MED 443
King Saud University

Biochemistry
MED443 | CNS

L4

Neuropsychiatry Block

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Pathogenesis of Cerebral Infarction at Cellular & Molecular Levels

Editing File

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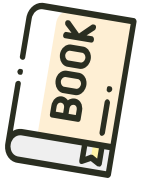
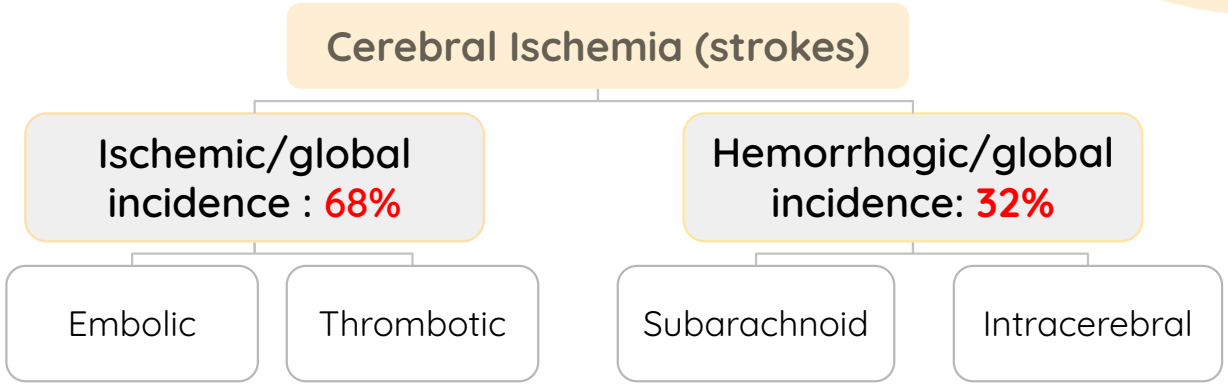
Objectives



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



Risk factors

There are a number of risk factors for stroke: Dr: you don't have to memorize all of them, **3 enough**

- Some increase the risk of one type of stroke (hemorrhagic or ischemic).
- Some increase the risk of both types.
- Occasionally, strokes occur in people who have no risk factors.

Hemorrhagic Stroke risk factors	Ischemic Stroke risk factors
- High blood pressure - Smoking - Illegal drug use (especially cocaine and "crystal meth") - Use of warfarin or other blood thinning medicines Aspirin is used by some individuals in low doses to prevent CVA but there's a debate whether its effective or not, in high doses it can cause hemorrhagic stroke.	- Age older than 40 years. - Heart disease. - High blood pressure. - Smoking. - Diabetes. - High blood cholesterol levels. -Illegal drug use. -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

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

Cell death mechanisms in cerebral ischemia: Necrosis and Apoptosis

Dr's Q: what is the difference between the two types of cell death?

Important!

1

Necrosis:
is commonly observed early after severe ischemic insults

2

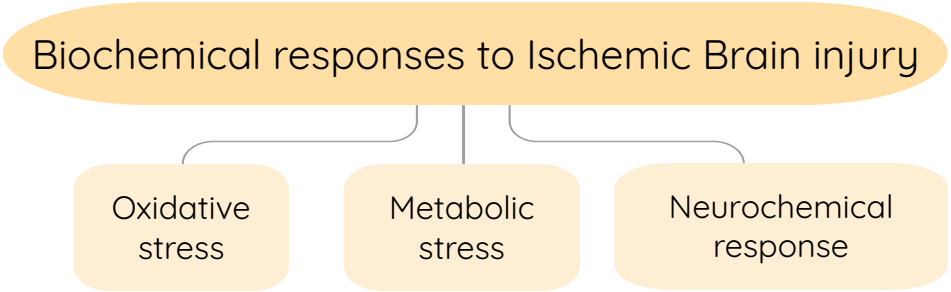
Apoptosis:
Occurs with more mild insults and with longer survival periods

3

Mechanism:
The mechanism of cell death involves calcium-induced calpain-mediated proteolysis of brain tissue Proteolysis means brain protein degradation by enzymes such as Ca-induced- Calpain-mediated proteolysis. This enzyme requires Ca to be activated

4

Substrates of calpain:
- Cytoskeletal proteins
- Membrane proteins
- Regulatory & signaling proteins



-Oxidative stress means when there is imbalance between oxidants and antioxidants inside the cell

