation of acute fatty liver.

- Both CCl4 and Acetaminophen are commonly used for suicidal attempts.

  Additional information: A third of 54,863 intentional overdoses presenting to the five Emergency Departments in the UK involved paracetamol without other drugs (N~ 18,011). This problem is more common in females and younger patients, all of which posit necessary reconsideration to restriction in prescription package size in countries and, more importantly, the mass implementation of national awareness against suicide.

CCL4 toxicity

- Both CCL4 and Acetaminophen have the same mechanism, they are first converted into an active metabolite within the SER of the hepatocytes. In the case of CCl4, it becomes CCl3* with an unpaired electron(free radical), it reacts ds with oxygen to begin its effects, mainly Lipid peroxidation affecting the rER's protein synthesis and plasma membrane damage which affects the ATP-dependant pathways (Ion exchange, causing cell swelling) and other organelle functions such as the mitochondria's



Endoplasmic Reticulum Stress

Due to accumulation of misfolded proteins

As we mentioned earlier, all proteins need to be folded by chaperons found in ER to function properly

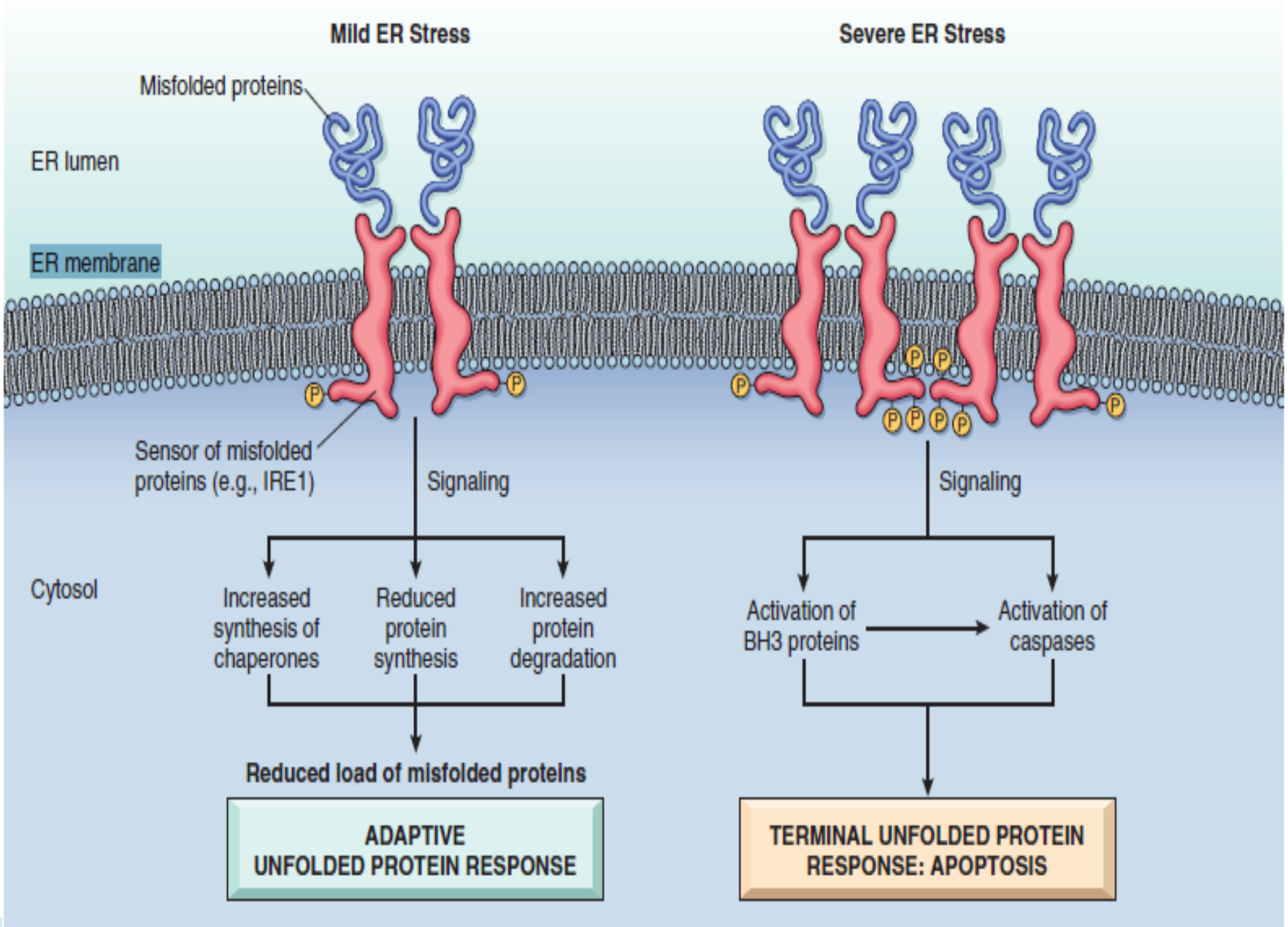
- Chaperones in ER control proper protein folding
- Misfolded proteins >> ubiquinated >> targeted to proteolysis

