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An overview of some ischemia/reperfusion models created in rats and the application procedure of these models

Volkan Gelen[1*]

[1]Department of Physiology, Faculty of Veterinary Medicine, Kafkas University, Kars, Türkiye
*Corresponding: gelen_volkan@hotmail.com

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Abstract
As a result of damage induced by ischemia-reperfusion in organs and tissues in various conditions such as sepsis, open surgeries, tissue transplantations, organ tumors, and shock, organ damage, and related dysfunction occur. Recently, various studies on experimental animals have tried to develop agents that can prevent or treat ischemia-reperfusion injury. Understanding the mechanism of damage caused by ischemia-reperfusion in tissues and organs will be useful in developing an effective treatment method in this case. In addition, the feasibility of ischemia-reperfusion models in rats and the correct application of the methods of these models will increase the probability of success in preventing or treating organ dysfunction that may occur in this case. For this reason, in this study, we tried to give a general summary of the mechanism of damage in the experimental ischemia-reperfusion model in rats and the method of applying the ischemia-reperfusion model created in various organs/tissues. Because fully understanding the complex cellular events from the onset of ischemia to the occurrence of acute or chronic damage and the correct application of the model can provide us with new perspectives on the methods to be followed in treatment.

Keywords: *Ischemia, reperfusion, rat, tissue damage*

1. Introduction

Ischemic injury of the cell is the most common type of cell injury in clinical practice and has therefore been investigated in many clinical and experimental studies.[1,2] It can occur with the occlusion of the vein that goes directly to the tissue, as well as with the decrease of systemic blood circulation to critical levels.[3,4] The cell renews itself if blood circulation is restored without irreversible damage.[5] However, if irreversible damage has occurred to the cell in relation to the severity and duration of ischemia, the restoration of blood flow may increase the damage rather than repair it. Thus, ischemia-reperfusion (I-R) injury occurs.[6,7] Ischemia-reperfusion injury; The sequence of cellular events triggered by insufficient oxygen (O_2) supply to the tissues, which concerns all organs and systems, and is one of the most researched subjects in recent years. Oxidative stress occurs as a result of insufficient O_2 and other metabolic requirements to be delivered to the tissues.[8,9] Oxidative stress can cause serious clinical consequences, up to multiple organ failure. Clinically, I-R injury; gaining importance in surgical procedures, organ transplantation, trauma, and vascular surgery.[10,11] Although reperfusion after ischemia is a prerequisite for the healing of damaged tissues, it can further increase the damage.[12] It has been demonstrated that free oxygen radicals formed in ischemic tissue by reperfusion are responsible for this damage, and it has been proven by a series of experimental studies that reperfusion-related damage can be reduced or prevented with some pharmacological agents.[13] Studies with experimental animals are of great importance in explaining the molecular mechanisms of ischemia-reperfusion injury in various tissues and in the development of medicine.[14] Therefore, in light of this information, we aimed to explain the mechanism of ischemia-reperfusion injury and the method of applying the ischemia-reperfusion model created in various organs/tissues in rats.

2. Molecular mechanism of cell damage resulting from ischemia-reperfusion

Hypoxia, which causes inhibition of oxidative phosphorylation in mitochondria, initiates anaerobic respiration in the cell.[15,16] Hypoxia slows down and stops the formation of adenosine triphosphate (ATP), causing insufficiency of the active sodium pump in the cell membrane and causing intracellular sodium accumulation and potassium excretion from the cell.[17,18] Simultaneous accumulation of solid material is accompanied by isoosmotic water accumulation, resulting in cellular swelling.[19,20] Cellular ATP decrease occurs together with adenosine monophosphate (AMP) increase.[21] This stimulates the phosphofructokinase enzyme and increases anaerobic glycolysis, resulting in the formation of ATP from glycogen. In this way, intracellular energy resources are preserved.[22] However, in glycolysis, intracellular pH decreases with the accumulation of inorganic phosphate formed as a result of hydrolysis from lactic acid and phosphate derivatives.[23] Subsequently, the rough endoplasmic reticulum within the cell is separated from the ribosomes. By increasing membrane permeability, budding occurs on the cell surface.[24] Concentric lamina originating from plasma, such as organelle membranes, is seen in or outside the cytoplasm.[25] The endoplasmic reticulum is enlarged and the whole cell is markedly

