REVIEW

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Lung damage reduction due to reperfusion injury of acute lower limb ischemia with and without pentoxifylline and vitamin c combination therapy: histopathological evaluation

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ABSTRACT

Several studies report an incidence of acute limb ischemia of approximately 1.5 cases per 10,000 persons per year. The mortality rate from acute limb ischemia ranges from 15-to 20% and the morbidity rate from 25% by amputation and 5-to 25% to fasciotomy. Revascularization is the main goal in the management of acute limb ischemia. Reperfusion of ischemic limbs can save limbs from amputation, but Ischemia-reperfusion injury can also cause systemic damage in the form of damage to body organs (lung, liver, kidney, heart, and brain tissue) which increases morbidity and mortality rates. Lung tissue is an easily affected target organ, and damage from Ischemia-reperfusion injury can cause acute lung injury and acute respiratory distress syndrome. These conditions damage the endothelium and epithelium of the lung parenchyma resulting in edema and impaired gas diffusion with a very high mortality rate of around 25-40%. The combination therapy of Pentoxifylline and Vitamin C in reducing the effect of Ischemia-reperfusion injury on lung tissues and have shown a protective effect of lung tissue damage due to ischemia-reperfusion injury in lower limbs.

Keywords: Acute limb Ischemia, reperfusion injury, lung, vitamin C, pentoxifylline.
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INTRODUCTION

Acute limb ischemia (ALI) is characterized by a rapid reduction in leg perfusion that threatens leg viability and frequently necessitates urgent revascularization.[1] Restoring blood flow or reperfusion to ischemic tissue is essential to prevent permanent cell injury.[2] Revascularization is the main goal in the management of acute limb ischemia. Reperfusion of ischemic limbs can save limbs from amputation, but Ischemia-reperfusion injury can also cause systemic damage in the form of damage to body organs (lung, liver, kidney, heart, and brain tissue) which increases morbidity and mortality rates. Lung tissue is an easily affected target organ, and damage from Ischemia-reperfusion injury can cause acute lung injury (ALO) and acute respiratory distress syndrome (ARDS).[3] These conditions damage the endothelium and epithelium of the lung parenchyma resulting in edema and impaired gas diffusion with a very high mortality rate of around 25-40%.

Pentoxifylline in particular inhibits neutrophil adhesion to the endothelium and prevents leukocyte degranulation.[4] Inhibition of neutrophil adhesion in lung tissue will reduce endothelial and epithelial cell damage in the lung parenchyma which can lead to pulmonary oedema and impaired gas diffusion in the lung parenchyma. Research by the University of Tehran showed a reduction in the inflammatory index after administration of pentoxifylline dissolved in 0.9% normal saline. Administration of pentoxifylline reduces ischemia-reperfusion injury due to skeletal muscle ischemia, as well as post-transplant lung and liver injury.[5-7]

Vitamin C is an endogenous antioxidant, which counteracts ROS, thereby reducing free radicals. Several experimental studies have shown that antioxidant vitamins, including ascorbic acid, produce cytoprotective effects by protecting microvascular endothelium against neutrophil adhesion. Several experimental studies show that antioxidant vitamins including ascorbic acid can produce cytoprotective due to the reduction of free radicals. Lehr et al have shown that vitamin C protects the microvascular endothelium from neutrophil adhesion therefore can prevent microvascular circulation injury. Herbaczynska-Cedro et al have shown that vitamin C reduces the production of free radical oxygen in human circulating neutrophils. According to him, the effect of giving vitamin C against augmentation of lipid peroxidation induced by ischemia reperfusion injury (IRI) at a dose of different (range 30-100 mg/kgBb).[6] In an animal study by Baltalarli et al with Albino rats, there was a decline in plasma leukocyte sequestration (a parameter for pulmonary injury) after pretreatment with iloprost and vitamin C. Kearns et al also noted a reduction in the microvascular pulmonary injury due

to ischemia-reperfusion and respiratory burst activity of circulating neutrophils after administration of vitamin C.[6,21]

From previous studies, no one has raised the effect of combination therapy Pentoxifylline and Vitamin C in reducing the effect of Ischemia-reperfusion injury on lung tissues. Several studies have shown a protective effect of giving pentoxifylline in combination with other therapies and vitamin C in combination with other therapies and vitamin C is expected to increase the protection of lung tissue due to ischemia-reperfusion injury in lower limbs.

