The Cellular Basis of Disease
Cell Injury 2

Mechanisms of Cell Injury

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"Details from today are continually revised and updated with research. Lots of it changes on a daily basis so today will be a broad overview"
Objectives

- Describe and understand mechanisms of cell injury including depletion of ATP; mitochondrial damage; entry of calcium into the cell; increased reactive oxygen species (ROS); membrane damage
- Describe and understand the pathogenesis and give examples of ischemic and hypoxic injury; ischemia-reperfusion injury; chemical injury and radiation injury
Mechanisms of Cell Injury

- Cellular response to injury depends on nature, duration and severity of injury.
- Consequences of injury depend on type, state and adaptability of the injured cell.
- Cell injury results from different biochemical mechanisms acting on essential cellular components.

Minor injuries with a long duration can have a more profound effect than a major injury with short duration.

Young people can respond better to injury than old people. The reason for this resides at cellular level.
This is critically important, and often underlies or coincides with other causes of injury.

We'll talk about each of these in turn.

Most Ca++ is kept outside cell or inside mitochondria.

May initiate apoptosis cascade.
Mechanisms of Cell Injury

- Depletion of ATP
- Mitochondrial Damage
- Entry of Calcium into the cell
- Increase reactive oxygen species (ROS)
- Membrane Damage
- DNA damage, Protein misfolding
Ischemia

Mitochondrion

↓ Oxidative phosphorylation

↓ ATP

↓ Na⁺ pump

↓ Influx of Ca²⁺, H₂O, and Na⁺

↓ Efflux of K⁺

ER swelling
Cellular swelling
Loss of microvilli
Blebs

↓ Anaerobic glycolysis

↑ Lactic acid

↓ Glycogen

↓ pH

Detachment of ribosomes

from ER

↓ Protein synthesis

Clumping of nuclear chromatin

Lipid deposition

in all the wrong places

Need proteins to transport lipids

problem in lots of enzyme function

Blockage of blood flow -> less O₂ to cell -> less oxidative phosphorylation

Clumping of nuclear chromatin caused by a more acidic pH generated by increased anaerobic glycolysis (since ox phos isn't happening)

ER swelling due to Ca and Na, H₂O influx due to inability to maintain gradient via Atp dependent pump

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Anaerobic versus Aerobic ATP Production

**Anaerobic Glycolysis:**
Glucose + 2 ADP ---> 2 Lactate + 2 ATP
Glycogen + 3 ADP ---> 2 Lactate + 3 ATP

**Aerobic Oxidative Phosphorylation:**
Glucose + 6O₂+ 36 ADP --> 6CO₂ + 6H₂O + 36 ATP

You should remember this from Mol and Cells. Make Newgaard proud!
Depletion of ATP

- ATP depletion and decreased ATP synthesis are common with both hypoxic and toxic (or chemical) injury.
- Na\textsuperscript{+}, K\textsuperscript{+}- ATPase pump activity is reduced.
- Cellular energy metabolism is changed.
- Failure of Ca\textsuperscript{++} pump.
- Reduced protein synthesis.
Mechanisms of Cell Injury

- Depletion of ATP
- **Mitochondrial Damage**
- Entry of Calcium into the cell
- Increase reactive oxygen species (ROS)
- Membrane Damage
- DNA damage, Protein misfolding

Often coincident w/ or cell damage
Consequences of Mitochondrial damage

• Loss of membrane potential via membrane permeability transition
• Results in failed oxidative phosphorylation and loss of ATP
• Membrane damage leads to leakage of Cytochrome c and other proteins which activate apoptotic pathways
Mitochondrial Damage

Mitochondrial injury or dysfunction
(Increased cytosolic Ca\(^{2+}\), oxidative stress, lipid peroxidation)

- ATP production
- H\(^+\)
- Cytochrome c
- Mitochondrial permeability transition (MPT)
- Cytochrome c, other pro-apoptotic proteins

