جامعة الملك سعود
King Saud University

"اللَّهُمَّ لا سَهْلَ إلاَّ ما جَعَلْتَهُ سَهْلاً، وأنْتَ تَجْعَلُ الحَزْنَ إذَا شِئْتَ سَهْلاً "

Biochemistry437

Pathogenesis of Cerebral Infarction

Biochemistry Team 437

Color index:
Doctors slides
Doctor's notes
Extra information
Highlights

Neuropsychiatry block

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MED437
KING SAUD UNIVERSITY

Objectives:

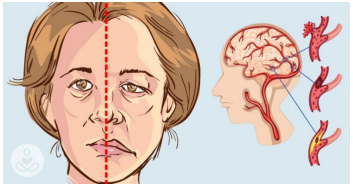
By the end of this lecture, the students should be able to:

- 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

Stroke

- **Hemorrhagic**
  Global incidence: 32%
  - Intracerebral
  - Subarachnoid
- **Ischemic**
  Global incidence: 68%
  Because of occlusion or blockage
  - Thrombotic[1]
  - Embolic[2]



1: A blood clot that forms in the brain, Most common cause is atherosclerosis.
2: Clot formed somewhere else in the body then travels to blood vessels that supply the brain.

Risk Factors of Strokes

There are a number of risk factors for stroke:

Some increase the risk of one type of stroke (hemorrhagic or ischemic).

Occasionally, strokes occur in people who have **no** risk factors.

Some increase the risk of both types.

Ischemic stroke risk factors

- Age older than 40 years
- Heart disease
- High blood pressure
- Smoking
- Diabetes
- High blood cholesterol levels
- Illegal drug use (like cocaine)
- Recent childbirth
- Previous history of transient ischemic attack
- Inactive lifestyle and lack of exercise
- Obesity
- Current or past history of blood clots
- Family history of cardiac disease and/or stroke

Hemorrhagic stroke risk factors

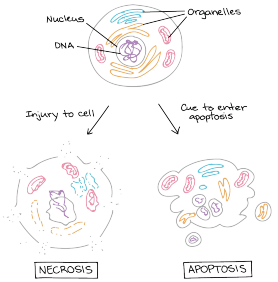
- High blood pressure
- Smoking
- Illegal drug use (especially cocaine and "crystal meth")
- Use of warfarin or other blood thinning medicines

Mechanisms in Cerebral Ischemia

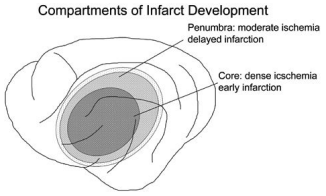
The cell death mechanisms implicated in the pathogenesis of ischemic brain injury includes necrosis and apoptosis

Necrosis and Apoptosis

- Necrosis[1]: is commonly observed early after severe ischemic insults
- Apoptosis[2]: occurs with more mild insults and with longer survival periods
- Which one happens in stroke? it depends upon severity and duration.
- The mechanism of cell death - in both cases - involves calcium-induced calpain-mediated proteolysis[3] of brain tissue
- Substrates for calpain include:
  - Cytoskeletal proteins
  - Membrane proteins
  - Regulatory and signaling proteins



~The pictures are extra



1: Cell death caused by disease, injury, failure of blood supply .
2: Programmed cell death caused by:
- Normal aging of the cells
- Changes in cell physiology, for example mitochondrial dysfunction can release apoptotic factors and initiate apoptosis

3: - Calpain is a calcium dependent protease present in the cytosol.
- Increase in calcium ions activates this enzymes and initiates calpain mediated proteolysis.
- These enzymes target proteins, And has severe consequences regarding cell death.

Presence of both necrosis and apoptosis in cerebral infarction:
- The cerebral infarct contain 2 compartments, a core of severe infarct, and around it an area of moderate infarction with a little blood supply called penumbra.
- The core usually contains necrosis -more severe- While the penumbra contain apoptosis.

