PATHOGENESIS OF CEREBRAL INFARCTION

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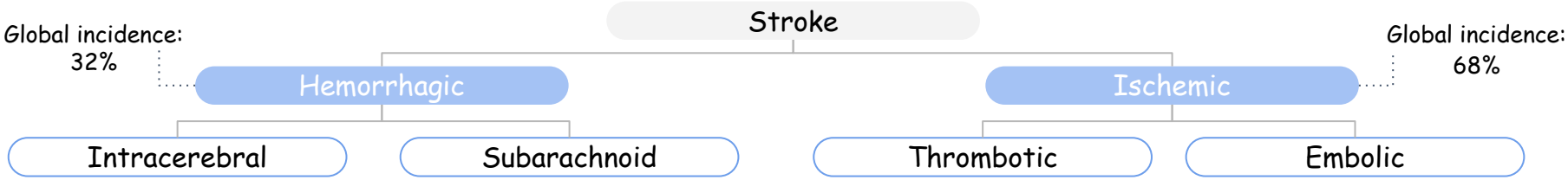
Editing File

Objectives:

- Identify the possible **cell death mechanisms** implicated in the pathogenesis of ischemic brain injury.
- Acquire the knowledge of the important role played by **oxidative stress and free radicals** in the pathogenesis of cerebral infarction.
- Understand the various factors involved in ischemia-induced metabolic stress.
- Identify the Neurochemical changes involved in cerebral ischemia.

Cerebral Ischemia (Strokes) subtypes



Risk factors of strokes

Some increase the risk of one type of stroke (hemorrhagic or ischemic), or both types, and stroke can occur with no risk factor.

Ischemic stroke risk factors		Hemorrhagic stroke risk factors	
Age older than 40 years	Heart disease	High blood pressure	
Diabetes	Illegal drug use	Smoking	
High blood cholesterol levels	Recent childbirth	Previous history of transient ischemic attack	Illegal drug use (especially cocaine and "crystal meth")
Inactive lifestyle + lack of exercise / Obesity	Current or past history of blood clots	Family history of cardiac disease and/or stroke	Use of warfarin or other blood thinning medicines

The cell death mechanisms implicated in the pathogenesis of ischemic brain injury

Cell death mechanisms in cerebral ischemia:

Necrosis

Commonly observed **early** after severe ischemic insults

Apoptosis

Apoptosis occurs with more **mild** insults and with **longer** survival periods

- The mechanism of cell death involves calcium-induced **calpain-mediated proteolysis** of brain tissue.[1]
- **Substrates for calpain include:**

  Cytoskeletal proteins, Membrane proteins and Regulatory and signaling proteins

Biochemical Responses to Ischemic Brain Injury

1. Oxidative stress
2. Metabolic stress
3. Neurochemical response

1. caplin enzyme is activated by Ca influx, this enzyme is responsible for degeneration of cellular proteins.

Dr note: In ischemic situations, necrosis and apoptosis take place in different percents. Depending on the duration and severity of ischemia.

Dr note: In the core of the ischemic area (immediate cell death happens): necrosis; around it is the area that is not dead yet and can be saved if perfusion is started fast (the peri-infarct ar

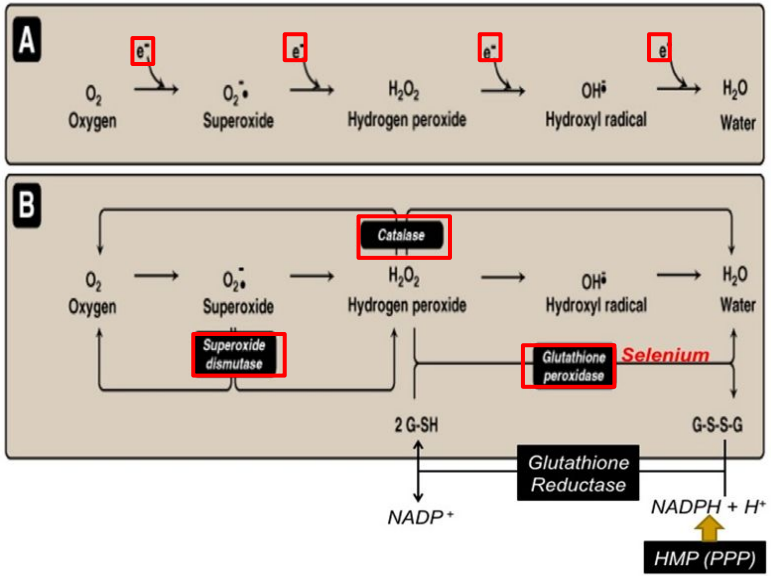
apoptosis

1 Oxidative stress

★ **Oxidative stress** is a condition in which cells are subjected to excessive levels of Reactive oxidizing species (ROS or RNS) & they are unable to counterbalance their deleterious effects with antioxidants.