-Mainly in **Apoptosis**

Oxidative stress

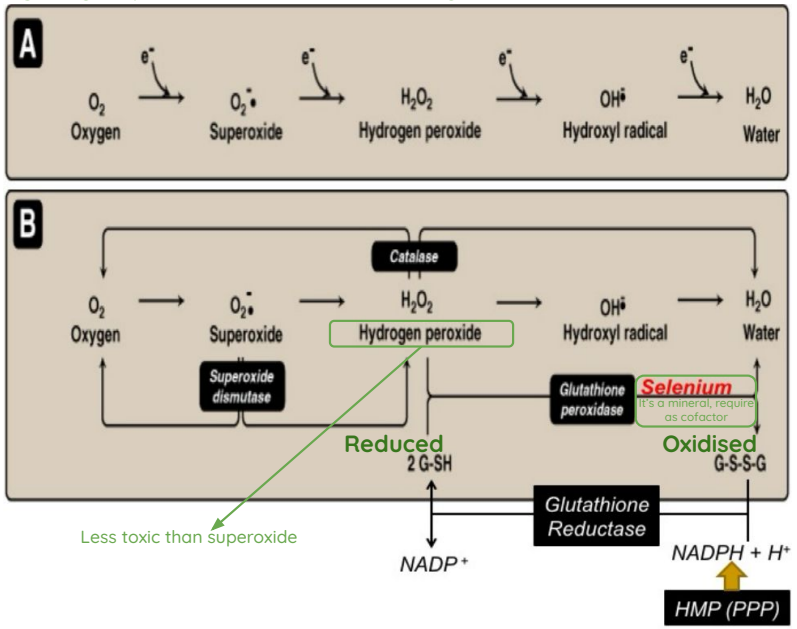
The Role of Reactive Oxygen Species (ROS) & Reactive Nitrative Species (RNS) in Normal Brain Physiology:

- 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

This figure shows antioxidant machinery which contains enzymes that take care of ROS and completely reduced them