Acute Limb Ischemia

Acute limb ischemia (ALI) is a condition that occurs due to a sudden decrease in arterial perfusion on the limbs, which could threaten the limb tissue and other organs' viability, and the onset time is less than two weeks. Several studies reported ALI incidence of approximately 1.5 cases per 10,000 persons per year. Populations at risk include smokers, people aged>50 years with diabetes mellitus, and kidney failure patients. Incidence in men compared to women is 2:1. The mortality rate from ALI ranges from 15-20% and the morbidity rate is 10-15% for bleeding, 25% for amputation, and 5-25% for fasciotomy. Comorbidities in ALI depend on the underlying etiology, namely due to embolism or due to thrombosis.[1-3]

Overall, the cause of ALI could be divided into emboli (38%) such as air, fat embolism, embolism due to arrhythmias and heart valve disease, thrombosis (41%) such as atherosclerosis, aortic dissection, hypercoagulable state, stent-graft (15%), external compression, iatrogenic (2%), and traumatic thrombosis (2%). In 90% of embolism cases, it's cardiogenic and most often obstructs the common femoral artery bifurcation and popliteal artery trifurcation. This occurs due to the detached thrombus in the atrium or ventricles in patients with atrial fibrillation or acute myocardial infarction. Acute leg ischemia due to thrombosis might be due to stent or graft occlusion in patients with chronic peripheral arterial disease undergoing angioplasty or peripheral artery bypass surgery.[4,5]

In ALI, metabolic changes occur from aerobic to anaerobic due to ischemia, resulting in the formation of reactive oxygen species (ROS), a reactive chemical molecule that is responsible for cell damage. In ischemia-reperfusion injury (IRI) of the lower extremity, the endothelium integrity is damaged, resulting in capillary leakage which leads to interstitial edema and eventually the limb necrosis. This is followed by the release of inflammatory mediators such as Interleukin (IL)-1, IL-6, IL-8, tumor necrosis factor (TNF), monocyte chemotactic protein-1, and systemic complement activation, causing multi-organ dysfunction.[6,7]

The purpose of ALI treatment is to prevent the formation of thrombi and the aggravation of ischemia. Endovascular revascularization using catheter-directed thrombolysis, percutaneous aspiration thrombectomy, percutaneous mechanical thrombectomy, surgical therapy with thromboembolectomy, and anticoagulant medication are all used to treat acute limb ischemia. Patients who come with category I (viable ischemia) receive intervention in the form of thrombolysis within 6-24 hours. Prompt revascularization within six hours should be performed in ALI category II patients. The treatment option for ALI category IIa is less aggressive, which is through endovascular thrombolysis, while in more severe and threatening ischemia (category IIb) the treatment option is more invasive, which is thromboembolectomy surgery. In irreversible ischemia (category III) there is no indication for revascularization because the limb is no longer viable, therefore amputation is the treatment of choice.[3,8,9]

Ischemic Reperfusion Injury

Ischemic reperfusion damage happens when blood supply to the tissue is interrupted for a few minutes to many hours (ischemia) and then restored (reperfusion). Complications might occur after revascularization due to life-threatening local and systemic reperfusion injuries. Local complications that occur are in the form of compartment syndrome and rhabdomyolysis. Data from 1992 to 2000 in the United States showed that approximately 5.3% of cases of ALI that were successfully revascularized required fasciotomy due to compartment syndrome. Reperfusion in the limbs causes an increase in capillary permeability resulting in local edema and hypertension in the compartment. This obstructs the venules, capillaries, and arterioles, and if the intercompartmental pressure exceeds 30 mm Hg, muscle and nerve necrosis might occur. Muscle necrosis that occurs leads to rhabdomyolysis, which is characterized by myoglobinuria. Myoglobin excreted through the kidneys triggers lipid peroxidation and renal vasoconstriction, resulting in tubular necrosis.[9]

Ischemic reperfusion injury in ALI could affect distant organs, causing systemic damage. It also contributes as a factor that increases morbidity and mortality due to multiple organ dysfunction syndromes (MODS). Distant organs that could be affected are lung tissue, heart, kidneys, gastrointestinal tract, and central nervous system. Myocardial dysfunction that occurs is reversible, although in some cases inotropic or mechanical support is required. Arrhythmias after reperfusion are caused by rapid changes in ion concentrations in previously ischemic tissues. In the central nervous system, there is damage to the blood-brain barrier, resulting in leukocyte migration that produces ROS and damages brain tissue. These events manifest as sensory, motor, or cognitive impairment, or even death. Translocation of intestinal bacteria into the systemic circulation occurs due to increased intestinal permeability caused by the activation of cytokines.[10]

The final effect of reperfusion injury is MODS. The mortality rate in the intensive care unit correlates with the number of injured organs, where the mortality is 30-40% if it affects 1 organ, 50-60% if 2 organs are affected, and 80-100% if more than 3 organs fail.[5,11]

