Ischemia-reperfusion injury in vascular disease

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Case presentation - Case I

- 21 yo BM from Lumberton with self-inflicted GSW (AK-47) to left lower extremity

- Transferred approximately 7 hours with an insensate foot without pulses

- Left popliteal fossa gunshot wound with popliteal artery injury, popliteal vein and superficial femoral artery injury
Case presentation - Case I
Case presentation - Case I
Case presentation - Case I

- Eighteen units of packed cells, 7 units FFP, 2 units platelets, 1 unit of cryo, 6.5 liters of crystalloid, 500 mL of Plasmanate.

- Vascular reconstruction SFA to the PT with contralateral reverse saphenous vein graft

- Profound hypotension due to reperfusion and hypovolemia

- Graft clinically patent with external fixation prior to leaving the operating room
Case presentation
Case presentation - Case I

- Graft clinically thrombosed the next morning
- Thrombectomy led to restoration of blood flow
- Above knee amputation 2 days later
Case presentation - Case II

- 62-year-old WF with a PMHx for bilateral arterial occlusive disease of her lower extremities

- PMHx:
  
  CAD s/p MI in Dec 2007
  Cardiomyopathy
  COPD
  Asthma/RAD
  HL

- Multiple lower extremities interventions
Case presentation - Case II
Case presentation - Case II

- The left SFA stent grafted throughout its length into the popliteal artery
Case presentation - Case II

- Angiojet thrombectomy was performed throughout the stent grafted segments, with substantial residual thrombus. SFA for overnight infusion of tPA.

- Because of critical ischemia in the foot which is cold, hypesthetic and extremely painful, she was taken to the operating room on emergency basis for revascularization. Femorotibial bypass using spliced arm vein.

- She developed good revascularization as evidenced by warm skin and in fact, palpable pulse.
Outline

- Definition
- Physiopathology
- Clinical implications
- Cerebral hyperperfusion after CEA
- Treatment
Definitions

- Ischemia refers to cessation or reduction of blood flow to and from a tissue and generally coincides with a reduced tissue oxygen delivery/demand ratio of 2:1 or below.

- Reperfusion injury refers to damage to tissue caused when blood supply returns to the tissue after a period of ischemia.

The absence of oxygen and nutrients from blood creates a condition in which the restoration of circulation results in inflammation and oxidative damage through the induction of oxidative stress rather than restoration of normal function.
First reports

- Bywaters EG. Crush injuries with impairment of renal injuries. BMJ 1941; 1: 427-432
- Hearse D. Reperfusion of the ischemic myocardium. Mol Cell Cardiology 1977; 9: 605-616
Myonephropathic-metabolic syndrome

“Following extraction of the thrombus, the previously cyanotic limb that had been anesthetic and paralyzed improved, although stiffness of the toes, ankle and knee persisted. Within 24 hr the limb became massively swollen and extremely hard, with non-pitting edema. The patient’s urine was cherry red and the pigment was identified as myoglobin.”

“On the third day, renal shutdown was complete and there was early gangrene of the foot. The patient died of renal failure on postoperative day 10th.”
Ischemia tissue injury accounted for the first and third leading causes of death in the US in the last decade (myocardial infarction and stroke, respectively)

Accounts for more than 2 million cardiothoracic, vascular and other surgical interventions per year.

Great relevance to the practice of surgery:
- Vascular Surgery
- Cardiac Surgery
- Transplant Surgery
Cellular consequences of ischemia

- High-energy phosphate production fails (ATP, GTP, creatine phosphate, etc)
- Slowing down and failure of \( \text{Na}^+-\text{K}^+ \) ATPase pump
- Altered membrane potential—loss of \( \text{K}^+ \) and gain of \( \text{Na}^+ \)
- Altered ion distribution (++ intracellular Ca/Na)
- Massive increase of intracellular Ca (10,000:1 extracellular/intracellular ratio)

- Damaging enzymatic cascades
- Cell swelling
- Cellular acidosis

Cell death

U.S. National Library of Medicine
Reperfusion injury

- Often more severe than damage incurred during the ischemic period itself

- Characterized by:
  - cellular edema
  - intracellular Ca\(^{2+}\) overload
  - activation of Ca\(^{2+}\)-dependent autolytic enzymes
  - disruption of lipid membranes
  - changes in mitochondrial structure and function
Mediators of reperfusion injury

Production of Partially Reduced Oxygen Species (PROS)
Role of endothelium

- Thin monolayer of cells, resting on a basement membrane, surface area 5000 m².