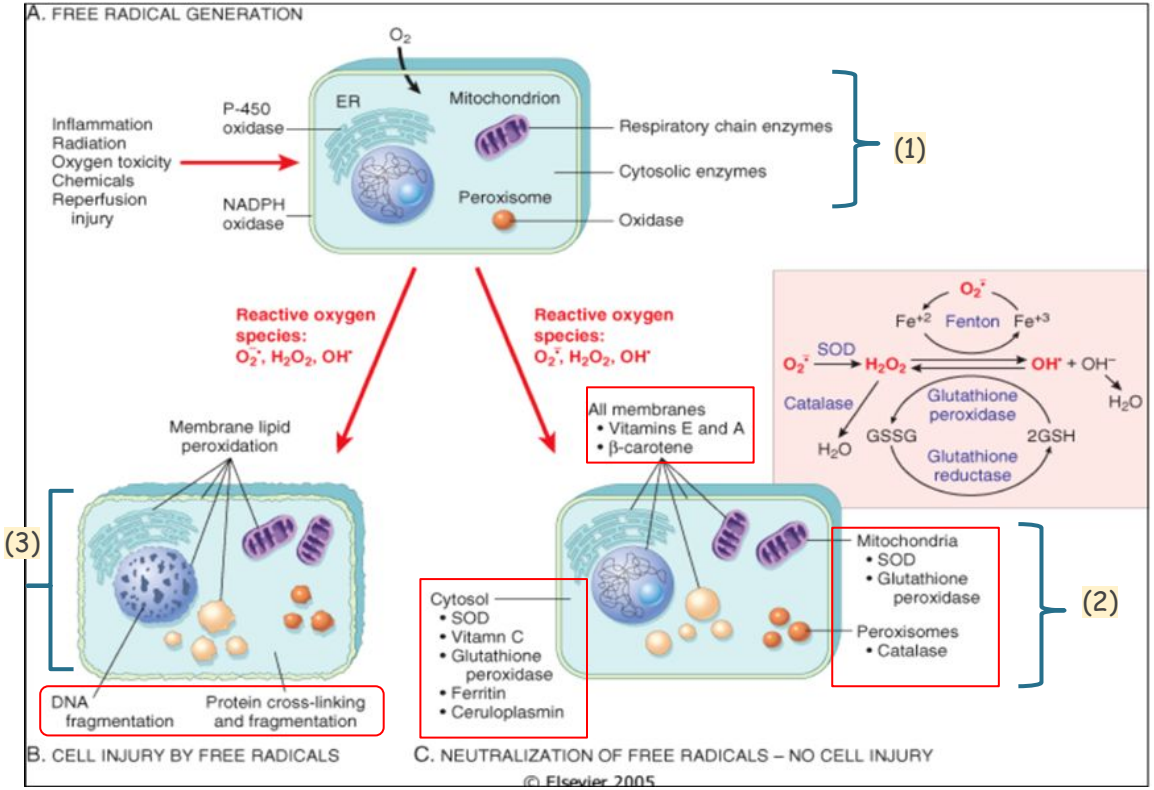
★ It has been implicated in the ageing process & in many diseases (e.g. atherosclerosis, cancer, neurodegenerative diseases, stroke)

These reactions are taking place in mitochondria in normal cell physiology



- By adding one electron to the oxygen it starts to be converted to ROS molecules
- Normally we have anti-oxidant system that can converts ROS to water or back to oxygen molecule as the following:
  1. Catalase enzyme can convert H2O2 into O2 or water.
  2. Superoxide dismutase enzyme can convert superoxide into oxygen or to hydrogen peroxide.
  3. Glutathione peroxidase can convert hydrogen peroxide into water.

