APROTININ ATTENUATES ISCHEMIA/REPERFUSION INJURY IN THE RAT LUNG AFTER INFRARENAL AORTIC OCCLUSION

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Objectives: *It is known that lower torso ischemia leads on reperfusion to sequestration of polymorphonuclear leukocytes (PMN) in the lungs. We hypothesized that the use of aprotinin would attenuate the reperfusion injury in the lung observed after infrarenal aortic occlusion because of a reduction in the inflammatory response.*
Material and Methods: *A rat model in which the infrarenal aorta was cross-clamped for 3 hours followed by 2 hours of reperfusion was used. Thirty rats were randomised into three groups: Group I (n=10), 30 000 KIU/kg aprotinin administered before the aorta was clamped; group II (n=10), similar volume of saline solution was used; group III (sham group, n=10), animals were anesthetized and subjected to the surgical procedures without aortic occlusion. At the end of the experiment, bronchoalveolar lavage (BAL) specimens were obtained from the left lung and PMN rate in the 100 cell were counted. The right lungs were histologically examined for evidence of injury. Lung injury was rated between Grade 0 and Grade 4; based on congestion, interstitial edema, PMN infiltration, and airspace hemorrhage.*
Results: *The rate of PMN in the BAL cytology was significantly lower in group I than group II (respectively, 4.3±1.82%, 16.7±4.32%, $p<0.05$). Ischemia/reperfusion resulted in a significant increase in lung injury scores in Group II (mean 2.9±0.54). The animals pretreated with aprotinin (Group I) had significantly lower score (mean 1.3±0.64, $p<0.05$).*
Conclusions: *The findings demonstrate that lower torso ischemia and reperfusion cause a significant lung injury. It can be attenuated by aprotinin.*

Key Words: *Aprotinin; Lower extremity; Ischemia; Reperfusion injury; Lung.*

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Acute aortic occlusion with subsequent ischemia/reperfusion (IR) of the lower extremities is known to predispose to lung injury.1-3 Polymorphonuclear neutrophil leucocytes (PMN) have been shown to have a central role in lung injury caused by IR of the lower extremities, and their depletion exerts a protective effect on the lungs under these conditions. 3

Aprotinin (Trasylol; Bayer Pharmaceuticals, Turkey) is a serine protease inhibitor that is presently in wide clinical use for minimizing perioperative blood loss in cardiac operations and has been shown to protect against the damage of ischemia and reperfusion by suppressing the release of lysosomal enzymes and inhibiting their activities.4, 5

In this experimental study, the effects of aprotinin on ischemia/reperfusion injury on the rat lung after infrarenal aortic occlusion were studied.

MATERIALS AND METHODS

The study was performed at the Experimental Animal Research Laboratory on female rats. All rats received humane care in compliance with the European Convention on Animal Care. The Study was approved by the Institutional Ethics Committee.

Thirty Wistar Albino rats weighing between 300-400 g were divided into three randomized groups. At the room temperature (200C) anesthesia was administered by intramuscular (IM) injection of ketamine HCl (Ketalar) of 30 mg/kg and xylosine HCl (Rompun) of 6 mg/kg to the left anterior foot. During the surgical procedures, anesthesia was maintained with IM ketamine at every 30-45 minutes and body temperature was maintained with a water-filled heating pad. A jugular venous line was established for intravenous fluid infusion through the neck incision. The animals were then given heparin (1000 units/kg) via the right jugular vein. The abdominal aorta was exposed through a midline abdominal incision, and the infrarenal aorta was cross-clamped for 3 hours followed by 2 hours of reperfusion. A bulldog clamp was used for the infrarenal aortic occlusion. 7n group I (Aprotinin group, n=10), 30 000 KIU/kg aprotinin was administered before the aorta was clamped; Group II (IR group, n=10), a similar volume of saline solution was used; Group III (Sham group, n=10), animals were anesthetized and subjected to the surgical procedures, aorta was dissected into visibility without aortic occlusion. Cessation of blood flow was verified by doppler ultrasound. Abdominal contents were replaced and covered with a damp swab for the 3-hour period of cross-clamping and the abdomen was resutured for the period of reperfusion.

After 3 hours of ischemia and 2 hours of reperfusion, both the lungs and trachea were harvested. Saline (6 ml) was then injected as 3 aliquots of 2 ml each. Each aliquot was injected quickly and then withdrawn slowly 3 times to obtain bronchoalveolar lavage (BAL) specimen. Fluid recovery was routinely %90 or greater. BAL fluid were centrifuged at 1000g for 10 minutes to remove cells. Polymorphonuclear neutrophil leucocytes (PMN) were counted in the most cellular ten high power field.

The right lung samples were fixed with a 10 % formaldehyde solution. The tissues were embedded in parafin, sectioned in 6 micron thick slices, and stained with routine hematoxylin and eosin. The specimens were examined using light microscopy and evaluated by the same pathologist who was blinded to the study. As suggested by Tassiopoulos6, 7 lung injury was rated with a semiquantative scoring system; based on congestion, interstitial edema, PMN infiltration, and airspace hemorhage, as follows: Grade 0: no changes; grade1: focal, mild, subtle changes; grade 2: multifocal mild changes; grade 3: multifocal prominent changes; and grade 4: extensive prominent changes.