- Exerts influence over:
  - Blood vessel tone
  - Permeability
  - Cell adhesion
  - Coagulation
  - Growth
Endothelial cell-mediated tone

**Vasodilatation:**
- Prostacyclin (PGI$_2$):
  - activates adenylate cyclase and protein kinase A
  - inhibits platelet aggregation by increasing cAMP
- Nitric Oxide (NO):
  - inhibits platelet aggregation
  - decreases vascular smooth muscle cell proliferation
- Adenosine:
  - inhibits platelet and neutrophil aggregation

**Vasoconstriction:**
- Thromboxane A$_2$ (TXA$_2$):
  - opposes prostacyclin and produces platelet adherence
- Endothelin-1:
  - most potent vasoconstrictor known, counteracts NO
Effects of ischemia on endothelium

- Decrease O2 in endothelium:
  - Alters production of soluble mediators
  - Alters barrier function
  - Decreases production of growth factors
  - Reduces extracellular matrix proteins

- Expression of pro-inflammatory gene products (leukocyte adhesion molecules, cytokines) bioactive agents (endothelin, TxA2)

- Repressing other “protective” gene products (constitutive NO synthase, thrombomodulin) and bioactive agents (prostacyclin, NO)
Endothelial cell during reperfusion

- Three phases:
  1. The hypoxic phase
  2. Initial reperfusion phase: PROS and activated complement fragments induce within seconds to minutes expression of pre-formed proteins that promote leukocyte-endothelial cell interaction
  3. Prolonged reperfusion phase: Transcriptional activation and protein expression on endothelial surface. Completed over the course of several hours.
Endothelial cell during reperfusion

- Mediators formed during reperfusion induce endothelial cells to express:
  - Intercellular adhesion molecules (ICAM 1 and 2)
  - Endothelial leukocyte adhesion molecule (ELAM)
  - Selectins

- These receptors bind the CD11/CD18 complex on activated neutrophils, facilitating adherence and migration across endothelium

- Secretion of soluble factors that promote vasoconstriction, platelet aggregation, PMN plugging of capillaries, and increased vascular permeability:
  - Platelet aggregating factor (PAF), LTB$_4$, TXA$_2$ and endothelin
Role of PMNs

- Reperfusion results in PMNs:
  - Activation
  - Chemotaxis
  - Leukocyte–endothelial cell adhesion
  - Transmigration
    - mechanical obstruction
    - activated leukocytes release toxic PROS, proteases, and elastases, resulting in increased microvascular permeability, edema, thrombosis, and parenchymal cell death

- Activated PMNs: major source of PROS
PMNs migration

1. Neutrophil
2. P-Selectin, CD11/CD18, ICAM-1
3. PGSL-1, PECAM-1

Blood Flow
Integrins
Selectins

Loose Adhesion ("Rolling")
Firm Adhesion/Aggregation
Diapedesis
PMNs in I/R injury

I/R injury is associated with marked neutrophil infiltration as indicated by the large increase in skeletal muscle myeloperoxidase (MPO) activity

Sources of PROS

- Free-fatty acid pathway:
  - Ischemia > Increase intracellular Ca > activation of Phospholipase C and A > generation of Aracidonic Acid > TxA2 and PROS

- Purine metabolites:
  - Ischemia > Adenine nucleotide products metabolized > Xantine Oxidase > generation of PROS

- Activation of PMNs

- Local pH effects:
  - Massive accumulation of CO2
  - Lactic acid production
Damaging effects of PROS

- Three different molecules to be aware of:
  - Superoxide anion $O_2^-$
  - Hydrogen peroxide $H_2O_2$
  - Hydroxyl radical $\cdot OH$