Free radical generation



- (1) ROS produced 90% in mitochondria and the remaining 10% by cytosolic enzymes: oxidase, P-450 oxidase, NADPH oxidase.
- (2) Normally we have anti-oxidant enzymes to take care of them→ no cell injury (you have to know at least 3 enzymes and other 2 molecules).
- (3) If ROS increased, they will enter the cells and degrade all proteins causing proteins DNA fragmentation which lead to cell death.

Some notes about the figure:

- When ROS are generated, they can cause membrane lipid peroxidation which means that the membrane gets degraded. If this happens to lysosomes (which contain proteases) their contents leak into the cell → cell death.
- The Fenton reaction: Fe interacts with H_2O_2 → hydroxyl radical (very reactive). Other ions like Cu can also lead to the generation of ROS.
- The brain has a lot of iron stores that are also involved in the formation of ROS.

The Role of Reactive Oxygen Species (ROS) & Reactive Nitrative Species (RNS) in Normal Brain Physiology :

- ★ They are mainly generated by microglia & astrocytes
- ★ They modulate synaptic transmission & non-synaptic communication between neurons & glia
- ★ During periods of increased neuronal activity, ROS & RNS diffuse to the myelin sheath of oligodendrocytes activating Protein kinase C (PKC) → posttranslational modification of myelin basic protein (MBP) by phosphorylation
- ★ They regulate neuronal signaling in both central & peripheral nervous systems
- ★ They are required for essential processes as learning & memory formation

The brain is highly susceptible to ROS-induced damage because of:

- High concentrations of peroxidisable lipids
- Low levels of protective antioxidants
- High oxygen consumption
- High levels of iron[1] (acts as pro-oxidants under pathological conditions)
- The occurrence of reactions involving dopamine & Glutamate oxidase in the brain

1. Dr note: The fenton cycle (in previous slide) regarding this point.

Dr note: ROS are normally produced and have many beneficial function and the anti-oxidant are taking care of them. If the level of ROS increases then they will start to damage the body.

Effects of ROS in ischemic stroke

Molecular

- **DNA** damage
- **Lipid** peroxidation of **unsaturated fatty acids**
- **Protein** denaturation
- Inactivation of **enzymes**
- Cell **signaling** effects (e.g. release of Ca^{2+} from intracellular stores)
- **Cytoskeletal** damage
- **Chemotaxis**

Vascular

- Altered vascular tone and cerebral blood flow
- Increased platelet aggregability
- Increased endothelial cell permeability

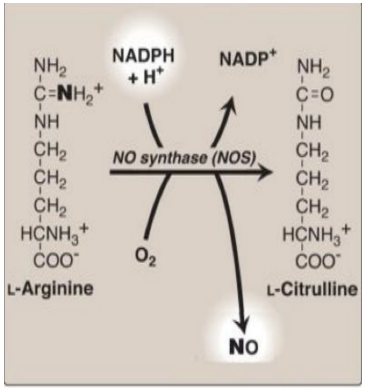
The role of NO in the pathophysiology of cerebral ischemia

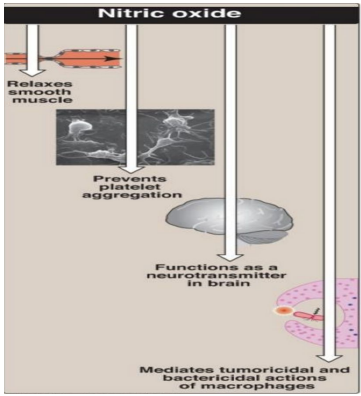
★ Ischemia leads to an <u>abnormal</u> NO production

★ Depending on where NO released, it can be **beneficial** or **detrimental**:

- NO produced by endothelial NOS (eNOS) improves vascular dilation & perfusion → **beneficial** (1st to be produced in stroke)
- NO produced by neuronal NOS (nNOS) or by the inducible form of NOS (iNOS) → **detrimental** (harmful) after some time, when neurons are hyper-excited they produce nNOS

★ Increased iNOS activity generally occurs in a delayed fashion after brain ischemia and trauma and is associated with inflammatory processes





Dr note: nNOs & iNOS are harmful because they react with ROS to produce reactive nitrogen species.

