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PATHOPHYSIOLOGY OF ISCHEMIA REPERFUSION INJURY: AN OVERVIEW

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ABSTRACT

The Ischemia Reperfusion (I/R) Injury is the leading cause of ischemic heart failure, congestive heart failure, renal failure, and stroke. The various studies explained the various types of pathological pathways associated problems. This study explain the numbers of pathological molecular pathways such as alteration of Na+/K+ pump, endothelial function, mitochondrial level, reactive oxygen species, closing of K_{ATP} channels including activation of leucocytes due to prolong ischemia followed by reperfusion resulting cell death. Most commonly I/R injury are concerned with the variety of organs like Heart, Liver, Lungs, Kidney, brain and Intestine. This review highlights the role of various basic pathological signaling mechanisms involved in the pathophysiology of ischemia/reperfusion injury which can be helpful as a roadmap for preconditioning and postconditioning type survival techniques.

INTRODUCTION

The Ischemia Reperfusion (I/R) Injury is the leading cause of death due to ischemic heart, congestive heart, renal failure and stroke. We have learned about the pathological pathways exacerbate I/R injury. It has been debated whether ischemia is responsible evoked at the start of reperfusion is responsible for the additional cell injuries [1]. However, it is stated that reperfusion has lethal qualities or abilities which evokes tissue injury is called as reperfusion injury [2]. Therefore, it is believed that the controversy has surrounded the destructive role played by reperfusion. Until recently the predominating review was that cell death occurred largely during ischemia, fundamentally as a result of ATP synthesis depletion and its multiplication consequences [3,4]. Although, for the past decades, it has been believed that reperfusion is a very important phenomenon for protecting the cell injury by restoring ATP depletion. However, the question arose at this statement what about the cell that died during reperfusion [5]. The existence of lethal reperfusion injury as a separate entity is controversial, with some comments suggesting that reperfusion exacerbates the cellular injury sustained during the ischemic period. Other studies indicate that the oxidative stress and abrupt metabolic changes that accompany reperfusion can initiate cellular injury. As, Ischemia reperfusion injury is the pathological conditions characterized by an initial restriction of blood supply to an organ followed by subsequent restoration of blood flow and oxygen supply to that organ [6]. Reperfusion participates in causing the serious injuries to the cells after the prolonged period of ischemia called as ischemia-reperfusion (I/R) injury [7]. Reperfusion initiates the pathways of acute inflammatory events which results in impairment of tissue functions and necrosis that leads to cell death. Most commonly I/R injury concerned with the organs like Heart, Liver, Lungs, Kidney, and Intestine. It also occurs in skeletal muscles which considers as a major problem results in local as well as systemic problems like crush syndrome [8]. I/R injury occur because of the interaction of inflammatory and immunological signaling pathways causes the accumulation of free radicals. These accumulated free radicals results in the migration of white blood cell which stimulates the inflammatory mediators at the time of reperfusion [8]. The major reason of I/R injury is the oxidative stress that would be originated due to the imbalance between accumulated free radicals and endogenous scavenging system [9]. Reperfusion causes micro vascular dysfunction that evokes the inflammatory responses after I/R which further lead to the systemic inflammatory response syndrome (SIRS) or multiple organ dysfunction syndromes (MODS) [10]. It has been noted swelling, mitochondrial clarification, calcium overload;

necrosis types morphological changes in I/R injury [11]. In the present review, the mechanisms involved in pathophysiology of I/R Injury are vitally discussed.

Pathophysiology of I/R-Injury

The pathophysiology of I/R injury is a roadmap for the new paradigms in the area of heart, brain, liver and kidney research. I/R-injury pathways involved in many situations such as re-plantation, free tissue transfer, myocardial infarction, stroke, organ transplantation [12].