Once the cell senses the accumulation of misfolded proteins, it starts this response:

- **Unfolded protein response (adaptive response):** increase chaperones production, decrease protein translation and increase destruction.
- If failed >> proapoptotic sensor activation (BH3-only family) + direct activation of caspases >> **apoptosis by the mitochondrial pathway.**

"Caspases" are the enzymes of apoptosis

- This illustration sums up the previous slide



Causes of misfolding

- Gene mutations
- Aging (decreased capacity to correct misfolding)
- Infections, especially viral infections (microbial proteins)
- Increased demand for secretory proteins such as insulin in insulin-resistant states
- Changes in intracellular pH
- Neurodegenerative diseases
- Deprivation of glucose and oxygen in ischemia and hypoxia.

Such as Alzheimer's

Type 2 diabetes. Increased demand over production of Insulin is met with pancreatic cells being unable to fold them quickly enough, which causes accumulation-> apoptosis

Protein misfolding causes disease by:

- **Deficiency of an essential protein due to degradation**
- Cystic fibrosis
- **Inducing apoptosis of the affected cells**
- Neurodegenerative disorders (Alzheimer disease, Huntington disease & Parkinson disease), type 2 diabetes and prions disease.
- **Inducing both:**
- Alpha 1 antitrypsin deficiency.
- **Improperly folded proteins accumulation in extracellular tissues**
- Amyloidosis

Type 2 Diabetes affects pancreatic cells as we mentioned. Neurodegenerative disorders are related to Neurons, which is why Alzheimer's patient's brains become atrophied due to the neuronal apoptosis.

DNA Damage

- Radiation
- Chemotherapeutic agents
- Intracellular generation of ROS
- Mutations

Uv light exposure/ xray/radiotherapy

- DNA damage >> p53 activation >>arrest cell cycle at G1 phase for repair >> if repair is impossible >> apoptosis.
- In P53 mutations >> mutated cells replicate >> neoplastic change.

"Cancer"

Inflammation

- Pathogens
- Necrotic cells,
- Dysregulated immune responses (autoimmune diseases and allergies)

- Inflammatory cells (neutrophils, macrophages, lymphocytes) secrete products that destroy microbes and damage host tissues.

These cause an inflammatory reaction which recruits inflammatory cells that produce products (lysosomal enzymes, toxic substances) which cause destruction to both the offending agent and the host's tissue

Common Events in Cell Injury From Diverse Causes

- Mitochondrial Dysfunction
- Defects in Membrane Permeability

These two events are the main causes of cellular swelling and death.

Mitochondrial Dysfunction

- Energy factory
- Hypoxia, toxins, radiation.
- In necrosis and apoptosis.

- because the mitochondria is an energy factory, it needs oxygen, so any agent that causes Hypoxia through toxins or radiation is bound to affect its function, eventually leading to either necrosis or apoptosis

- **Consequences:**
- Failure of oxidative phosphorylation, ATP depletion.
- Abnormal oxidative phosphorylation, formation of ROS
- Mitochondrial permeability transition pores, loss of membrane potential.
- Release of cytochrome c >> apoptosis

- No ATP-> No cellular transport, No protein synthesis.

- Defects in those mitochondrial permeability transition pores causes the release of cytochrome C

Vital(all mechanisms lead to)