Apoptosis

Has secondary effects on other organelles and the cell itself

MPT→apoptosis so not only is the MT injured but the cell and other organelles are injured because of the "toxic" stuff released in the cell.
Mechanisms of Cell Injury

- Depletion of ATP
- Mitochondrial Damage
- **Entry of Calcium into the cell**
- Increase reactive oxygen species (ROS)
- Membrane Damage
- DNA damage, Protein misfolding
Entry of Calcium into the cell

- Intracellular Ca\(^{++}\) is low and is sequestered in mitochondria and endoplasmic reticulum
- Extracellular Ca\(^{++}\) is high
- Gradients are maintained by Ca\(^{++}\) Mg\(^{++}\) ATPases
- Increased cytosolic Ca\(^{++}\) activates enzymes: ATPases, phospholipases, proteases, endonucleases.

starts breaking apart ATP, lipid membrane, cellular proteins, and DNA, respectively
Injurious agent → Extracellular Ca^{2+} → Mitochondrion → Increased cytosolic Ca^{2+} → Activation of cellular enzymes → Phospholipase ↓ Phospholipids → Disruption of membrane and cytoskeletal proteins → Membrane damage → Protease → Disruption of membrane and cytoskeletal proteins → Nucleus damage → Endonuclease → ATP → ATPase → Mitochondrial permeability transition → ATP
What cellular processes consume the most energy on an ongoing basis?

A. protein synthesis
B. DNA synthesis
C. DNA repair
D. phospholipid synthesis
E. ion transport

Answer on next page

Ca++ is important!!!
Mechanisms of Cell Injury

- Depletion of ATP
- Mitochondrial Damage
- Entry of Calcium into the cell
- **Increase reactive oxygen species (ROS)**
- Membrane Damage
- DNA damage, Protein misfolding

E. Ion transport
You have to keep Ca++ out of the cell!
Accumulation of oxygen-derived free radicals (Oxidative stress)

- Reactive oxygen species (ROS)
- Biologically Important ROS
- Generation of ROS

Function of ROS
- Removal of ROS
- Pathologic effects of ROS
- Cellular defense against ROS

Not all ROS are free radicals
Reactive Oxygen Species

- React with and modify cellular constituents.
- Initiate self perpetuating processes when they react with atoms and molecules.
- Electrons are frequently added to O₂ to create biologically important ROS.
Biologically Important ROS

3 major ROS to remember:

- **Superoxide anion radical** $O_2 + e^- \rightarrow O_2^-$
- Produced by phagocyte oxidase, damages lipids, proteins and DNA. (in neutrophils and macrophages)
- **Hydrogen peroxide** $H_2O_2$
  - Generated by SOD and by oxidases, destroys microbes, may act at distant sites.
- **Hydroxyl radical** $\cdot OH$
  - Generated from $H_2O$ by hydrolysis, most reactive, damages lipids, proteins and DNA.
Normally this is all maintained in balance; they are all biologically important. When the balance is tipped, you get injury.

Fenton reaction in the presence of Fe^{2+}

Inflammation
Radiation
Chemicals
Reperfusion injury

all these things can make superoxide radical

PATHOLOGIC EFFECTS OF ROS: CELL INJURY AND DEATH

ROS react with:
- Fatty acids → oxidation → generation of lipid peroxidases → disruption of plasma membrane, organelles
- Proteins → oxidation → loss of enzymatic activity, abnormal folding
- DNA → oxidation → mutations, breaks

REMoval of FREE radicals

Antioxidant mechanisms:
- SOD (in mitochondria) converts \( \cdot O_2 \rightarrow H_2O_2 \)
- Glutathione peroxidase (in mitochondria) converts \( \cdot OH \rightarrow H_2O_2 \rightarrow H_2O + O_2 \)
- Catalase (in peroxisomes) converts \( H_2O_2 \rightarrow H_2O + O_2 \)

Can be a good thing or a bad thing depending on if you wanted to kill the cell.
Generation of ROS

• Free radical is an unpaired electron which makes the atom or molecule extremely reactive.