Role of Na+/K+ Pump in I/R- Injury: It is noted that inhibition of Na^+/K^+ pump during ischemia resulting sustained increased of intracellular concentration of Na^+. Now cell tries to regain its ion homeostasis by activating Na^+/Ca^{2+} exchanger which expels Na+ out of the cell but it increases the intracellular concentration of Ca^{2+}. At the time of reperfusion, the re-oxygenation of cells at risk can precipitate an abrupt worsening of cation control, mainly by intracellular acidosis correction mechanisms that further worsen cytosolic Na^+ overload [13]. The return of blood flow rapidly washes out the catabolites (basically H^+) from the extracellular matrix, which leads to a pH gradient between the cells and their environment, thereby activating the mechanisms of correction of intracellular acidosis. This corrective response to intracellular acidosis further worsens the cytosolic Na^+ overload by activating reverse Na^+/Ca^{2+} exchange which causes an additional influx of Ca^{2+}. The Ca^{2+} influx through reverse mode Na^+/Ca^{2+} exchange is of little relevance in physiological conditions in human myocytes, but can be detrimental when the cell is overloaded with Na^+ [14]. As a result of this chain, a great amount of Ca^{2+} accumulates in the re-oxygenated cell, thus seriously compromising its own survival [15].

Role of Mechanical fragility in I/R-Injury: Ischemia developed the mechanical fragility which can further reduce the resistance of the cell against the mechanical stress developed during reperfusion. It is noted that calpain activation leads to the accumulation of calcium and proteolysis of the sarcolemma cytoskeleton during reperfusion resulting membrane fragility. Moreover, calpain degrades the ankyrin (a protein that helps in anchoring Na^+/K^+ATPase) resulting destruction of Na^+ pump due to Na^+ overload activates of Na^+/Ca^{2+} exchanger leads to intracellular accumulation of calcium leads to the opening of the mitochondrial permeability transition pores (MPTP) which results in apoptosis or necrosis of cell lead to cell death (Fig. 1) [16,17].