-superoxide and hydroxyl radical: very reactive and they are free radicals

-hydrogen peroxide: second messenger and it's not a free radical

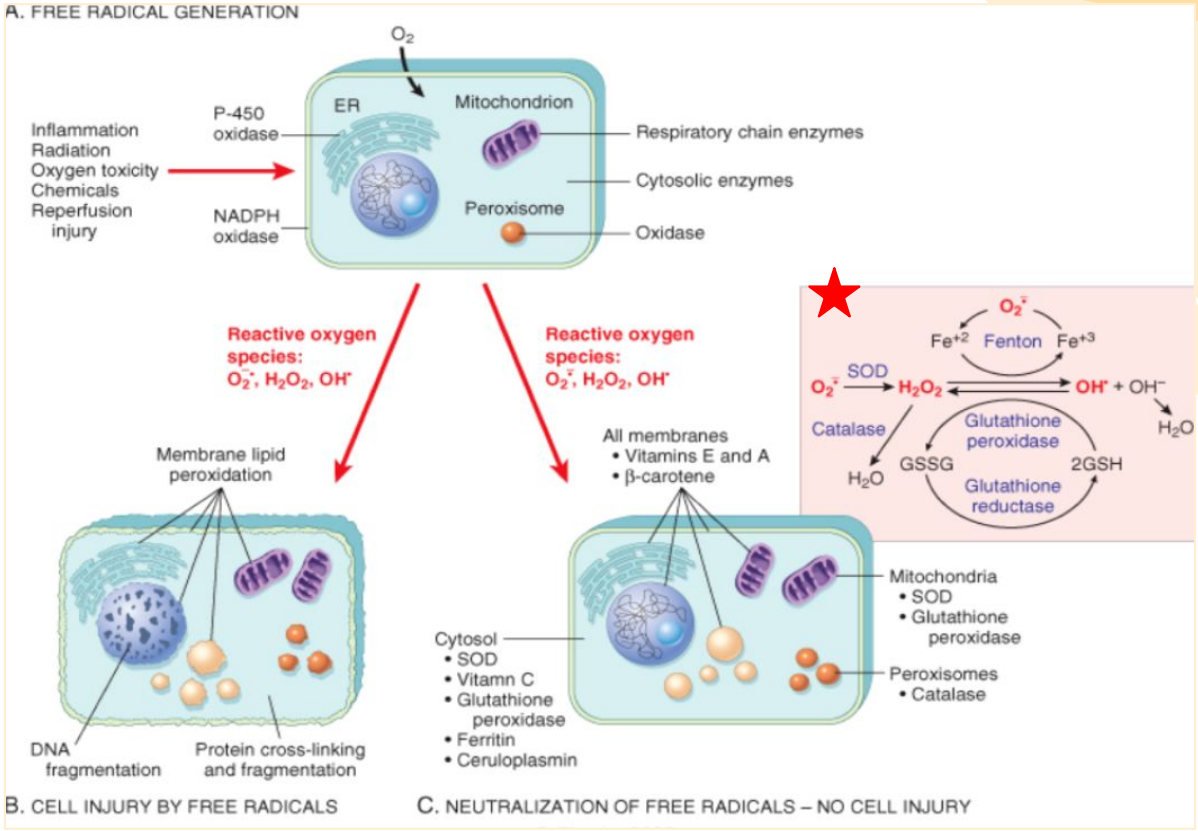


Oxidative stress

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)

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 H2O2 → 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.
-Special thanks to team 438



The brain and oxidative stress

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

Dr: Presence of highly unsaturated fatty acid in the brain tissue that lead to increase the susceptibility of damage by ROS

1. High concentrations of peroxidizable lipids
2. Low levels of protective antioxidants
3. High oxygen consumption
4. The occurrence of reactions involving dopamine & Glutamate oxidase in the brain
5. High levels of iron (acts as pro-oxidants under pathological conditions) Iron in normal amounts helps in anti-oxidation, but it can lead to production of hydroxyl free radicals.

Molecular & Vascular effects of ROS in ischemic stroke

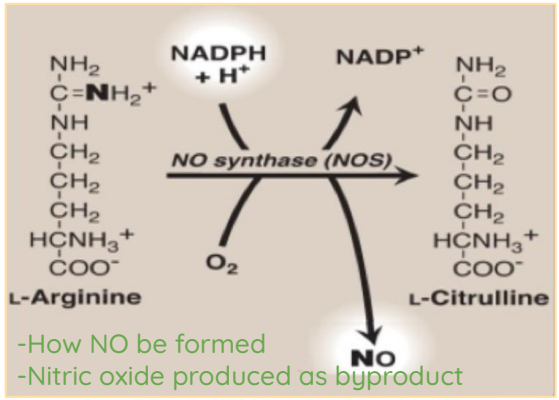
Molecular	Vascular
● **DNA** damage ● **Lipid** peroxidation of **unsaturated fatty acids** ● **Protein** denaturation ● Inactivation of **enzymes** ● Cell **signaling** effects (e.g., release of Ca2+ from intracellular stores) ● **Cytoskeletal** damage ● **Chemotaxis**	● Altered vascular tone and cerebral blood flow ● Increased platelet aggregability ● Increased endothelial cell permeability

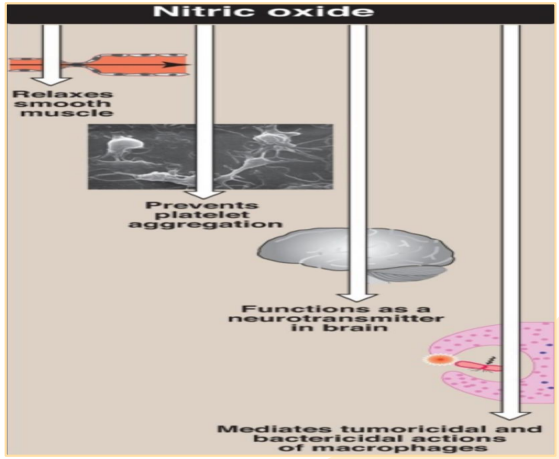
Important in red!

The role of NO in the pathophysiology of cerebral ischemia

NO is a vasodilator

- Ischemia-> **abnormal** NO production
- This may be both **beneficial** and **detrimental**, <u>depending upon when and where NO is released</u>
- ★ 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





Important!
It might come as **MCQ**.