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swollen.[26] All of the above disorders are reversible until oxygen is given, but if ischemia continues, irreversible damage begins.[27] Hypoxia affects oxidative phosphorylation and stops the production of vital ATP.[28] It causes lethal membrane damage after the critical point. At the same time, the formation of free radicals originating from xanthine oxidase in the cell during reperfusion, Increased adhesion of neutrophils to the damaged endothelium, Ca^{2+} transport to the organ with energy loss, Insufficient supply of adenine nucleotides in the post-ischemic period, energy deficit in the cell,[29] ATP, ADP, AMP, inosine in ischemia It is broken down to e and hypoxanthine,[30] Normally, hypoxanthine is oxidized to xanthine and uric acid by xanthine oxidase,[31] This accumulation creates an excess of substrate for hypoxanthine oxygenation Since a sudden and large amount of O_2 is provided in reperfusion, oxidation of hypoxanthine to xanthine causes the emergence of superoxide radicals [32,33] (Figure 1).

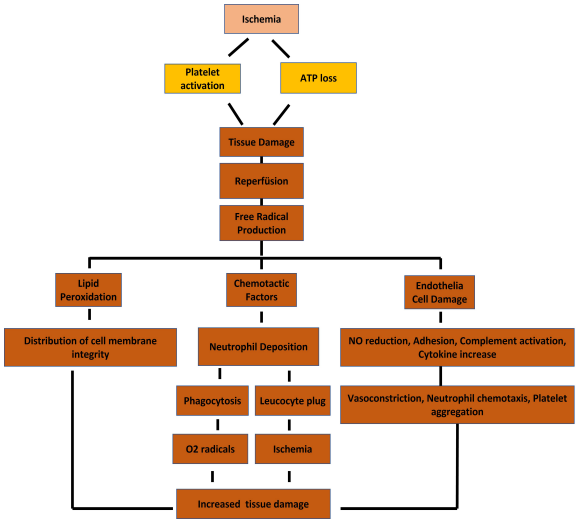

Figure 1. *Mechanism of cell damage resulting from ischemia-reperfusion.[27]*

3. Some ischemia-reperfusion models and application procedures created in rats

When creating an experimental animal model, attention should be paid to model selection.[34] It is very important that the selected model can be best adapted to the clinic.[35] In order for the research to be carried out in accordance with its purpose, it is necessary to know the anatomical, physiological, and behavioral characteristics of the species planned to be used in the experimental research.[36] Rats are among the most used experimental animals in experimental studies.[37] Broadcasting is used in the laboratory due to a number of reasons such as the short breeding period of rats, the fact that many offspring are obtained in one litter and they are easy to find.[38] In addition, they are very suitable for use in such studies in terms of the ease of application of ischemia-reperfusion models in rats and the clinical adaptability of these models.[39] Experimental ischemia-reperfusion models in rats have been developed for many organs and tissues.[39] The most common among these models are the brain, testis, lower extremity, liver, kidney, ovary, intestine, and heart ischemia-reperfusion models. The application procedure for these mentioned modules is as follows.

3.1. Transient focal cerebral ischemia/reperfusion model application procedure in rats

Rats are anesthetized with a combination of intraperitoneal (i.p.) ketamine hydrochloride 75 mg/kg + Xylazine 8 mg/kg. Then, the rats are fixed on the operating table in the supine position. Then the midline of the neck is shaved. After the operation area is disinfected, a midline incision is made. After superficial microdissection, it is advanced to the right common carotid artery with deep microdissection. The common carotid artery is reached by visualizing the trachea and dissecting the paratracheal muscles. Two aneurysm clips are placed on the common carotid artery from 1 cm to 3 cm proximal to the carotid bifurcation to provide proximal and distal control [40] (Figure 2). Clips are kept closed for ten minutes. The opened incisions of the rats are sutured after ten minutes.