NO synthase produces NO from Arginine

2 Metabolic stress

★ Biochemical changes in the brain during ischemia:

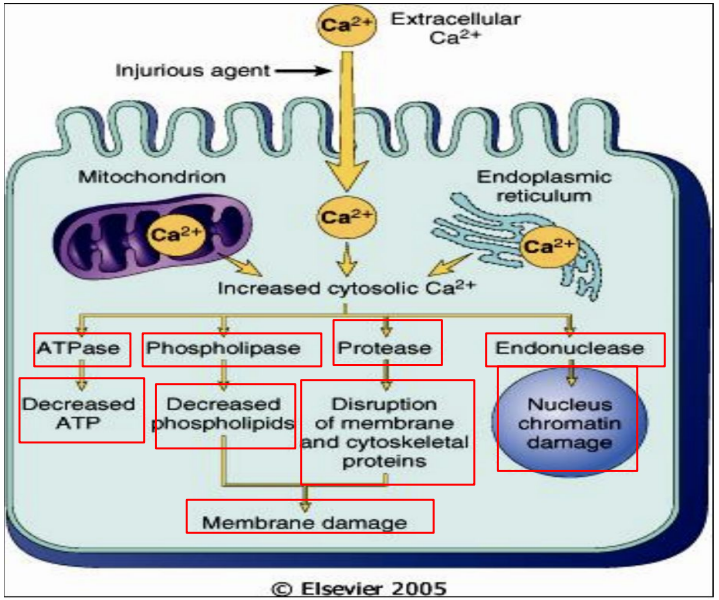
ischemia → interruption or severe reduction of blood flow, O_2 & nutrients in cerebral arteries → Energy depletion (depletion of ATP & creatine phosphate)

★ As a result on energy depletion:

1) Inhibition of ATP-dependent ion pumps ⇒ **membrane depolarization** & **perturbance of transmembrane ion gradients:-**
   - Na+ influx
   - K+ efflux (this causes increased release of excitatory amino acids) → activation of neurotransmitters
   - Ca2+ influx[1] (translocation from extracellular to intracellular spaces) → activation of cellular proteases (Calpins) & lipases → breakdown of cerebral tissue

2) Increased lactic acid in neurons → acidosis → promotion of the pro-oxidant effect → increased rate of conversion of $^{\cdot}O^{-}_2$ to H_2O_2 or to hydroperoxyl radical

When cells shift to anaerobic respiration, they produce ATP but in low amounts. This ATP depletion causes closure of ATP pumps; such as Na/K ATPase (leading to K efflux & Na influx) as well as Na/Ca pump (leading to Na influx which is accompanied by water, resulting in neuronal swelling + Ca buildup which causes the generation of glutamate neurotransmitter, and when glutamate is released, it activates nearby neurons that excite their nearby neurons. This is called hyperexcitation/excitotoxicity and it is cytotoxic for the neurons)



1. Sources & consequences of increased cytosolic calcium in cell injury: Ca comes in from extracellular stores & intracellular stores (mitochondria+endoplasmic reticulum) and affects different enzymes causing the following changes

3 Neurochemical response

★ Following cerebral ischemia, extracellular levels of various neurotransmitters are increased e.g.

- Glutamate
- Glycine
- GABA
- Dopamine

Blood tests in patients with brain ischemia or hemorrhage

★ Complete blood count (CBC); including hemoglobin, hematocrit, white blood cell count, and platelet count
★ Prothrombin time, international normalized ratio (INR), and activated partial thromboplastin time
★ Thrombin time and/or ecarin clotting time, **if** patient is known or suspected to be taking a direct thrombin inhibitor or a direct factor Xa inhibitor
★ Blood lipids; including total, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol, and triglycerides
★ Cardiac enzymes and troponin[1]

1. Dr note: We check cardiac enzymes & troponin because a lot of times patients are having a stroke and a myocardial infarction at the same time

Biochemical basis of pharmacological intervention

★ Examples of potential biochemical intervention in cerebral ischemia:

- Inhibitors of glutamate release
- Ca2+ channel blockers
- Nitric oxide synthase inhibitors & free radical inhibition
- Calpain inhibitors

Dr note: We give inhibitors of glutamate release to prevent hyperexcitiation of neurons; Ca channel blockers to stop cell death.