- Cause widespread damage to cellular macromolecules

- Most damaging effect is on lipid membranes, impairs normal fluidity and permeability of cell membranes leading to cellular edema, massive $Ca^{2+}$ and $Na^+$ overload and cell lysis

- Peroxidation of lipid membranes, protein degradation, nucleic acid damage, cytochrome inactivation and neutralization of nitric oxide
Molecular pathways

Localized I/R injury

Systemic Mediator Release

- PAF, C5a, LTB4
- Neutrophil Activation Adhesion Molecule Expression
- O-, H2O2, CD11/CD18

Cytokines, enteric LPS

- Endothelial Adhesion Molecule Expression
- ICAM-1, P-Selectin

Neutrophil-endothelial Cell Adhesion

Neutrophil-Mediated Remote Organ Vascular Dysfunction and Tissue Injury
Systemic consequences of I/R injury

- **Myocardial injury:**
  - Release of myocardial depressant factors: C3a, TxA2, LTD4, PAF

- **Remote lung injury:**
  - Non-cardiogenic pulmonary edema – activation of PMNs, endothelial injury
  - ARDS

- **Renal injury:**
  - Myoglobin deposition in renal tubules
  - Acute tubular necrosis

- **Splanchnic ischemia:**
  - 5-24% pts undergoing Ao surgery
  - PROS have been associated with:
    - increased capillary permeability
    - reduced reabsorption of nutrients
    - protein leak
    - translocation of bacteria
    - release of endotoxin and gut enzymes into portal circulation
Local consequences of I/R injury in skeletal muscle

- Critical tissue ischemic times

<table>
<thead>
<tr>
<th>Tissue</th>
<th>Timing</th>
</tr>
</thead>
<tbody>
<tr>
<td>Muscle</td>
<td>4 hours</td>
</tr>
<tr>
<td>Nerve</td>
<td>8 hours</td>
</tr>
<tr>
<td>Fat</td>
<td>13 hours</td>
</tr>
<tr>
<td>Skin</td>
<td>24 hours</td>
</tr>
<tr>
<td>Bone</td>
<td>4 days</td>
</tr>
</tbody>
</table>

- Revascularization of ischemic skeletal muscle:
  - Release of K+
  - Hydrogen ions
  - Myoglobin
  - Acid phosphatase
  - Amino Acids
  - Nucleotides
  - Purine bases
It’s all about timing!!!
Molecular events in skeletal muscle I/R injury
PROS defense and therapy

Endogenous antioxidants:
- Glutathione: donates hydrogen atoms to radial oxygen species
- Carotenoids: Vitamin A, β-Carotene
- Tocopherols inhibit membrane lipid peroxidation
- Ascobic acid (Vit C)
- Superoxide Dismutase: enzyme present in cytosol that reduces superoxide radical to hydrogen
- Catalase

Exogenous antioxidants:
- Vit E
- DMSO
- Lazaroids
Enzyme inhibition and other agents

- Allopurinol: inhibits xanthine oxydase
- Deferoxamine: chelates free metals; removes an essential cofactor for the generation of hydroxyl radical
- N-acetylcysteine - pretreatment 30 minutes before infrarenal aortic clamping may help prevent reperfusion injury
Anticomplement therapy

- C3 convertase inhibitor

- Soluble complement receptor 1 decreases infarct size by 44% in a rat model of myocardial I-R.

- “Humanized,” recombinant, single-chain antibody specific for human C5 (h5G1.1-scFv)
  - Attenuates complement activation
  - Leukocyte activation
  - Myocardial injury
  - Blood loss
  - Cognitive dysfunction in humans undergoing CABG with cardiopulmonary bypass
Antileukocyte therapy

- Inhibition of inflammatory mediator release or receptor engagement, leukocyte adhesion molecule synthesis, or leukocyte–endothelial adhesion
  - Leukocyte depletion/ Filtration
  - Soluble interleukin-1 receptor antagonists, anti–tumor necrosis factor antibodies, or platelet activation factor–leukotriene B4 antagonists
  - Aspirin-triggered lipoxins prevent chemotaxis, adhesion, and transmigration of neutrophils
Antileukocyte therapy

Immunoneutralization of common P subunit CCDIS of leukocyte glycoprotein adhesion complex CD11KD18 is as effective as fasciotomy in reducing muscle necrosis in canine gracilis muscles subjected to prolonged I/R.