Metabolic Stress

(Biochemical changes in The brain during ischemia)

Ischemia

↓

Interruption or severe reduction of blood flow, O2 & nutrients in cerebral arteries

↓

Energy depletion (depletion of ATP & creatine phosphate)

- Inhibition of ATP-dependent ion pumps
- Membranes depolarization
- Perturbance of transmembrane ion gradients

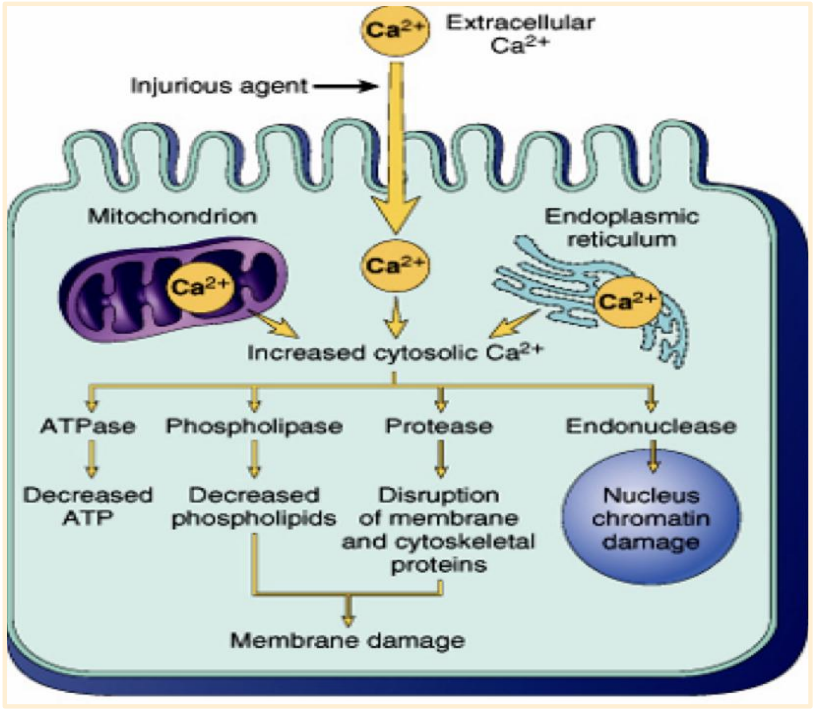
↑ Lactic acid in neurons—> **acidosis**—>promotes the pro-oxidant effect—>↑ the rate of conversion of O2.- to H2O2 or to hydroxyperoxyl radical

Ca2+ Influx (translocation from extracellular to intracellular spaces) -> activation of cellular proteases (Calpains) & lipases-> breakdown of cerebral tissue (calcium-induced calpain-mediated proteolysis)

Na+ influx

K+ efflux
K+ induced release of **excitatory** amino acids

Sources and consequences of increased cytosolic Calcium in cell injury



442 team:Ca comes in from extracellular stores & intracellular stores (mitochondria+endoplasmic reticulum) and affects different enzymes causing the death of cell

Important! Focus on this slide
It might come as MCQ or SAQ.

Neurochemical response to cerebral ischemia

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

- Glutamate
- Glycine
- GABA
- 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

international normalized ratio (INR), and activated partial thromboplastin time(PTT)

Thrombin time and/or ecarin clotting time

if patient is known or suspected to be taking a direct thrombin inhibitor(warfarin)or a direct factor Xa inhibitor

Blood lipids

including total, high-density lipoprotein (HDL), and low-density lipoprotein (LDL) cholesterol, and triglycerides.