Lung Tissue Damage Due to Ischemia-Reperfusion Injury

Lung tissue is the most commonly affected organ, where lung tissue injury could rapidly progress to respiratory failure. Lung tissue is an organ that is susceptible to damage due to neutrophil activation by ROS and metabolites produced by IRI, which in turn causes a fatal effect in the form of acute respiratory distress

syndrome (ARDS). The mechanism underlying the occurrence of lung tissue damage due to IRI is very complex and related to the role of polymorphonuclear (PMN) cells. In lung tissue, leukocyte infiltration, damage to the lung capillary barrier, pulmonary epithelial cell edema, and apoptosis occur.[11]

During the ischemia period, metabolites of inflammatory mediators are formed, such as Leukotriene B4 (LTB4), Thromboxane 2 (TXA2), and complement C5, which then activate neutrophils. When reperfusion occurs, these neutrophils accumulate in the pulmonary circulation, causing edema and damage to lung tissue. Several proinflammatory cytokines produced during IRI, particularly TNF-α and IL-1, also mediate lung tissue damage. TXA2 causes pulmonary vasoconstriction resulting in increased neutrophil and endothelial interactions. During the ischemia-reperfusion period, IL-8 produced by alveolar macrophages and lung tissue endothelial cells also plays a role in neutrophil activation in lung tissue.[5,11]

Damages to lung tissue are in the form of edema, alveoli congestion, bleeding, infiltration or aggregation of neutrophils in the alveoli or blood vessel walls, and thickening of the alveoli walls or locations with hyaline membranes. Lung tissue damage due to ischemia-reperfusion injury into 4 grades as follows:

- Grade 1 (normal): normal lung tissue parenchyma
- Grade 2 (mild): focal edema of a small amount of alveolar septum, mild congestion, less than 50 neutrophils in the alveolar septum per large visual field.
- Grade 3 (moderate): moderate edema of the alveolar septum or mild edema of multiple septums, moderate congestion, neutrophils in the alveolar septum 50 – 100 per large visual field.
- Grade 4 (severe): severe edema of the alveolar septum or mild edema of multiple septums, moderate congestion, neutrophils in the alveolar septum more than 100 per large visual field (5).

Treatment of Ischemia-Reperfusion Injury (IRI)

The formation of ROS is one of the events that play an important role in IRI occurrence, therefore the objective of the given therapy is to reduce oxidative stress. Many therapeutic strategies to prevent or limit organ damage due to IRI have been conducted and have given good clinical results in humans, although several strategies are still under research that has not yet reached a clinical trial stage in humans. The treatment strategy could be in the form of surgical intervention such as ischemic preconditioning or pharmacological therapy for example with Aspirin-Triggered Lipoxin (ATL) analogs, anti-complements, and anti-cytokines, endothelin receptor antagonists, Leukotriene-B4 antagonists, or antioxidants such as vitamin E, vitamin C, allopurinol. However, reperfusion of ischemic tissue is still the therapy choice for preventing IRI in clinical practice.[6,9]

The ischemic preconditioning method is carried out by giving ischemia exposure for a short period, where this method could protect the tissue from the harmful effects of prolonged ischemia-reperfusion. Although the positive effects of this method have been demonstrated in many experimental animals, clinical data in humans are limited. Currently, the protective effects shown by this method are the restoration of cardiac contractility after coronary artery bypass surgery and reduction of liver injury in patients undergoing liver resection.[7]

The hypothermic strategy is intended to increase tissue tolerance to ischemia. Tissue hypothermia has been experimentally shown to decrease the inflammatory response and oxidative stress due to CIR.[11]

Pharmacological therapy that could be given is anti-complement, anti-leukocyte, and antioxidant. An experiment in rats that were given a C3 convertase inhibitor showed a reduction in myocardial infarction size by 44%. In addition, the administration of single-chain C5-specific antibodies in patients undergoing coronary artery bypass surgery has shown a significant reduction in complement and leukocyte activation, myocardial injury, and cognitive dysfunction.[6,12]

Antileukocyte therapy is one of the therapies to reduce damage due to leukocyte activation during IRI. Its mechanism of action is to block the release of inflammatory mediators or endothelium and leukocyte attachment. The release of inflammatory mediators could be inhibited using IL-1 receptor antagonists, anti-TNF- antibodies, or platelet activation factor-LTB4 antagonists. One study showed that aspirin might induce lipoxin biosynthesis, a lipoxygenase product derived from arachidonic acid, thereby preventing neutrophil chemotaxis and transmigration. Administration of an aspirin-triggered lipoxin analog could prevent changes in vascular permeability and other organ damage mediated by neutrophils in rats subjected to limb IRI. Although this anti-leukocyte strategy has received limited clinical attention, it is effective in experimental animals with IRI.[9,13,14]