Fasciotomy and CD18 antibody treatment (Combined) produced a further reduction on postischemic muscle necrosis.

Clinical presentation

- Local findings:
  - Limb edema
  - Compartment syndrome

- Systemic
  - Hyperkalemia
  - Rhythm disturbances
  - Myoglobinuria with renal failure
  - Pulmonary complications
Treatment approaches

Mortality rates may vary from 7.5% to 41% in publications from the 1960s and 1970s


Prevention:

Time is the single most important determinant confounding a successful outcome and preventing reperfusion syndrome

Ischemia preconditioning

- Exposure of tissues to brief periods of ischemia protects them from the harmful effects of prolonged I-R
  - coronary artery bypass grafting
  - reduce liver injury undergoing hepatic resection

- Increases cellular adenosine production and confers protection by augmenting cellular energy stores and/or inhibiting leukocyte adherence
Controlled limb reperfusion

Control of the conditions of reperfusion:

<table>
<thead>
<tr>
<th>Condition</th>
<th>Principle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Temperature 34-35°C</td>
<td>Normothermia to slight hypothermia</td>
</tr>
<tr>
<td>Pressure 60-70 mmHg</td>
<td>Reduce edema formation</td>
</tr>
<tr>
<td>Leukocyte filter</td>
<td>Reduce neutrophil count</td>
</tr>
</tbody>
</table>

Control of the composition of the reperfusate:

<table>
<thead>
<tr>
<th>Substance</th>
<th>Amount (mL)</th>
<th>Principle</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glucose 5%</td>
<td>500</td>
<td>Hyperosmolarity</td>
</tr>
<tr>
<td>Citrate-phosphate-dextrose</td>
<td>150</td>
<td>Reduce Ca++</td>
</tr>
<tr>
<td>Tromethamine</td>
<td>0.3 mL/L200</td>
<td>Buffer</td>
</tr>
<tr>
<td>Glutamate/aspartate</td>
<td>150</td>
<td>Substrate</td>
</tr>
<tr>
<td>Allopurinol</td>
<td>2.5</td>
<td>Reduction of free radicals</td>
</tr>
</tbody>
</table>
Controlled limb reperfusion

- Animal studies using controlled limb reperfusion:
  - Restore oxygen consumption to control levels,
  - Avoid tissue edema
  - Limit CK and potassium release
  - Restore limb flow to greater than control values
  - Restore a normal range of motion to the knee joint

Surgical technique for controlled limb reperfusion

- Fluid, electrolytes, and cardiovascular abnormalities are corrected as much as possible before the operation.
- Heparin dose (100 IU/kg) after induction of general anesthesia.
- Fluid replacement is restricted to account for the crystalloid load infused with the controlled reperfusion, and furosemide (10 mg) is given intravenously as needed.
Surgical Technique for Controlled Limb Reperfusion

- Six-hundred milliliters oxygenated blood is collected first in the blood bag.

- In the reperfusion bag, the 600 mL oxygenated blood is mixed with 100 mL asanguineous solution (ratio 6:1) to form the required controlled limb reperfusate.

- After the reperfusion line is deaired, the controlled limb reperfusate is given via the reperfusion line into one or two distal reperfusion cannulas.

- The reperfusion pressure should not exceed 60 mmHg.