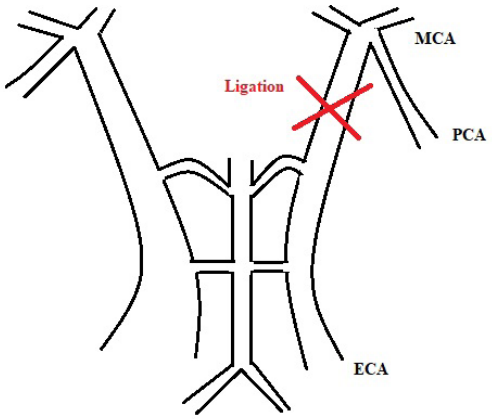

Figure 2. *The carotid region with focal cerebral ischemia. MCA: Middle cerebral artery, PCA: Posterior cerebral artery, ECA: External carotid artery.*

3.2. Renal ischemia/reperfusion model application procedure in rats

Rats are anesthetized with a combination of intraperitoneal (i.p.) ketamine hydrochloride 75 mg/kg + Xylazine 8 mg/kg. In animals whose abdomen is shaved approximately 2 minutes (min) before the operation, the operation area is cleaned with 10% Povidone Iodine. Only the area where the incision will be made is covered in a sterile manner, leaving it open. The right pedicle (artery and vein) is ligated with a right dorsolateral incision and a right nephrectomy is performed. After the right nephrectomy, renal IR injury is created by clamping the left kidney pedicle and applying ischemia for 60 minutes and reperfusion for 24 hours, [41] (Figure 3).

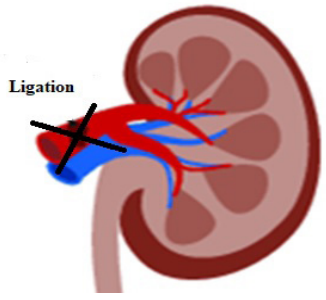

Figure 3. *Renal ischemia/reperfusion model.*

3.3. Procedure for applying lower extremity ischemia/reperfusion model in rats

Rats are anesthetized with a combination of intraperitoneal (i.p.) ketamine hydrochloride 75 mg/kg + Xylazine 8 mg/kg. After anesthesia, a tourniquet is applied to the left or right lower extremity and is created by providing ischemia for 3 hours and then reperfusion for 24 hours,[42] (Figure 4).

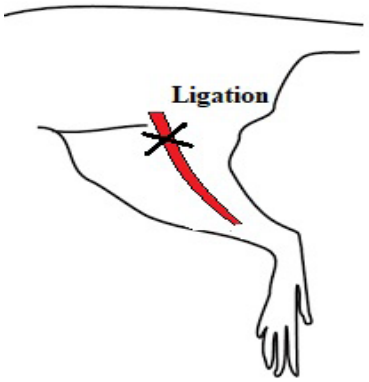

Figure 4. *Lower extremity ischemia/reperfusion model.*

3.4. Ovarian ischemia/reperfusion model application procedure in rats

Rats are anesthetized with a combination of intraperitoneal (i.p.) ketamine hydrochloride 75 mg/kg + Xylazine 8 mg/kg. After dividing the abdominal region, the ovaries are reached by entering the tars. Vascular clamps are placed approximately 1 cm below the adnexal structure containing the right tuba and ovarian vessels, and the incision line is closed with 4-0 nylon (Figure 5). Two hours later, a relaparotomy is performed to ensure that the ovary, where reperfusion takes place, turns pink.[43]

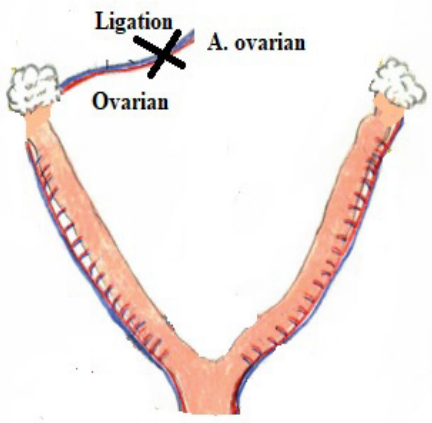

Figure 5. *Ovarian ischemia/reperfusion model application.*

3.5. Intestinal ischemia/reperfusion model application procedure in rats

Rats are anesthetized with a combination of intraperitoneal (i.p.) ketamine hydrochloride 75 mg/kg + Xylazine 8 mg/kg. After shaving the abdominal skin, it is cleaned with povidone-iodine. A laparotomy is performed with a vertical incision of approximately 6 cm from the xiphoid. For the intestinal I-R model, after the intestinal structures are retracted, the SMA is located from the aorta and clamped (Figure 6). After one hour of ischemia, the clamp is opened and reperfusion is provided for 2 hours.[44]