Biochemical Responses to Ischemic Brain Injury

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Oxidative Stress | Metabolic Stress | Neurochemical Response

Oxidative Stress

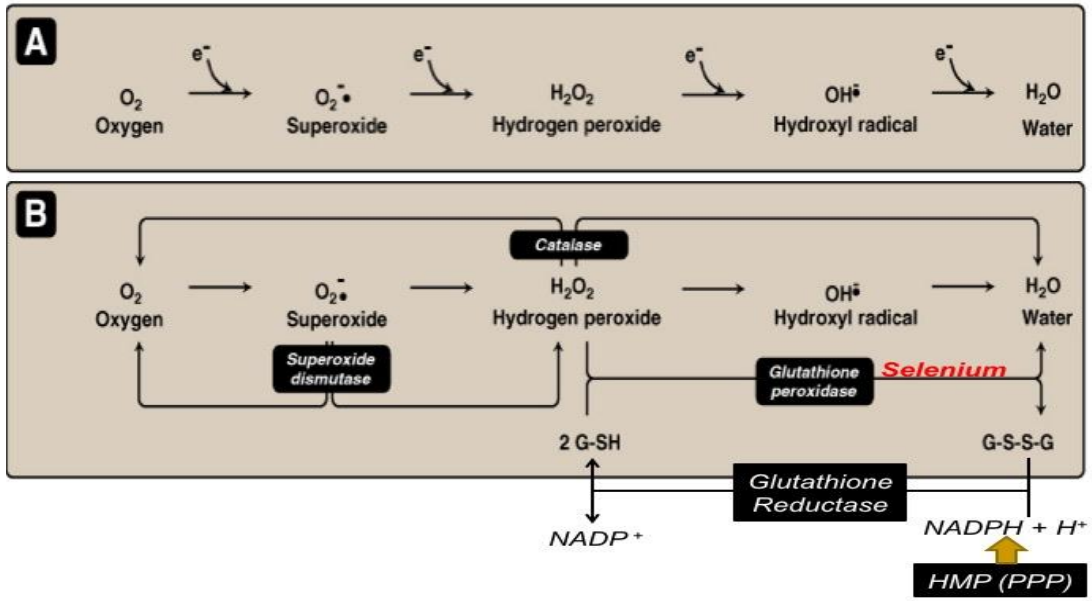
The Role of Reactive Oxygen Species (ROS) & Reactive Nitrate Species (RNS) in Normal Brain Physiology:

- 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

You don't need to memorize the oxidative pathway

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)
- The enzymes required for the cells antioxidant machinery are superoxide dismutase, catalase, and glutathione peroxidase



- Oxygen takes electrons and becomes superoxide which is an anion with an unpaired electron. [unpaired electron = free radical]
- The superoxide takes an electron and becomes hydrogen peroxide which is not a free radical but a ROS and can cause damage, although free radicals are more active than hydrogen peroxide.
- Another electron is given to hydrogen peroxide to give hydroxyl radical.

Antioxidant machinery depends on a number of enzymes:

- **Superoxide dismutase** turns superoxide to hydrogen peroxide and a molecule of oxygen.
- Then the enzyme **Catalase** converts H2O2 to oxygen or water.
- **Glutathione peroxidase** transfers hydrogen peroxide into water "the specific mechanism is explained later"
- Hydroxyl radical has no enzyme to break it only antioxidants and it is short lived.

Generation of Free Radicals

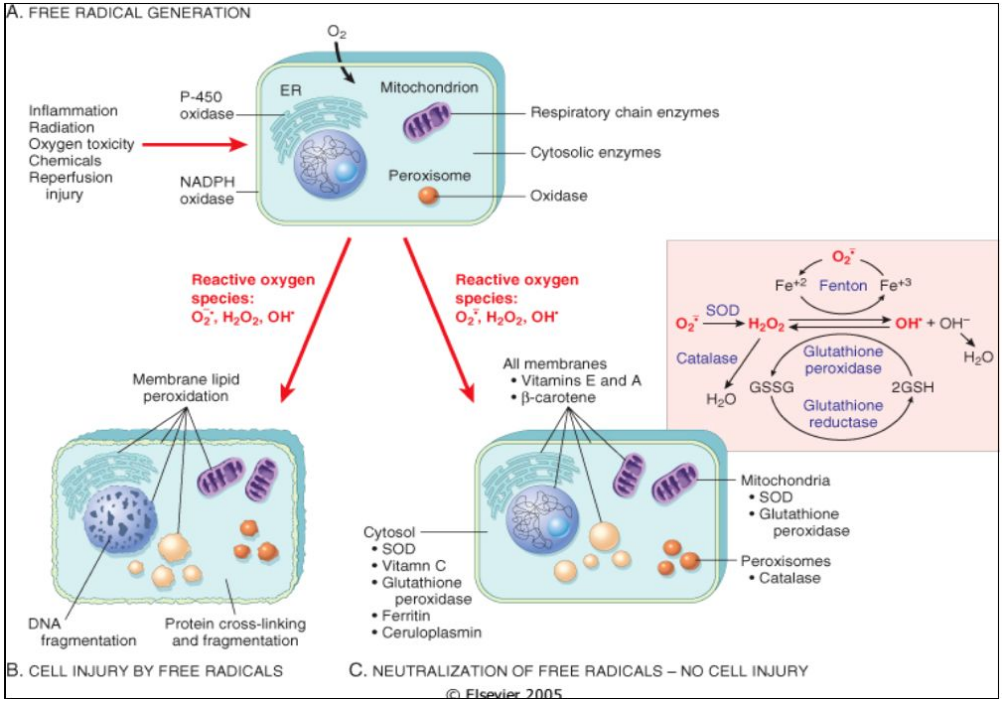
- Because the brain requires a lot of oxygen, it can develop oxygen toxicity. And produce more reactive oxygen species.
- When antioxidants cannot counterbalance oxidants, oxidants (ROS) react with DNA, lipids and proteins producing harmful effects
- These ROS target :
  - The DNA: causing fragmentation
  - Proteins: causing protein cross linking* and fragmentation
  - Membrane lipid: they cause their peroxidation, which starts a chain of autocatalytic reactions that leads to degradation of membrane lipids and ultimately cell death