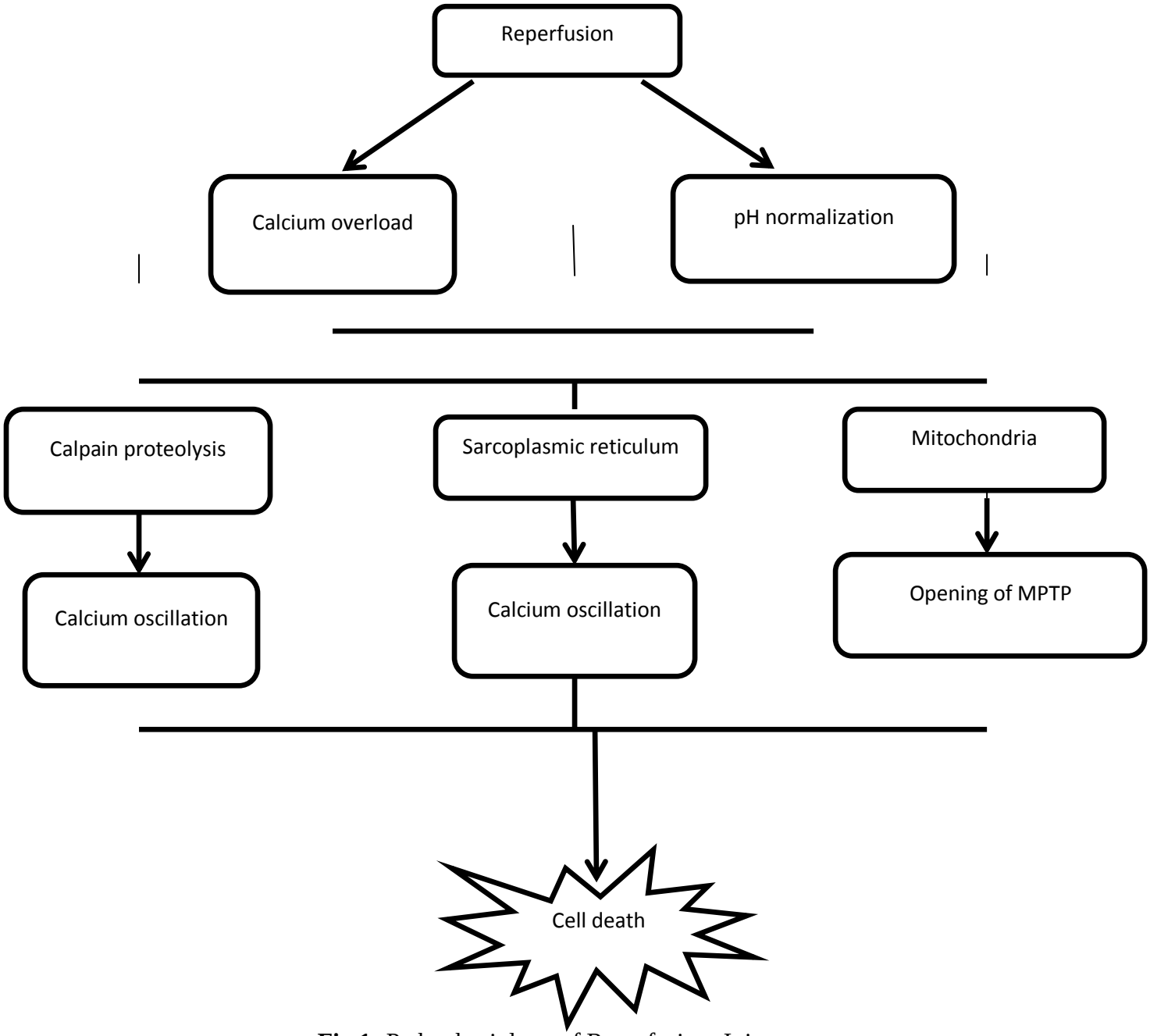

Fig 1: Pathophysiology of Reperfusion- Injury

MPTP indicates mitochondrial permeability transition pores

Role of Endothelial Cell and Reactive Oxygen Species in I/R-Injury: The high level of ROS is noted in I/R Injury. The episode of ischemia and reperfusion activates injury to endothelial cells, mitochondria, muscle fibers and neuronal axons due to high oxidative stress. These injuries cause functional and structural changes of the cells due to restriction in the blood flow at a particular area [18]. ROS is an important signaling molecule in I/R-Injury and increased level of ROS destructs the structure of DNA, vascular tissue, oxidation of protein, lipids, inhibits the growth of cell and vascular injury leads to cell death [19].

Superoxide is one of the free radical which plays a major role in the progression of diseases like atherosclerosis, coronary artery disease, hypertension and diabetes mellitus [20]. However, the ROS that would be generated at the time of oxidative stress are superoxide anion, hydroxyl radicals, peroxynitrite, nitric oxide, lipid peroxyl and lipid alkoxyl radicals. The major elevator of oxidative stress is tripeptide glutathione. However, reactive species are able to modify cell function by inhibiting ion channels, calcium pump and Na^+/K^+ATPase [16]. Moreover, mitochondria play a major role in the formation of ROS result in impairment of electron transport chain which leads in mitochondrial dysfunction. However, I/R injury further responsible for endothelial destruction. It is noted that reperfusion caused microvascular dysfunction, increased fluid filtration, axonal destruction, inflammation and leukocyte plugging in capillaries. Moreover, ROS activate neuronal cell cyclooxegenase and induced electrolyte imbalance at the cellular level [21,22].

Role of KATP channels (Kir6) in I/R-Injury: KATP channels are the molecular sensitivity channel that plays the important role in the cardioprotective effect when the cardiac muscles are under the stress of I/R injury [23]. KATP composed of Kir6.2 subunit is localized in myocyte (inner mitochondria) and Kir6.1 subunit is localized in smooth muscle cells [24,25]. These channels are known to be very sensitive for production of oxidative stress in case of ischemia [26]. The study reported that ischemia closes the KATP channels leads to inhibit the inward rectifies potassium current, result increase the intracellular ADP/ATP and calcium concentration. Moreover, the down-regulation of KATP leads to activation of neutrophils and inflammatory mediators due to high oxidative stress leads to apoptotic cell death [27].