Mitochondrial Damage and Dysfunction

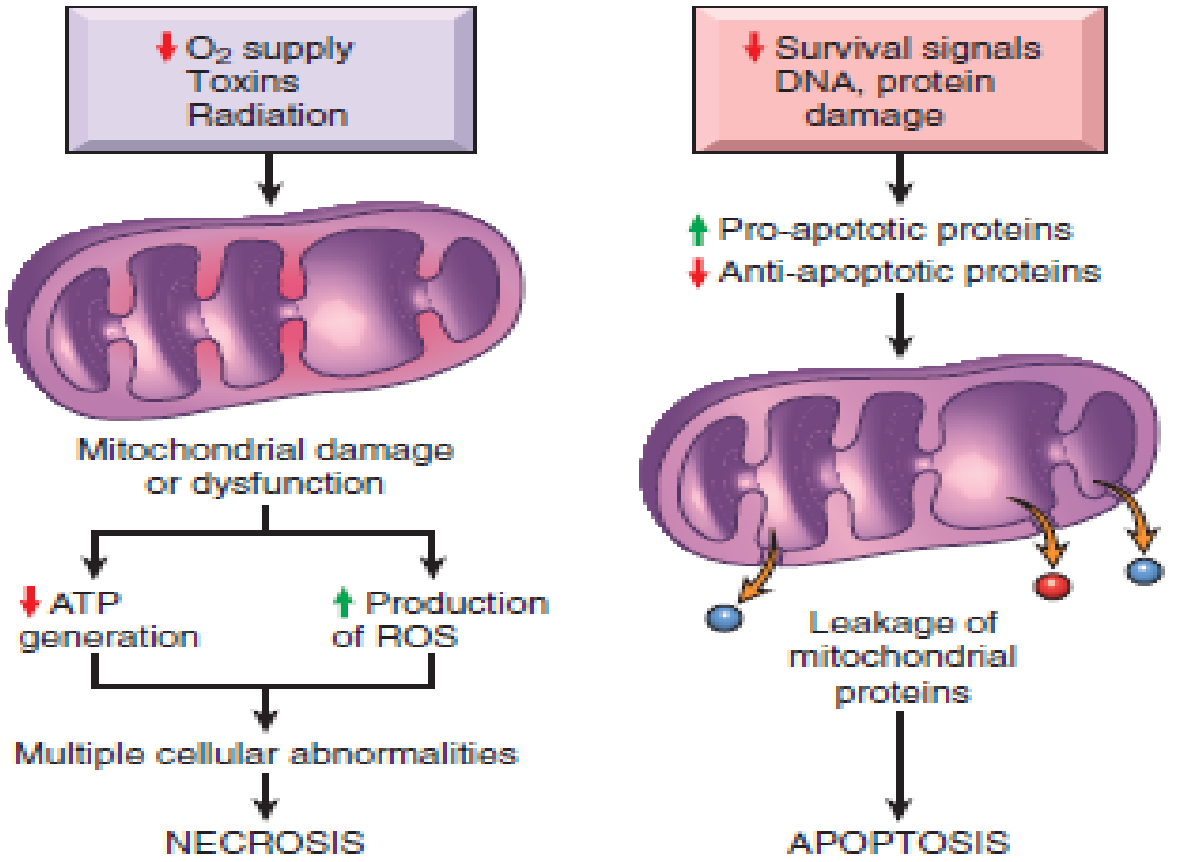


Figure 1–16 Role of mitochondria in cell injury and death. Mitochondria are affected by a variety of injurious stimuli and their abnormalities lead to necrosis or apoptosis. This pathway of apoptosis is described in more detail later. ATP, adenosine triphosphate; ROS, reactive oxygen species.

Depletion of ATP

- The professor read through them quickly, but I still advise on memorizing these effects as most of them you've already studied in previous slides.