Protective mechanisms against ROS:

- We have many antioxidants in our body distributed in the membrane, the cytosol and the mitochondria.
- Examples of these antioxidants are:
  - Vitamin A,C,E. - Glutathione peroxidases
  - B-carotene

Fenton reaction:

- Brain cells have a lot of iron
- When this iron reacts with hydrogen peroxide in certain pathological conditions, it produces harmful free radical (it becomes a prooxidant)



*It is where more than one proteins are joined together by covalent bond. And this causes them to be dysfunctional and precipitate

The Brain and Oxidative Stress

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

- High concentration of peroxidizable lipids "saturated fatty acids that can produce free radicals"
- Low levels of protective antioxidants
- High oxygen consumption (due to the metabolism)
- High levels of iron (acts as pro-oxidants under pathological conditions)
- The occurrence of reactions involving dopamine & Glutamate oxidise in the brain

Molecular & Vascular Effects of ROS in Ischemic Stroke

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- DNA damage
- Lipid peroxidation of unsaturated fatty acids
- Protein denaturation
- Inactivation of enzymes
- Cell signaling effects (eg. release of Ca2+ from intracellular stores)
- Cytoskeletal damage
- Chemotaxis

Molecular effects

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

Vascular effects

The Role of NO in the Pathophysiology of Cerebral Ischemia

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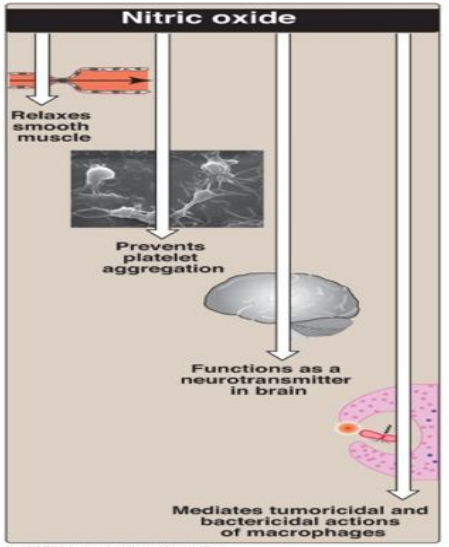
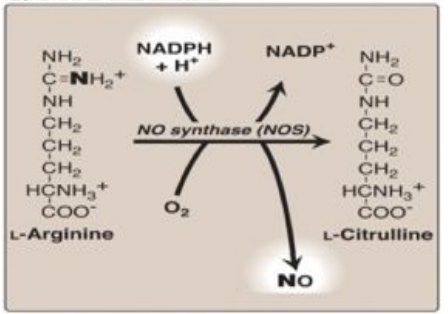
- Ischemia leads to abnormal NO production
  When there is an increase in ROS, Nitric oxide synthase activity also increases. This will lead to increase production of NO from the enzyme arginine.
- This may be both **beneficial** and **detrimental** "harmful", depending upon when and where NO is released
  - NO produced by endothelial NOS (eNOS) improving vascular dilation and perfusion (i.e **beneficial**)
  - In contrast, NO production by neuronal NOS (nNOS) or by the inducible form of NOS (iNOS) has **detrimental** (harmful) effects
- Increased iNOS activity generally occurs in a delayed fashion after brain ischemia and trauma and is associated with inflammatory processes and cell death.