• When a free radical reacts with another atom or molecule, the result is usually another free radical.

• $\text{H}_2\text{O}_2$ is not a free radical but it is reactive, thus the term reactive oxygen species. It is generated by SOD from $\text{O}_2^-$ and by oxidases.

• Common oxidases are P450 in the ER and NADPH oxidase in the plasma membrane.
Function of ROS

• Normal metabolism and respiration
• Absorption of radiant energy
• Inflammation
• Enzymatic metabolism of chemicals or drugs
• Nitric oxide synthesis
Removal of free radicals

- **Antioxidants** - Vitamins A and E, glutathione and ascorbic acid.
- Iron and Copper ions catalyze formation of ROS and are bound to transport proteins - transferrin, ferritin, ceruloplasmin.
- Enzymes scavenge free radicals - Catalase in peroxisomes; Superoxide dismutase in mitochondria and cytosol; Glutathione peroxidase in cytosol.
Pathologic effects of reactive oxygen species (ROS)

- **Fatty acids** - lipid peroxidation of plasma membranes and organelles
- **Proteins** - oxidation with loss of enzyme activity, protein misfolding
- **DNA** - oxidation, mutations, breaks
Lipid damage

- Plasma membranes and organelles have a high lipid content.
- **Double bonds** of unsaturated fatty acids are attacked by \( \text{O}_2^- \) derived free radicals.
- This yields peroxides which are unstable and **propagate the injury** which leads to membrane injury.
Protein damage

Cysteine residues (with SH groups) in proteins can be oxidized, resulting in the formation of disulfide (S--S) bonds. This results in conformational changes in proteins, loss of enzyme activity, and protein cross linking.
Protein cross linking – Contraction band  Necrosis

Pathology-speak for abnormally cross-linked proteins; myosin in this case
Injury to DNA

- Free radicals cause single and double strand breaks.
- Free radicals cause cross-linking of DNA strands.
- Free radicals cause adducts.
- Cells may be able to repair DNA injury if damage is minor.
- These changes are implicated in cellular aging and malignant transformation.
Mechanisms of Cell Injury

- Depletion of ATP
- Mitochondrial Damage
- Entry of Calcium into the cell
- Increase reactive oxygen species (ROS)
- Membrane Damage
- DNA damage, Protein misfolding
Mechanisms of Membrane Damage

• Reactive oxygen species
• Decrease phospholipid synthesis
• Increase phospholipid breakdown
• Cytoskeletal abnormalities
Membrane Damage

- Mitochondrial dysfunction
  - Decrease in oxygen ($O_2$)
  - Decrease in ATP
- Phospholipid reacylation/synthesis:
  - Decrease
- Phospholipase activation:
  - Increase
- Protease activation:
  - Increase
- Cytosolic calcium ($Ca^{2+}$):
  - Increase
- Phospholipid degradation:
  - Increase
  - Phospholipid loss
  - Lipid breakdown products
- Cytoskeletal damage:

Ca pump failure
Consequences of Membrane Damage

- Mitochondrial membrane damage causes increased cytosolic Ca$^{++}$, oxidative stress, lipid peroxidation, phospholipase activity, loss of membrane potential, leakage of Cytochrome c.
- Plasma membrane damage causes loss of osmotic balance, loss of proteins, enzymes and nucleic acids.
- Injury to lysosome membranes causes leakage of enzymes with destruction of cellular components.
- Leading to Cell Death
Mechanisms of Cell Injury

- Depletion of ATP
- Mitochondrial Damage
- Entry of Calcium into the cell
- Increase reactive oxygen species (ROS)
- Membrane Damage
- DNA damage, Protein misfolding
DNA damage
Protein misfolding

• If DNA damage to cell is too severe, apoptosis is initiated.
• Improperly folded proteins can initiate apoptosis.
• Cell Injury 3

tomorrow’s lecture. we’ll worry about it then
Examples of Cell Injury

- Ischemic and Hypoxic Injury
- Ischemia-Reperfusion Injury
- Chemical Injury
- Radiation injury

Only recently understood in the last 15-20 yrs
Ischemic and Hypoxic Injury

- Most common type of injury in modern medical practice
- **Hypoxia** = reduced oxygen availability
- **Ischemia** = reduced blood flow usually due to atherosclerosis
- **Ischemia** may also be caused by reduced venous return

 KNOW THE DIFFERENCE!