Role of I/R injury in Spinal Cord: Spinal Cord I/R injury is the most devastating complications encountered in many pathophysiological situations such as Hypotension, Surgical procedures on thoracic, thoracoabdominal aneurysms and the Spine [28, 29]. It remains as a widespread and persistent problem, because it's debilitating injuries to the CNS. The blood in spinal cord act as barrier surrounds by astrocyte and perivascular microglial, consist of continuous capillary endothelium with the tight junctions between the cells and barrier disrupt in I/R injury [30]. Researchers have expanded into the glial/neuronal transmission and immune responses of resident glial cells to spinal cord injury. Spinal glial activation involves important components of the immune system and triggers a rapid signal transduction cascade of transcription factor nuclear factor κB, during gene expression of proinflammatory cytokines e.g. IL-1 in the course of pathological changes that occurs after brain injury. During pathogenic cascade after CNS injury, microglial are thought to be the

first non-neuronal cells to express a plethora of growth factors, chemokine as well as free radicals and other toxic mediators [31]. In microglial, toll like receptors (TLRs) have been shown to recognize various microbial products and to initiate innate immune responses upon interaction with infectious agents or endogenous ligands present in the spinal cord [32]. Ischemia is regarded as a powerful stimulus that disabled the endogenous inhibitory signaling and triggers microglial activation.

Upon activation, microglial could exhibit plenty of phenotypes and release both pro and anti-inflammatory mediators to exacerbate I/R injury [31].

Role of I/R in liver transplantation: The noted high degree oxidative stress in I/R-Injury activates the hydrolytic enzymes and swelling of kupffer cell sinusoidal endothelial cells [33]. Ischemia of hepatocytes also contributes to the imbalance between nitric oxide and endothelin production, causes narrowing of sinusoidal lumen, accumulation of neutrophils results in microcirculatory dysfunction. The Kupffer cells are activated resulting high release of ROS, proinflammatory cytokines, adhesion molecule, chemokine, neutrophils, TNF-alpha, Platelet activating factors, LTB4, IL-1 in I/R Injury leads cellular injury (Fig. 2) [34,35,36,37].