Effects of NO in the body





Metabolic Stress

Biochemical changes in the brain during ischemia

Ischemia → interruption or sever reduction of blood flow, O_2 & nutrients in cerebral arteries which leads to → energy depletion (depletion of ATP & creatine phosphate)

Energy depletion leads to:

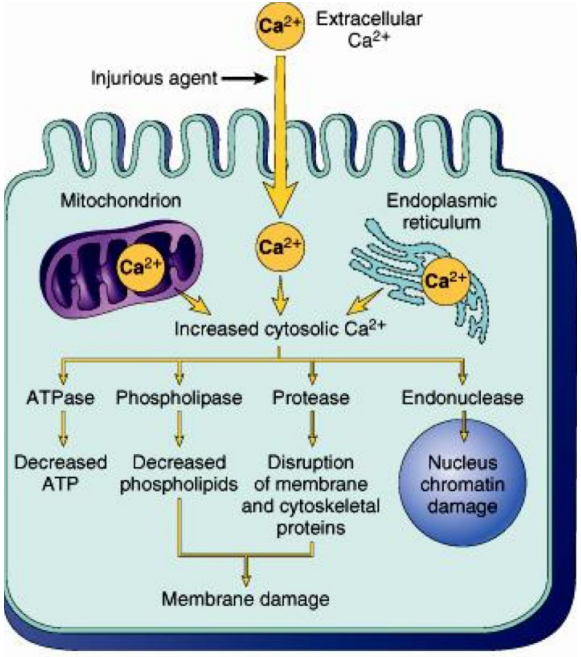
1- Inhibition of ATP-dependent ion pumps, which affect membranes depolarization and Perturbance (disturbance of equilibrium) of transmembrane ion gradients.

- Since there is no ATP, two ATP dependant pumps are going to be affected, the NA/k pump, and the CA^{+2}/NA pump.
- Inhibition of NA/K pump will lead to increase Na^+ Influx and K^+ Efflux.
- Increased NA influx will cause water retention, leading to cytotoxic edema.
- Increased K efflux will cause increase release of excitatory amino acids "mainly glutamate" which will cause neuronal hyperexcitation.
- Inhibition of CA^{+2}/NA channels will cause Ca^{2+} Influx (translocation from extracellular to intracellular spaces) leading to Activation of cellular proteases (Calpins) & lipases leading to breakdown of cerebral tissue.

2- In this case, the cell will have to switch to anaerobic respiration to provide energy, which causes Increased lactic acid in neurons leading to acidosis which promotes the pro-oxidant effect and increases the rate of conversion of O_2^- to H_2O_2 or to hydroperoxyl radical.

Sources & Consequences of Increased Cytosolic Calcium in Cell Injury

Increased intracellular calcium leads to activation of different enzymes which has multiple consequences on the cell “seen in the picture” ultimately leading to membrane damage and cell death.



Neurochemical Response

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The neurochemical response to cerebral ischemia

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

- Glutamate "main excitatory"
- Glycine
- GABA "main inhibitory"
- Dopamine

The Blood tests in patients with brain ischemia or hemorrhage

- Complete blood count, including hemoglobin, hematocrit, white blood cell count, and platelet count
- Prothrombin time[1] , 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[2]
- Blood lipids, including total, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol, and triglycerides.[3]
- Cardiac enzymes and troponin
- Other tests may include MIRIs, motor tests ..etc.

1- Is a blood test that measures how long it takes blood to clot.
2- Anticoagulants
3- To test for atherosclerosis

Biochemical Basis of Pharmacological Intervention

Based on the type of stroke, we use one of these interventions, or a combination of them

Examples of Potential Biochemical Intervention in Cerebral Ischemia:

- Inhibitors of glutamate release
- Ca^{2+} channel blockers
- Nitric oxide synthase inhibitors & free radical inhibition
- Calpain inhibitors