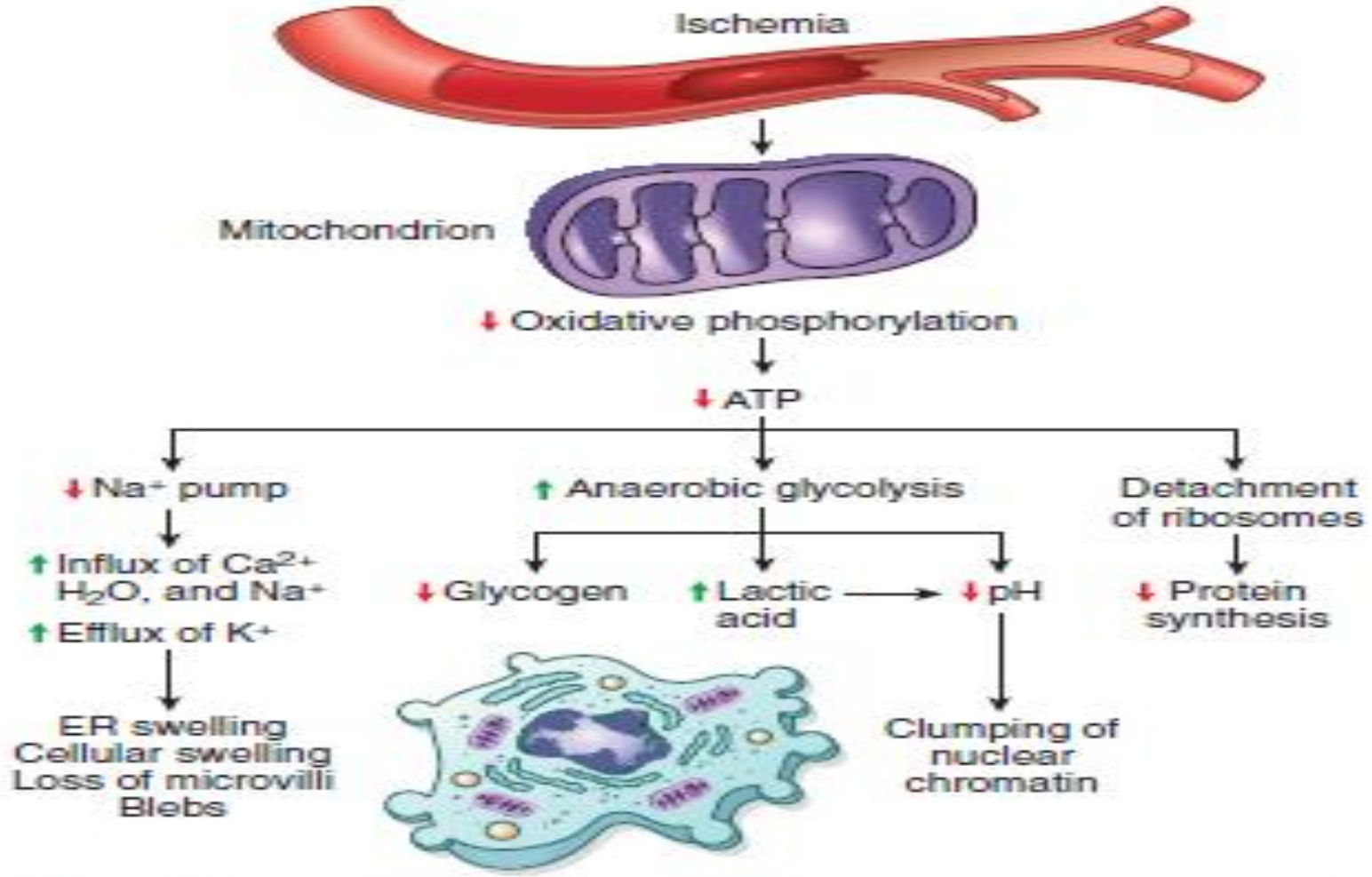


Figure 1–15 The functional and morphologic consequences of depletion of intracellular adenosine triphosphate (ATP). ER, endoplasmic reticulum.

Defects in Membrane Permeability

- Mitochondrial membrane damage: decreased ATP
- Plasma membrane damage: loss of osmotic balance, influx of fluids, leak of contents
- Lysosomal membranes: leakage of enzymes >> cellular digestion.

- A 20-year-old male is involved in a motor vehicle accident. The left femoral artery is lacerated (torn, or deeply cut) resulting in extensive blood loss. He is hypotensive (which means decreased perfusion) for hours during transport to ER. Which of the following tissues is most likely to withstand the impact of these events with the least damage? (Answer and explanation in the next slide)

- A- Intestinal Epithelium
- B- Skeletal Muscle
- C- Retina
- D- Cerebral Cortex
- E- Renal Tubules

- The answer is B. Skeletal Muscle

  Intestinal epithelium get affected quickly as we said before.

  Retina is from the neural tissues, which immediately gets affected

  Cerebral cortex is also neural, gets affected quickly

  Renal Tubules are also epithelial (used for reabsorption and excretion of stuff entering the kidney. Hypotensive shock usually causes renal failure.

- A 50-year-old female suffers an acute myocardial infarction. Thrombolytic agents are used to restore coronary blood flow. In spite of this therapy, the degree of myocardial fiber injury may increase because of which of the following cellular abnormalities?

- A- Increased production of ATP
- B- Decreased intracellular pH from anerobic glycolysis
- C- Increased free radical formation
- D- Mitochondrial swelling
- E- Decreased phospholipid peroxidation

- The answer is C. Increased free radical formation

  It isn't increased ATP production أكيد, all injuries include a Decrease* in ATP production

  it isn't decreased intracellular pH from anaerobic glycolysis because the heart doesn't function through the anaerobic pathway

  Mitochondrial swelling isn't the reason for increased injury

  Phospholipid peroxidation actually increases* during cell injury

In an experiment, cells are subjected to oxidant stress. There are increased numbers of free radicals generated within the cells. Generation of which of the following enzymes within these cells is most likely a protective mechanism to reduce the number of free radicals?

A- Phospholipase

B- Endonuclease

C- Glutathione Peroxidase

D- Myeloperoxidase

E- Cytochrome P450

- The answer is C. Glutathione Peroxidase

  Phospholipase simply doesn't have to do with free radicals

  Endonuclease is related to DNA rather than free radicals

  Myeloperoxidase is a free radical in itself

  Cytochrome P450 مش

- A cellular mutation results in a protein that does not fold properly. The misfolded protein remains within the cell and is not excreted. Activation of which of the following cytoplasmic enzymes is most likely to occur?

- A- NADPH Oxidase
- B- Glutathione Peroxidase
- C- Ribonuclease
- D- Caspase
- E- Telomerase

- The answer is D. Caspase

  Misfolded protein accumulation (if not reversed) causes the apoptosis of the cell, Caspase is the only enzyme within the options that is related to apoptosis

V2:تم إضافة توضيحات بلون الأحمر
V3: SLIDE 15