Enzymes

Cardiac enzymes and troponin

Important!
Dr: it came before in the exam

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

Biochemical basis of pharmacological intervention

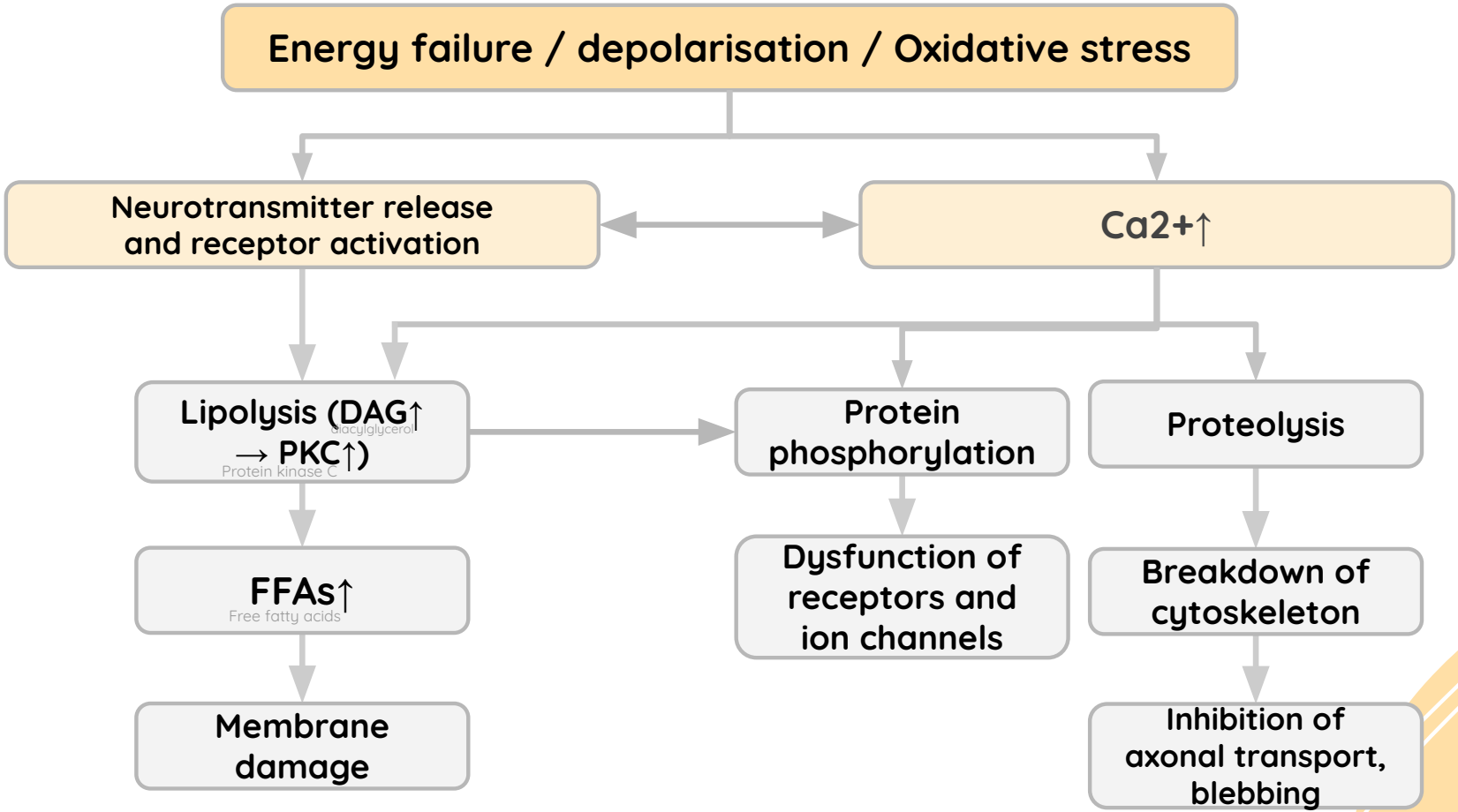
Targets the inhibitors of Glutamate release

Examples of potential biochemical intervention in cerebral ischemia:

- **Inhibitors of glutamate release.**
- **Nitric oxide synthase inhibitors & free radical inhibition.**
- **Ca2+ channel blockers.**
- **Calpain inhibitors.** It cause cell damage

Dr: just for recap what we have studied

Consequences of brain ischemia



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!