The parametric data (the rate of PMN in BAL fluid) were expressed as mean ± standard deviation, and compared with student t test. Nonparametric values of lung injury scores were analyzed with Mann Whitney U test. A p-value of less than 0.05 was considered significant.

RESULTS

All animals have completed the study and there was no mortality. In the sham group (Group III), there was no congestion and neutrophil infiltration in the lung histology (Figure 1). In the BAL cytology, there were no neutrophils; dominating cells were macrophages.

Figure 1: Normal histologic appearance of the rat lung. (Group III, hematoxylin-eosin stain, original magnification X20, injury score: 0+)

Table 2. Lung injury scores of Group I and Group II

	Lung Injury Score					Mean Score
	0	1	2	3	4	
Group I (n=10)	1	5	4	-	-	1.3±0.64
Group II (n=10)	-	-	2	7	1	2.9±0.54

Ischemia/reperfusion resulted in a significant increase in lung injury scores in Gorup II (ranging from 2 to 4+, mean 2.9±0.54) (Figure 2,3). The animals pretreated with aprotinin (Grup I) had a significantly lower score (ranging from 0 to 2+, mean 1.3±0.64, $p<0.05$). In the BAL cytology, the rate of PMN was also significantly lower in group I than group II (respectively, 4.3±1.82%, 16.7±4.32%, $p<0.05$)

Figure 2: Histologic picture of the lung, minimal vascular congestion and cellularity in the lung interstitium. (Group I, hematoxylin-eosin stain, original magnification X20, injury score: 1+)

Figure 3: Histologic picture of the severe lung injury, There is alveolar flooding, indicating pulmonary edema. (Group II, hematoxylin-eosin stain, original magnification X20, injury score: 4+)

DISCUSSION

During ischemia of lower extremities, muscle cells cannot keep their membrane integrity and this causes releasing of calcium, phospholipid A2 and formation of polyunsaturated fatty acids and

fatty acid radicals. If the oxygenation is re-established at that stage of ischemia, fatty acid radicals react with oxygen and perform the lipid peroxidation reaction. This reaction increases the membrane permeability and also stimulates chemotaxis of leukocytes, which can release oxygen-derived free radicals and proteolytic enzymes when activated. As a result, ischemic cell injury is worsened by reperfusion.8 Sometimes reperfusion injury leads to life-threatening metabolic abnormalities and high mortality and morbidity rates.9 Accumulating evidence suggests that the specific reperfusion component of the injury cascade is mediated in large part by neutrophil-endothelial adherence and subsequent neutrophil-mediated organ injury10 .

It is known that acute transient ischemia of the lower extremities in rats predispose to lung injury.1, 6 The lung injury process begins once the blood supply to the lower extremities is interrupted and aggravated during reperfusion. 6 Ischemia/reperfusion of lower extremity causes lung injury by PMN sequestreration in pulmonary microvasculature, increased endothelial permeability, and interstitial edema.2 The end result is then the profound structural and functional breakdown of delicate lung parenchyma.10 .

Previous studies with aprotinin have shown it to improve myocardial, hepatic, and renal viability after a period of ischemia followed by reperfusion.11-13 Beyond its antiproteolytic effects as a serine protease inhibitor, aprotinin has shown to decrease the release of lysosomal enzymes, increase intracellular adenine nucleotides (adenosine triphosphate and adenosine diphosphate), and effect the levels of cyclic monophosphates. Decreased levels of cyclic guanosine monophosphate and increased levels of cyclic adenosine monophosphate have been shown to inhibit lysosomal enzyme release.4, 14

Tumor necrosis factor-· is synthesized as a membrane-bound precursor and is released after cleavage by a serine protease inhibitor; thus , when aprotinin is used it precludes the release of this proinflammatory cytokine. Other studies have shown that tumor necrosis factor- · enhances endogenous nitric oxide production, whereas aprotinin deters nitric oxide production.15

Aprotinin appears to have hemostatic and antiinflammatory effects when the drug is at a kallikrein-inhibiting concentration. Aprotinin inhibits the initiation of both coagulation and fibrinolysis, as well as the release of the vasoactive peptide bradykinin. It appears that when kallikrein inhibition occurs, the production of the direct precursor of bradykinin, high molecular weight kininogen, is blocked. According to recent reports, because bradykinin increases during the ischemia-reperfusion period, its suppression by aprotinin should ameliorate reperfusion injury.16

There has been some previous literature regarding aprotinin and lung reperfusion injury. One study noted that after aprotinin administration, there was improved oxygenation, reduced edema formation, and significantly increased compliance.14

Our study shows that transient infrarenal aortic occlusion with subsequent ischemia/reperfusion of the lower extremities cause a significant lung injury. It can be attenuated by aprotinin pretreatment.

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