- This procedure is repeated for a total of 30 minutes.
Reperfusion after CEA

- Intracerebral hemorrhage after cerebral revascularization
  - Incidence ranges from 0.4-2%
    
    Piepgras DG. J Neurosurg 1988; 68: 532-543
    Mansoor GA. J vasc Surg 1996; 23: 147-152

- Mortality up to 36% in these pts
  

- Risks factors:
  - History of stroke
  - Relief of stenotic lesion >90%
  - Severe intraoperative or postoperative hypertension
  - Anticoagulant use
  - Severe chronic cerebral ischemia
  - Occlusion of contralateral carotid artery
Pronounced increase in cerebral blood flow:
- As much as 37% in ipsilateral side and 33% in contralateral side
  
  *Schroder T. J Neurosurg 1987; 66:824-829*

2-4 days after reperfusion

Hemorrhage can occur in areas of previously health brain tissue

Lack of autoregulation > increase perfusion pressure > vessels fixed to dilation > hemorrhage

*Ouriel K. J Vasc Surg 1999; 29: 82-89*
Clinical manifestations

- Headache (unilateral and ipsilateral to CEA)
- Bradycardia
- Massive hemorrhage with herniation
- HTN
- Seizures
- Neurologic symptoms
Intracerebral hemorrhage after carotid endarterectomy: Incidence, contribution to neurologic morbidity, and predictive factors

Kenneth Ouriel, MD, Cynthia K. Shortell, MD, Karl A. Illig, MD, Roy IC Greenberg, MD, and Richard M. Green, MD, Rochester, NY

Methods:
- Patients undergoing CEA between 1992-1997, in whom intracerebral hemorrhage associated with neurologic deterioration within 30 days of operation
- Control group of 50 patients who did not experience intracranial bleeding
Hemorrhage after CEA

<table>
<thead>
<tr>
<th>Class</th>
<th>Size of hemorrhagic area</th>
<th># of hemorrhagic areas</th>
<th>Mass effect</th>
<th>Herniation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Petechial</td>
<td>Small</td>
<td>Many</td>
<td>None</td>
<td>None</td>
</tr>
<tr>
<td>Moderate</td>
<td>Moderate</td>
<td>Usually 1</td>
<td>None to mild</td>
<td>None</td>
</tr>
<tr>
<td>Massive</td>
<td>Large</td>
<td>Usually 1</td>
<td>Significant</td>
<td>Impending or frank</td>
</tr>
</tbody>
</table>

- Intracranial hemorrhage with neurologic deficit occurred in 11 of the 1471 patients (0.75%)
- All events were ipsilateral to the operated carotid artery
- Bleeding occurred a median of 3 days after surgery (range, 0 to 18 days)
Hemorrhage after CEA

- **Symptoms:**
  - Headache in 7 of 7
  - Severe hypertension (systolic blood pressure greater than 180 mm Hg, diastolic blood pressure greater than 100 mm Hg) in all 11 patients
  - Bradycardia (heart rate less than 60) in 6 of the patients.
  - No patient experienced seizures before hemorrhage, but 3 of the patients had seizures after the event

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Hemorrhage (n=11)</th>
<th>No Hemorrhage (n=50)</th>
<th>P value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>66 ± 2.3</td>
<td>73 ± 1.1</td>
<td>.02</td>
</tr>
<tr>
<td>History of HTN</td>
<td>82%</td>
<td>64%</td>
<td>.05</td>
</tr>
<tr>
<td>Ipsilateral stenosis</td>
<td>92%</td>
<td>77%</td>
<td>.006</td>
</tr>
<tr>
<td>Contralateral Stenosis</td>
<td>78%</td>
<td>51%</td>
<td>.003</td>
</tr>
<tr>
<td>Contralateral occlusion</td>
<td>64%</td>
<td>12%</td>
<td>.05</td>
</tr>
</tbody>
</table>
Treatment

- Avoid intra and postop hypertension
- Judicious use of anticoagulants and antiplatelets
- Anticonvulsants if seizures present
- Diuretics if significant edema
- Early imaging if clinical suspicion
Conclusions

- Ischemia/reperfusion injury has local and systemic consequences
- High morbidity and mortality
- Timing to reperfusion is key
- NO available adequate therapy once developed
- Fasciotomy always indicated when suspicion high
Conclusions

- Reperfusion after CEA relatively uncommon
- High morbidity and mortality
- Can have delayed presentation
- Imaging indicated with postoperative symptoms