Administration of allopurinol, which aims to protect tissue from IRI, has been studied both in experimental animals and in humans. Administration of allopurinol reduces serum TNF- α concentrations in IRI thereby reducing inflammation caused by IRI.[13,15]

A large number of animal studies have shown the success of therapies that act as antioxidants such as superoxide dismutase (SOD) enzymes, catalase enzymes, GPx enzymes, vitamin E, vitamin C, and β-carotene to reduce IRI damage by scavenging free radicals formed. The role of oxidative stress in IRI which is supported by clinical and experimental data makes antioxidant therapy very important as a defense mechanism to protect tissues.[6,7,16,17]

Pentoxifylline is a methylxanthine derivative with several hemorheological properties. Administration of pentoxifylline reduces ischemia-reperfusion injury due to skeletal muscle ischemia, as well as post-transplant lung and liver injury. It has various mechanisms of action, both at the molecular or cellular level, through inhibiting leukocyte interaction with endothelium.

Pentoxifylline could enhance neutrophils' chemotaxis response and also inhibit phagocytosis and superoxide production by neutrophils and monocytes. Pentoxifylline in particular inhibits neutrophil adhesion to the endothelium and prevents leukocyte degranulation. Inhibition of neutrophil adhesion in lung tissue will reduce damage to endothelial and epithelial cells in the lung parenchyma which might lead to pulmonary edema and impaired gas diffusion. Pentoxifylline has a beneficial effect in patients with ischemic reperfusion injury to the lung. This potential local effect is important for acute lung injury manifestation by inflammatory mediator's activation that circulates through the blood, lymph, and free radicals. Research by the University of Tehran showed that the administration of 40 mg/kg of pentoxifylline dissolved in 0.9% normal saline to 2 ml affected reducing the inflammatory index in ischemic reperfusion injury. Pentoxifylline should be administered after the ischemia period to reduce inflammatory mediators and free radical release.[9,18,19]

Vitamin C serves as an antioxidant by lowering the impact of ROS-free radicals. Several studies have indicated that antioxidant vitamins, particularly ascorbic acid, have a cytoprotective impact by reducing free radicals. Lehr et al. demonstrated that vitamin C protects the microvascular endothelium against neutrophil adhesion, implying that vitamin C might preserve the microvascular circulation. Furthermore, Herbaczynska-Cedro et al.[6] discovered that vitamin C inhibits the generation of oxygen free radicals in human circulating neutrophils. According to them, the effect of vitamin C on lipid peroxidation augmentation induced by IRI depended on the dosage (range 30-100 mg/kg bb).[7,15,20]

CONCLUSION

Acute limb ischemia causes decreased blood flow to the lower limbs. Decreased blood flow results in hypoxia or anoxia of the tissues and stasis of the microcirculation. Reperfusion is done so that blood flow can immediately return to the tissues, which is very important to prevent permanent cell injury. Reperfusion restores blood flow in the lower limbs, but is also accompanied by the release of inflammatory mediators (Il-1,6; TNF-α, Complomen C5, LTB4, TXA2) to the systemic which will trigger vascular dysfunction and ROS release. Vascular dysfunction and the release of ROS due to reperfusion injury will cause reperfusion injury to distant organs, namely lungs, kidneys, liver, heart.

The release of inflammatory mediators and neutrophil PMN activation which will activate ROS will accumulate in the pulmonary circulation, causing edema and lung damage. Lung tissue is an organ that is susceptible to damage due to neutrophil activation by ROS and metabolites produced by ischemic reperfusion injury, which in turn causes a fatal effect in the form of acute respiratory distress syndrome (ARDS). The mechanism underlying the occurrence of lung tissue damage due to CIR is very complex and this is related to the role of polymorphonuclear cells (PMN). In the lung tissue, leukocyte infiltration occurs, damage to the lung capillary barrier, pulmonary epithelial cell edema and apoptosis. Damage that occurs in lung tissue in the form of edema and/or infiltration or aggregation of neutrophils in the alveoli of the lung tissue. Based on the literature, it is explained that the administration of combination therapy with vitamin C and pentoxyfillyline is expected to reduce lung tissue damage due to acute lower limb ischemia reperfusion injury. Vitamin C serves as an antioxidant by lowering the impact of ROS-free radicals and Pentoxifylline in particular inhibits neutrophil adhesion to the endothelium and prevents leukocyte degranulation. Inhibition of neutrophil adhesion in lung tissue will reduce damage to endothelial and epithelial cells in the lung parenchyma.

CONFLICT OF INTEREST

The author declares there is no conflict of interest regarding publication of current review.

ETHICAL CONSIDERATION

Ethical clearance is not mandatory for review article.

AUTHOR CONTRIBUTION

All author had contributed for manuscript writing and agreed for the final version of the manuscript for publication.

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