Mechanisms of Ischemia-Reperfusion Injury (IRI)

To make a complex story simple

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The Bad
Ischemia: the occlusion of a coronary artery

http://amazingworldofbiol.wix.com/
The Good

REPERFUSION: the reopening of a coronary artery

Balloon Angioplasty
Stent with Balloon Angioplasty

1. Plaque
2. Balloon
3. Expanded Stent
4. Inflated Balloon

Artery
The Ugly
Reperfusion Injury: at the re-opening of a coronary artery

Diagram depicts critical events in cardiac ischemia reperfusion injury (Suleiman et al. 2001)
Important Factors to Determine Ischemia and Reperfusion Injury

- Duration of ischemia
- Collateral circulation formation

Dependency on Condition of Reperfusion and Oxygen Supply

- The speed/modality of reperfusion
- The components of reperfusion solution
Why Reperfusion Injury?
Experimental study showed the importance of

- Calcium overload
- pH recovery (pH paradox)
- The Central Role of Mitochondria,

*mitochondrial Permeability Transition Pore* (mPTP) and «Reactive Oxygen Species» (ROS)
Why Coronary Ischemia?
10 Factors That Increase the Risk of Heart Disease and Heart Attack:

1) Tobacco Smoke
2) High Blood Cholesterol
3) High Blood Pressure
4) Physical Inactivity
5) Obesity and Overweight
6) Diabetes Mellitus
7) Stress
8) Alcohol
9) Diet and Nutrition
10) Age
Ischemic Heart Disease (IHD)

The leading cause of death in human population

- **It is the most common type of heart disease.**
- **IHD occurs when the coronary arteries, that supply blood to the myocardium, become hardened and narrowed due to the plaque buildup, reducing the flow of blood and oxygen to the heart.**
- **IHD can weaken the heart muscle and lead slowly (chronic) or rapidly (acute) to heart failure.**

http://www.who.int/mediacentre/factsheets/fs310/en/
The Bad

Ischemia:
the occlusion of a coronary artery
Reversible injury

Ischemia

Mitochondrion

Oxidative phosphorylation

ATP

Na pump

Influx of Ca++, H₂O, and Na+
Efflux of K+

ER swelling
Cellular swelling
Loss of microvilli
Blebs

Anaerobic glycolysis

Glycogen

pH

Clumping of nuclear chromatin

Other effects

Detachment of ribosomes, etc.

Protein synthesis

Lipid deposition
Troponin

Alteration of ion exchanges

Reversible Injury

- Ischemia
- Mitochondria
  - ↓ Oxidative phosphorylation
  - ↓ Na pump
  - ↑ Influx of Ca²⁺, H₂O, and Na⁺
  - Efflux of K⁺
- ATP
- Glycolysis
- ↓ Glycogen
- Detachment of ribosomes
- ↓ Protein synthesis
- Lipid deposition

Other effects

Irreversible Injury (Cell death)

- Membrane injury
  - Loss of phospholipids
  - Cytoskeletal alterations
  - Free radicals
  - Lipid breakdown
  - Others
- ↑ Leakage of enzymes (CK, LDH)
- ↑ Ca²⁺ influx

- Intracellular release and activation of lysosomal enzymes
- ↓ Basophilia (↓ RNP)
- Nuclear changes
- Protein digestion
• Cessation of oxygen supply in ischemia leads to a loss of ATP production and an increase of reactive oxygen species (ROS) in the mitochondria

• Reduced activity of the ATP consuming Na\(^+\)-K\(^+\)-pump leads to Na\(^+\) accumulation in the myocyte and the resting membrane potential is lowered

• With the development of acidosis, the Na\(^+\)-H\(^+\)-exchanger (NHX) further increases intracellular Na\(^+\)

• Under these conditions the 3Na\(^+\)-1Ca\(^{2+}\)-exchanger (NCX) slows down due to acidic pH and intracellular accumulation of Na\(^+\) or may even operate in the reverse mode, letting Ca\(^{2+}\) into the cell

• Ca\(^{2+}\) also enters through the sarcolemmal L-type voltage-gated Ca\(^{2+}\) -channel (L) as the resting membrane potential is low

• The increased Ca\(^{2+}\) is taken up into the sarcoplasmic reticulum (SR) by the SR Ca\(^{2+}\) pump SERCA2 (P) and released (leak) from there via RYR, leading to contraction and contracture.
Mitochondria may accumulate enormous amounts of Ca$^{2+}$ (crucial for buffering cytosolic calcium). Ca$^{2+}$ overload may favor opening of mPTP, which may be kept closed by low pH. Depressed mitochondrial calcium uptake secondary to accelerated mitochondrial depolarization during ischemia, and impaired potential recovery during reperfusion, aggravates cytosolic calcium overload and contracture.

During ischemia contracture development can be defined as an impairment of relaxation with an increase in diastolic ventricular pressure (LVEDP) of 4 mmHg above pre-ischemic LEDVP values. This together with ATP shortage determine a loss of contractility.
The Ugly

Reperfusion Injury: at the re-opening of a coronary artery
If an Infarcting Ischemia is Followed by *Reperfusion*:

- *a)* the extension of the myocardial infarction increases;
- *b)* the contractility dysfunctions are more severe;
- *c)* the incidence of arrhythmias may increase.
Theoretical infarct size in absence of PPCI (AAR)

Myocardium salvaged by timely and effective PPCI

Actual infarct size after PPCI

Infarction due to reperfusion injury

Theoretical infarct size if reperfusion injury prevented

Infarction due to ischemic injury

Reperfusion time

Time

Ischemic time

PPCI

Onset of chest pain

Infarct size

Onset of chest pain
Major components of myocardial reperfusion injury (RI).