Take Home Messages

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

- Cell death
- Oxidative stress
- Metabolic stress and neurochemical changes

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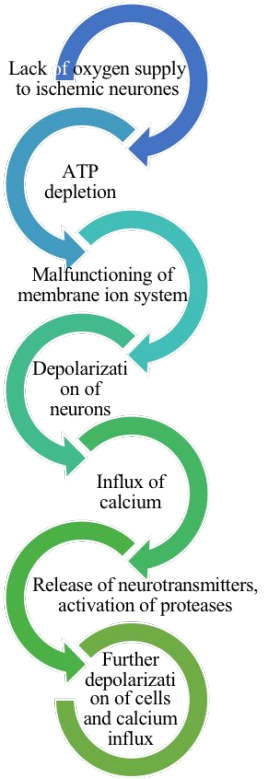
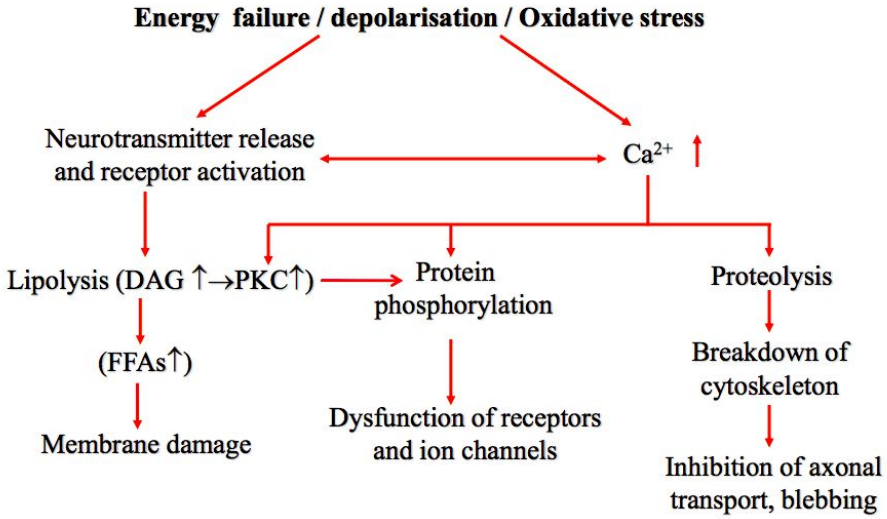
Ischemic cascade



Consequences of brain ischemia



Stroke	Hemorrhagic	Ischemic
Types	1- Intracerebral 2- Subarachnoid	1- Thrombotic 2- Embolic
Risk Factors	1. Hypertension 2. Smoking 3. Illegal drug use	
	✓ Blood thinning medications like Warfarin	✓ Has much more risk factors, thus it occurs more commonly than the hemorrhagic type.

Necrosis	Apoptosis
observed early after severe ischemic insults	In more mild insults and with longer survival periods
Involve **calcium-induced calpain-mediated proteolysis** of brain tissue, and **Calpain** includes many proteins; cytoskeletal, membranous, regulatory, and signaling.	

Oxidative stress	ROS & RNS have important functions in the nervous system. - When cells are exposed to amounts of ROS and RNS, and can't fight them with antioxidants, oxidative stress occurs. - The brain is highly susceptible to ROS damage. - ROS has both molecular and cellular damaging effects. - NO has beneficial vascular effects but harmful neural effects.
Metabolic stress	- Ischemia eventually leads to energy depletion mainly due to inhibition of ATP dependent ion pumps which affects the cell membrane. - Influx: Ca^{2+}, Na^{+} Outflux: K^{+} - Increased lactic acid ➤ acidosis ➤increases conversion of O_2^- to H_2O_2.
Neuro-chemical response	- Extracellular NTs are increased: Glutamate - Glycine - GABA - Dopamine - So as intervention we give inhibitors to Ca^{2+}, Glutamate, NO, free radicals, and calpain.
Required Blood tests	- Complete blood count - Prothrombin time, INR, Activated partial thromboplastin time - Thrombin time, Ecarin clotting time - Blood lipids (HDL, LDL) - Cardiac enzymes and troponin

MCQs: from 436

1) Which of the following cell death mechanisms occurs with more mild insults and with longer survival periods ?

a) Necrosis
b) Phagocytosis
c) Apoptosis
d) None of them

2) Which of the following is not a risk factor for ischemic stroke ?

a) Recent child birth
b) Past history of blood clots
c) Warfarin usage
d) Heart disease

3) The enzyme that converts superoxide to hydrogen peroxide is ?

a) NADPH oxidase
b) Superoxide dismutase
c) Catalase
d) Glutathione peroxidase

4) 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

5) ROS & RNS are mainly generated by ?

a) Microglia and astrocytes
b) Oligodendrocytes
c) Schwann cells
d) Myelin sheath

1-B
2-C
3-B
4-B
5-A

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