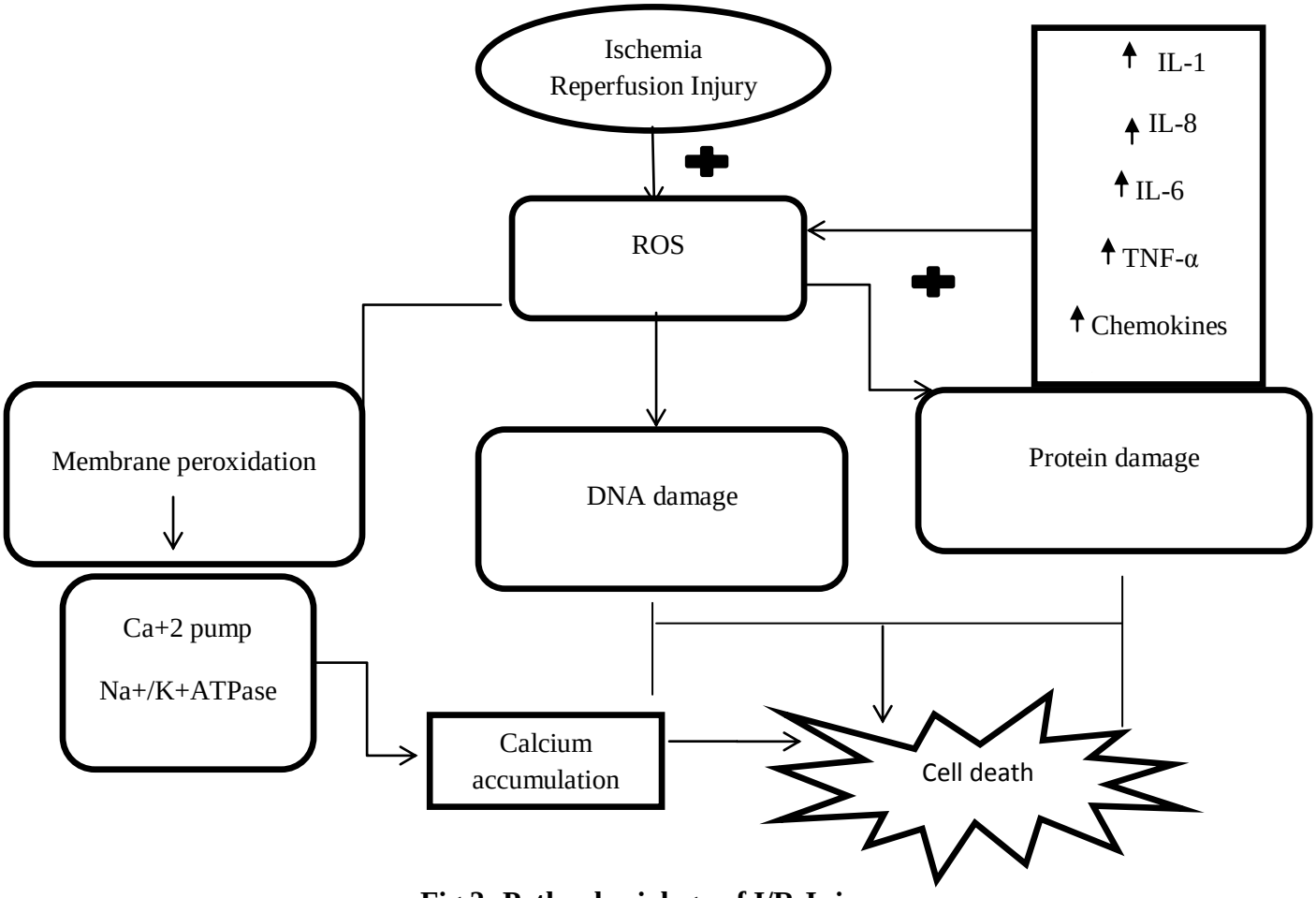

Fig 2: Pathophysiology of I/R-Injury

Role of Oxidative Stress in Mitochondria of Cell: Energy is produced from one of the organelles present in cytosol are Mitochondria. Mitochondria are membrane-enclosed cellular structure. However, the "Inner Mitochondrial Membrane" participates in the energy generation process which is also called as Oxidative Phosphorylation. Moreover, the inner mitochondrial membrane consists of redox complexes for respiratory system and phosphorylation apparatus for oxidative phosphorylation [38]. Therefore, inside mitochondria the ATP synthesis is feasible from the oxidation of nutrients like FFA and pyruvate, which are byproducts of glycolysis or lactate [39]. However, I/R injury results in increasing the lipid peroxidation in the cell leads to destruction of cell membrane. Moreover, Lipid peroxidation destructs the mitochondrial structure results in inhibiting the ATP synthesis within the mitochondria [40]. The production of excessive free radical worsens cells by DNA damage, chromosomal divergence and unusual cell death. Therefore, mitochondria predominantly show a remarkable increase in malondialdehyde (MDA) and calcium overload during I/R injury and decreases reduced glutathione. However, mitochondrial injury appreciably decreased the oxidative phosphorylation of cells, eventually inhibits the synthesis of ATP [41]. Moreover, the major source of apoptotic cell death is mitochondria. Apoptosis due to activation of Fas signaling pathway induced the mitochondrial cell death which is further caused by the release of pro-apoptotic proteins that are located in the inner membrane of mitochondria [42].

Role of leukocyte in I/R Injury: Leukocyte plays a vital role in innate immunity. It is increased in case of infection, inflammation, infarction and I/R Injury because its activation brings a series of changes, i.e. Chemotaxis, Leukocyte Endothelial Cell adhesion, Transmigration [43]. I/R Injury results in raising the level of Endothelial P-Selectin (CD62P), it interacts with the leukocyte counter receptor P-Selectin Glycoprotein-1 (PSGL-1), which causes "leukocyte rolling" [44]. When Leucocyte β2 integrins CD11a/CD18 and CD11b/CD18 interacts with the intracellular adhesion molecule-1 (ICAM-1) causes adhesion of leukocyte to endothelium [45]. Platelet endothelial cell adhesion molecule-1 (PECAM-1) facilitates the leukocyte transmigration via endothelial cell junction in the interstitial fluid of the cell [46, 47]. After the transmigration, activated leukocyte promotes generation of toxic ROS, Proteases and Elastases, resulting increases the microvascular permeability which causes accumulation of fluid that results in edema and thrombosis results parenchymal cell death. It is noted that hypoxial tissues secrete IL-8 which activates the accumulation of polymorphonuclear leukocyte (PMN) in extravascular compartment [10,48].