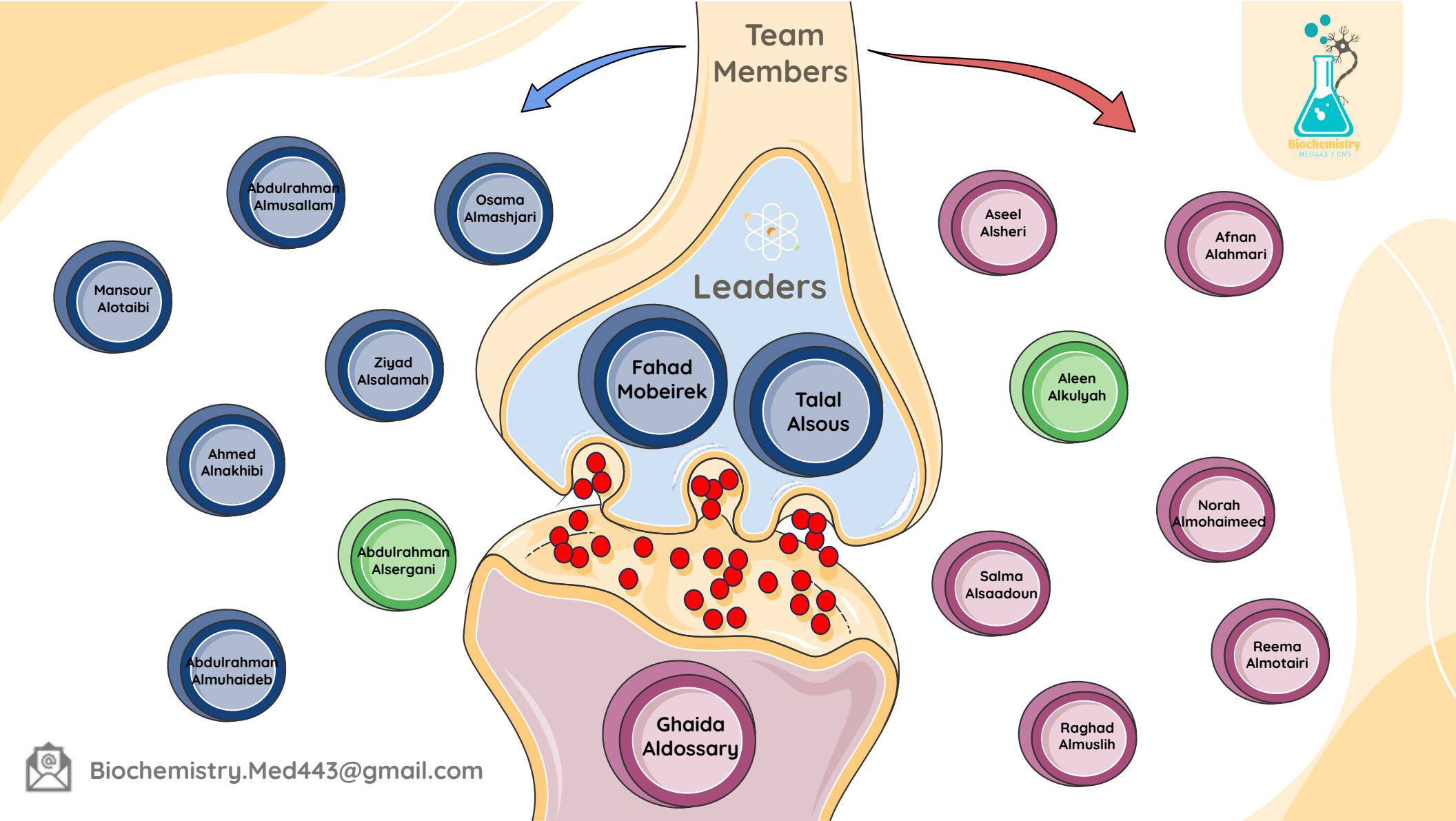
MCQs

Q1: Which of the following is the main event following brain ischemia?							
A	Calcium Influx	**B**	Sodium Influx	**C**	Elevation of PH	**D**	ATP excess
Q2: Which ONE of the following is affected by oxidative free radicals?							
A	Folded proteins	**B**	Ribosomes	**C**	Cell membrane	**D**	DNA
Q3: During periods of increased neuronal activity, reactive oxygen species activate protein kinase C (PKC). Which ONE of the following proteins will be subsequently phosphorylated?							
A	Calpin	**B**	Nitric Oxide	**C**	Myelin basic protein	**D**	Phospholipase
Q4: Severe cerebral ischemic insults lead to a complex cascade of biochemical and molecular events, including:							
A	Cell death	**B**	Oxidative stress	**C**	Metabolic stress and neurochemical changes	**D**	All
Q5:cerebral ischemia, extracellular levels of various neurotransmitters are increased, including:							
A	Glutamate	**B**	Ach	**C**	Norepinephrine	**D**	All

1) A
2) D
3) C
4) D
5) A

SAQ

Q6: List 3 biochemical changes that occur during cerebral Ischemia. (3 changes)	
Answer:	1- Cell death 2- Oxidative stress 3- Metabolic stress
Q7: mention extracellular neurotransmitters that increased in cerebral ischemia	
Answer: slide 11	-Glutamate -Glycine -GABA -Dopamine
Q8: Give tree Examples of Potential Biochemical Intervention in Cerebral Ischemia:	
Answer: slide 14	- Inhibitors of glutamate release. - Ca2+ channel blockers. - Calpain inhibitors.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