- Inflammation (neutrophils)
- Oxidative Stress
- Calcium overload
- pH Correction

Hypercontracture | mPTP opening

Letal myocardial RI

During the First Minutes of Reperfusion
Ion exchanges at reperfusion:

1) robust excretion of H⁺ due to prompt recovery of extracellular pH, 2) “reverse mode” excretion of accumulated Na⁺ and Ca²⁺ influx in turn, and 3) reexcretion of Ca²⁺ followed by recovery of ATP synthesis.

• Reperfusion is intended to produce ATP and to reactivate the Na\(^+\)-K\(^+\)-pump to slowly restore the sodium gradient leading to normal cation fluxes with the NCX eventually extruding the excess of cytosolic Ca\(^{2+}\).

• However reoxygenation during reperfusion will slowly restore ATP production with a further burst of ROS.

• Moreover, during the early reperfusion phase when the intracellular Ca\(^{2+}\) level is still high (NCX reverse mode and Ca\(^{2+}\) oscillations), myocardial hypercontracture (supercontraction of myocytes) may develop and irreversible injury occur (Ca\(^{2+}\) overload and rigor).
The consequences of Calcium overload
Effects of excess calcium in the cytosol

Cut-off point between reversible cell injury and cell death??
Mechanisms and consequences of altered Ca2+ handling in cardiomyocytes during initial reperfusion.


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Schematic diagram showing the proposed mechanisms by which calpains participate in reperfusion injury and in the cardioprotective effects of preconditioning and postconditioning.
ROS are double-edged swords
(They can be good or bad)

The bad-ugly consequences of ROS overproduction
Sources of ROS

In the cardio-circulatory system, ROS can be generated by:
- cardiomyocytes,
- endothelial cells, and
- neutrophils in inflammatory processes.

Different enzyme/mechanisms can produce ROS, including:
- electrons leaked from mitochondrial complexes,
- NADPH oxidase,
- xanthine dehydrogenase/xanthine oxidase,
- lipoxygenases,
- cyclooxygenases,
- peroxidases, and
- uncoupled nitric oxide synthase (NOS).
Mitochondria represent 36-40% of cardiomyocytes mass

O₂

Mitochondria

Incomplete reduction

H₂O₂

Superoxide

SOD

H₂O

Hydrogen peroxide

Fenton reaction

OH⁻

Hydroxyl radical

Reactive oxygen species

Reperfusion injury

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 O₂⁻ → H₂O₂
- Glutathione peroxidase (in mitochondria) converts °OH → H₂O₂ → H₂O + O₂
- Catalase (in peroxisomes) converts H₂O₂ → H₂O + O₂

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**ROS INDUCED ROS RELEASE (RIRR)**

- **Ischemia and reperfusion** raise the production of ROS which may activate the mPTP, especially in reperfusion, when pH recovers, and may be involved in the conversion of signaling to pathological ROS (RIRR).

- **RIRR** is a process originating in mitochondria responding to an increased oxidative stress by a positive feedback loop resulting in a regenerative, autocatalytic cascade.

- When **RIRR** is inappropriately not terminated, it may lead to unwanted cell loss such as after myocardial infarction.

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Zorov DB
Seen just before cell death

Mitochondrial ROS rise simultaneously with the mPTP-induced drop of $\Delta \Psi$

mPTP: forms from the FATP synthase, which would switch from an energy-producing to an energy-dissipating dimer (?)
Mechanisms of ROS-induced Ischemia/Reperfusion Injury

ROS are extremely reactive to interact with lipids, proteins and nucleic acids.

- The increase of membrane lipid peroxidation (MLP)

ROS interact with non-saturated fatty acids from membrane lipids and further induce lipid peroxidation reaction, which results in the structural alteration and dysfunction of membrane.

ROS induces oxidation of lips, proteins and nucleic acid.
ROS-mediated Membrane damage

The integrity, permeability and function of membrane are impaired during ischemia-reperfusion due to ROS-induced MLP.

- Deactivation and malfunction of membrane receptors and ionic pumps
- Mitochondrial dysfunction and further decreases ATP generation

These damages do not occur only in sarcolemma, but also in sarcoplasmic reticulum, mitochondria, lysosomes and other intracellular membranes.

Therefore, Ca^{2+} can flow into the cytoplasm through damaged membrane according to the gradient.
During the Subsequent Hours of Reperfusion

- With the reperfusion, the **endothelial and vessels become permeable**, thus causing interstitial edema.

- **Activated Endothelial cells** in reperfused myocardium express **adhesion proteins**, release **cytokines**, and **reduce production of NO**.

- These promote **adherence, activation, and accumulation of neutrophils** and monocytes in the ischemic-reperfused tissue.

• The release of **reactive oxygen species** and proteolytic enzymes from these **activated leukocytes** can contribute to the **damage of myocytes and vascular cells**.

• **Vascular plugging by adherent leukocytes and aggregated platelets** can also promote a **slow- or no-reflow phenomenon**, already favored by tissue contracture and increased pressure of interstitial edema.

• These additional reperfusion-induced noxes contribute to infarct development predominantly during the **first 2 hours of reperfusion**, as myocardial necrosis almost reaches its final size during this period.

Summary

Ischemia/Reperfusion Injury (IRI) occurring with ischemia and restoration of blood flow to post-ischemic tissue, is associated with arrhythmias, myocardial necrosis and apoptosis resulting in increased mortality and morbidity.

Calcium overload,
pH recovery,
and ROS overproduction are major players in determining IRI

Mitochondria play a pivotal role in life and death
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THANKS!
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