Role of complements in I/R-Injury: Complement activation occurs during I/R. I/R injury promote the formation of inflammatory mediators, including anaphylotoxins, C3a and C5a. Other complement components which are more potent than C3a and C5a include iC3b, C5b [49]. Complementary components directly stimulates leukocyte activation and chemotaxis, C5a involved in the inflammatory response to I/R Injury results in the formation and secretion of several proinflammatory cytokines i.e. IL-1, IL-6, monocyte, chemoattarctant protein-1 (MCP-1) and tumor necrosis factor α (TNF-α). C5b-9 and iC3b affects the functions of vascular endothelium [50,51,52]. Leukocyte adhesion is induced specifically by iC3b which is the cleaved form of C3b. However, C5b-9 inhibits the endothelium dependent relaxation and decreases endothelial cyclic guanoside monophosphate [53].

Role of Apoptosis in I/R Injury: Apoptosis plays major and essential role in the pathophysiology of I/R injury. Generally, Ischemia of long duration results in necrosis of cells and reperfusion promotes the apoptosis of the cell. Intrinsic and Extrinsic pathways of apoptosis are equally involved in I/R injury [54]. During apoptosis, cell starts shrinking and enhances the phagocytosis of the cell by extracellular secretion of ATP from apoptotic cells through Pannexin Hemichannels which will attract the phagocytes. In case of kidney's I/R injury, causes the secretion of Matricellular Protein Thrombospondin 1 from proximal tubular cells that will act as the activator of apoptosis [55]. However, usually under the normal conditions the components of the apoptosis resist inside the every living cell as inactive component. Activation of the caspase cascade is essential for preceding the apoptosis. Moreover, damaged mitochondrial membrane secretes proapoptotic factors like Bcl, Bax and Bim that would lead to the release of cytochrome C, smac and Omi in the cell [42]. However, apoptosome is formed when cytochrome C interacts to the apoptotic protease activating factor 1 (APAF-1). The Apoptosome is the heptamer complex that is also known as Apoptotic Proteases Factor 1 Cytochrome C complex. Caspase 9 is the principal component for apoptosis, which on cleavages produces caspase 3 and caspase 7 which also mediates the induction of apoptosis (Fig. 3)[56,57].

Role of GADD34 in I/R Injury: Growth arrest and DNA protein 34 (GADD34) is a cell cycle protein. It impairs the function of cell cycle. It is noted that increase level of GADD34 leads cell cycle arrest, DNA damage and endothelial dysfunction. It has been concluded that GADD34 dephosphorylate stress associated protein synthesis inhibition. However, Ischemia regulates the expression of GADD34 [58,59]. On the other hand, recent study showed that expression of GADD34 was increased at the time of I/R injury [60]. Furthermore, study also

detected that this gene expression reached at the peak after one hour of reperfusion, which further proved that reperfusion could induced endoplasmic reticulum stress [61].