Less O2 and also less nutrients are delivered

Less common cause, but know it too
Ischemic injury
Kidney with multiple embolic infarcts
Ischemic injury

Acute Myocardial infarct

Less damage in the tissue immediately adjacent to the chamber b/c still perfused by the blood in the heart chamber
Ischemia-Reperfusion Injury

- Blood flow restored to ischemic cells which are injured but have not died.
- Injured cells may die when they are re-perfused. You'd think they'd be happy, but noooo. Mechanism not well understood.
- Other dead cells will release cellular contents into the restored blood stream.
- New damaging processes mediated by ROS become activated.
- Inflammation and complement activation add to damage.

Common in coronary bypass grafts or stints.
A 53 year old man has had marked chest pain for the past 3 hours. Laboratory findings include elevated serum creatine kinase-MB. He is given a thrombolytic drug and the CK-MB rises further. Which of the following is the most likely biochemical basis for this observed rise in CK-MB?

A. Reduced protein synthesis
B. Generation of reactive oxygen species
C. Increased activity of Catalase
D. Reduced oxidative phosphorylation
E. Release of calcium from endoplasmic reticulum
Chemical Injury

• **Direct injury** by combining with a critical molecule or organelle
  - Mercuric chloride
    - Direct covalent binding
  - Arsenic
    - Binds -SH groups of cell membrane proteins -> increased permeability and inhibits ion transport. Especially hurts GI and kidneys

• **Indirect injury** by conversion to toxic metabolites via P-450 mixed function oxidase
  - Reactive free radicals and lipid peroxidation
CCl₄ → SER
CCl₃ → Microsomal polyenoic fatty acid
Lipid radicals + O₂

**LIPID PEROXIDATION**
Autocatalytic spread along microsomal membrane

- Membrane damage to RER
- Polysome detachment
- † Apoprotein synthesis

**Fatty liver**

- Release of products of lipid peroxidation
- Damage to plasma membrane
- † Permeability to Na⁺, H₂O, Ca²⁺
- Cell swelling
- Massive influx of Ca²⁺
- Inactivation of mitochondria, cell enzymes, and denaturation of proteins

**death**
Radiation injury

Will be discussed more thoroughly later in the course

HEMATOPOIETIC
- Hypoplastic bone marrow
- Leukopenia
- Thrombocytopenia

INTESTINAL
- Petechiae
- Infection, e.g., Pneumonia, Septicemia
- Purpura

BRAIN
- Epithelial necrosis and ulceration
- Infection
- Diarrhea
- Septicemia
- Cerebral edema
- Neuronal necrosis
- Vasculitis
- Coma

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<tr>
<td>Latency</td>
<td>2 weeks</td>
<td>3 days</td>
<td>1 hour</td>
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<tr>
<td>Death</td>
<td>3 weeks</td>
<td>2 weeks</td>
<td>1 day</td>
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</tbody>
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Relatively low dose: 300 rad
Higher dose: 1000 rad
Really high doses: 2000 rad
Mechanisms and Types of Cell Injury

- Depletion of ATP
- Mitochondrial Damage
- Entry of Calcium into the cell
- Increase reactive oxygen species (ROS)
- Membrane Damage
- DNA damage, Protein misfolding

- Ischemic/Hypoxic injury
- Chemical Injury
- Radiation Injury

Q: Radiation injury causes double strand breaks and ROS generation?
A: Yes