3.6. Testicular ischemia/reperfusion model application procedure in rats

Rats are anesthetized with a combination of intraperitoneal (i.p.) ketamine hydrochloride 75 mg/kg + Xylazine 8 mg/kg. The left testis is torsioned by turning 720° clockwise. To maintain the torsion, the testis is fixed from the tunica albuginea to the scrotum with a 3/0 silk suture. The skin is closed temporarily with sutures. After one hour, the skin sutures are opened and the fixation sutures are removed, and the torsion is corrected by turning the left testis counterclockwise. The incision is closed again with 3/0 silk sutures and reperfusion is achieved for 4 hours [45] (Figure 7).

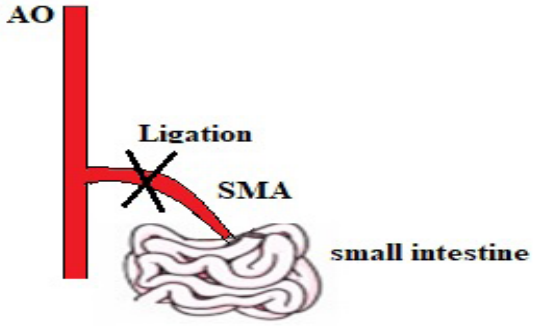

Figure 6. *Intestinal ischemia/reperfusion. AO: Aorta, SMA: superior mesenteric artery.*

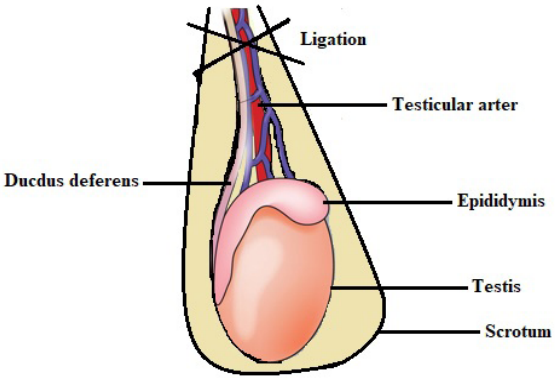

Figure 7. *Testicular ischemia/reperfusion.*

3.7. Liver ischemia-reperfusion model

Rats are anesthetized with a combination of intraperitoneal (i.p.) ketamine hydrochloride 75 mg/kg + Xylazine 8 mg/kg. The hepatic portal vein leading to the liver and hepatic artery is clamped with a bulldog clamp using the pringle maneuver method (Figure 8), which is opened with a midline incision in rats. After 60 minutes of ischemia, 60 minutes of reperfusion is achieved.[46]

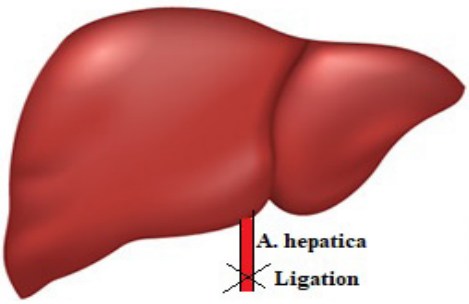

Figure 8. *Liver ischemia-reperfusion.*

4. Conclusion

As can be seen, ischemia-reperfusion injury is a condition that can occur in many tissues as a result of various factors. Knowing the molecular mechanism of ischemia-reperfusion injury occurring in these tissues is important for the development of new treatment options. In addition, learning the most appropriate application procedure of ischemia-reperfusion models that can be created in rats will enable the development of new agents to prevent or treat ischemia-reperfusion injury. In this review article, we tried to explain the molecular mechanism of tissue damage caused by ischemia-reperfusion and the application procedure of some experimentally created ischemia-reperfusion models in rats, using current articles.

Ethical approval

None

Authors contribution

Volkan Gelen: Research, planning, article scanning, writing-original draft & review.

Conflict of interest

There are no conflicts of interest associated with this research publication, according to the authors.

Data availability

Data will be made available on request

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