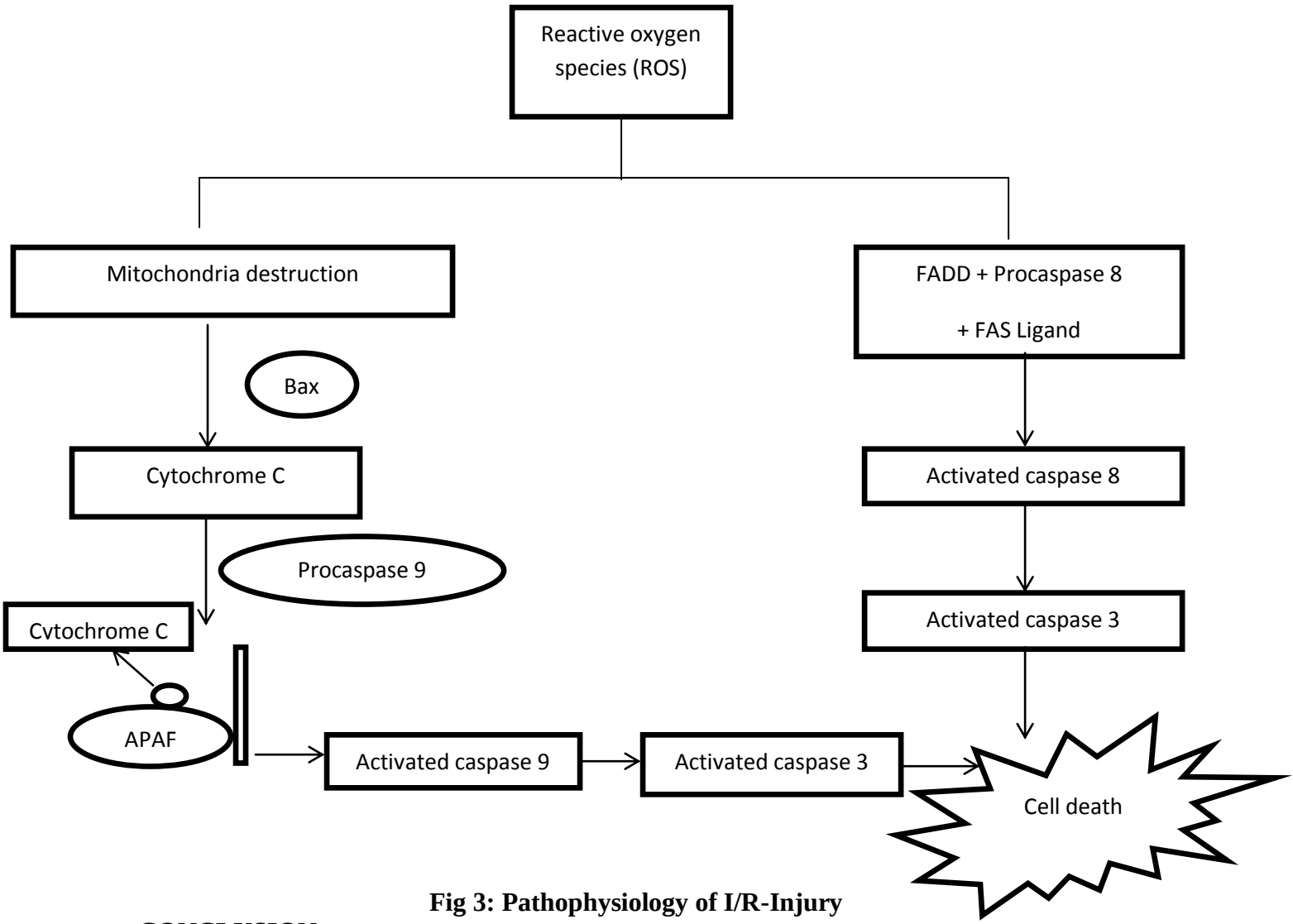

Fig 3: Pathophysiology of I/R-Injury

CONCLUSION

In the present study we have concluded that I/R injury has contributed to adverse outcomes that can be seen in various organs like heart, brain, lungs, liver, kidney, intestine, and skeletal muscles. There are various pathological pathways i.e. Na+/K+ pump inhibition, endothelial dysfunction, activation of ROS, leukocytes, complements, closing of K_{ATP} channels, mitochondrial damage, prolong ischemia, reperfusion induced apoptosis involved in necrosis of the cells leads to cell death. In addition, studies are obligatory to recognize the major signaling mechanisms involved in the pathological effects of I/R- injury, which may open a novel vista to use pharmacological interventions in the name of pharmacological preconditioning to limit lethal I/R or reperfusion injury during cardiovascular related surgery and stroke like complications.

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