Take Home Messages

Severe cerebral ischemic insults lead to a complex cascade of biochemical and molecular events, including:

1. **Cell death**
2. **Oxidative stress**
3. **Metabolic stress and neurochemical changes**

Summary from the doctors' slides

Ischemic cascade

Lack of oxygen supply to ischemic neurons

↓

ATP depletion

↓

Malfunctioning of membrane ion system

↓

Depolarisation of neurons

↓

Influx of calcium

↓

Release of neurotransmitters, activation of proteases

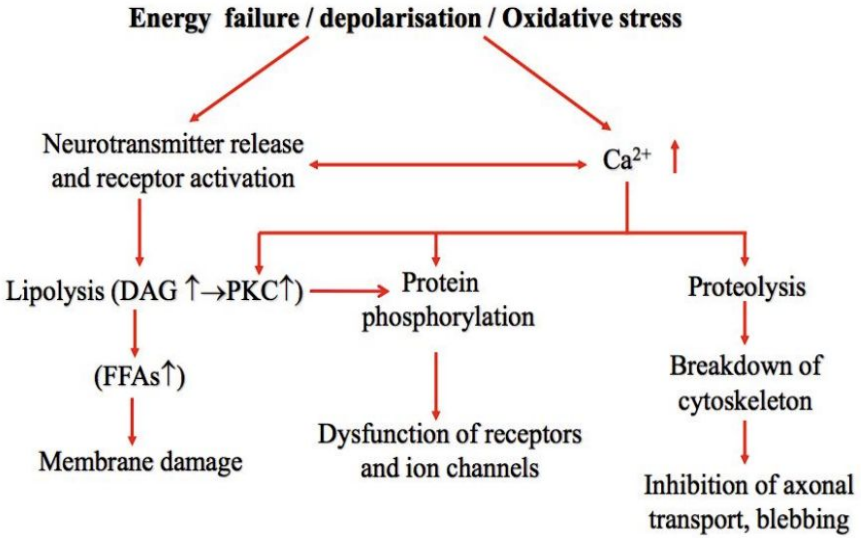
↓

Further depolarisation of cells

↓

Further calcium influx

Consequences of brain ischemia



Quiz

MCQs :

Q1: The brain is highly susceptible to ROS-induced damage because of:
a) High levels of antioxidants b) High oxygen consumption c) Low levels of iron

Q2: The (ROS) & (RNS) are mainly generated by:
a) Microglia b) Macrophages c) Astrocytes d) A and C

Q3: Which of the following NOS isoforms produces beneficial NO?
a) eNOS b) iNOS c) nNOS d) All of them

Q4: A result on energy depletion due to ischemia is:
a) Alkalosis b) Acidosis c) Na+ efflux d) K+ influx

Q5: Which of the following is not an effect of ROS in an ischemic stroke?
a) DNA damage b) Decrease platelet aggregability
c) Increased endothelial permeability d) Inactivation of enzymes

Q6: The enzyme that converts superoxide to hydrogen peroxide is?
a) NADPH oxidase b) Superoxide dismutase
c) Catalase d) Glutathione peroxidase

SAQs :

Q1: enumerate 2 risk factors of ischemic stroke

Q2: enumerate 3 molecular & vascular effects of ROS in ischemic stroke

Q3: Name 2 neurotransmitters that increase following cerebral ischemia?

Q4: Describe the ischemic cascade

★ MCQs Answer key:

1) B 2) D 3) A 4) B 5) B 6) B

★ SAQs Answer key:

1) Smoking, high BP

2) Molecular: DNA damage, protein denaturation, enzyme inactivation / vascular: altered vascular tone & cerebral blood flow, increased platelet aggregability, increased endothelial cell permeability

3) Glycine, GABA

4) Lack of oxygen supply to ischemic neurons → ATP depletion → Malfunctioning of membrane ion system → Depolarisation of neurons → Influx of calcium → Release of neurotransmitters, activation of proteases → Further depolarisation of cells → Further calcium influx

Team members

Girls Team:

- Ajeed Al-Rashoud
- Alwateen Albalawi
- Amira AlDakhilallah
- ★ Arwa Al Emam
- Deema Almaziad
- Ghaliah Alnufaei
- ★ Haifa Alwaily
- ★ Leena Alnassar
- Lama Aldakhil
- Lamiss Alzahrani
- Nouf Alhumaidhi
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- Sarah Alkhalife
- Shahd Alsalamah
- Taif Alotaibi

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- Alkassem Binobaid
- Naif Alsolais
- Omar Alyabis
- Rayyan Almousa
- Sultan Alhammad
- Tariq Alanezi

Team Leaders

Lina Alosaimi

Mohannad Alqarni

★ Excuses sound best to the person who's making them up.

We hear you