EFFECT OF APROTININ ON RENAL INJURY CAUSED BY ISCHEMIA/REPERFUSION OF THE LOWER EXTREMITIES IN THE RATS

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Objectives: *Acute aortic occlusion with subsequent ischemia/reperfusion (IR) of the lower extremities is known to predispose to renal injury. In addition to its well-known clinical effect of reducing perioperative blood loss, aprotinin has been shown to protect against the damage of ischemia and reperfusion. In the present experimental study, we studied the role of aprotinin in renal injury caused by lower extremity IR.*

Methods: *Thirty wistar albino rats were randomized into three groups: sham group (n=10), aorta exposed but was not clamped; IR group (n=10), infrarenal abdominal aorta was clamped for 3 hours, followed by 2 hours of reperfusion; aprotinin group (n=10), 30000 KIU/kg aprotinin administered before the aorta was clamped. At the end of the experiment, the left kidneys were removed and histologically examined for evidence of renal injury. The renal injury was rated according to acute tubular necrosis with a quantitative scoring system between 0-4. Grade 0: normal kidney; grade 1: minimal ATN.<5%; grade 2: mild ATN, 5-25%; grade 3: moderate ATN, 25-75 %; grade4: severe ATN,>75 %. Cell swelling, vacuolization, nuclear pyknosis, inflammatory infiltrate, and medullary congestion were also evaluated.*

Results: *There was a significant difference between sham and IR groups according to their renal injury scores (mean 0.2±0.4, 1.2±0.7 respectively, p<0.05), whereas no significant difference existed between sham and aprotinin groups (p>0.05). Renal injury scores were higher in IR group as compared with aprotinin group (mean 1.2±0.7, 0.7±0.6 respectively) but there was no significantly difference (p>0.05).*

Conclusions: *It was demonstrated that acute ischemia of the lower extremities in rats results in a renal injury. Aprotinin displays a moderate protective effect on renal injury when administered before IR of lower extremities.*

Key Words: *Aprotinin; Lower extremity; ischemia; reperfusion injury; kidney.*

Extremity ischemia is an unavoidable clinical symptom during peripheral vascular surgery, aortic aneurysm surgery, reimplantation of extremities or during peripheral vascular injury. Although the primary process is to recover the blood circulation of the ischemic limbs during resuscitation, there are strong clinical and experimental evidences that the reperfused ischemic tissues can induce renal dysfunction.1

Accumulating evidence suggests that the specific reperfusion component of the injury cascade is mediated in large part by neutrophil adherence and subsequent neutrophil-mediated organ injury. When the neutrophils are activated by adherence to the endothelial tissue, they secrete reactive oxygen species and proteolytic enzymes.2

Aprotinin (Trasylol; Bayer Pharmaceuticals, Turkey) is a serine protease inhibitor that is presently in wide clinical use for minimizing perioperative blood loss in cardiovascular 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.3, 4

Aim of this experimental study was to investigate effect of aprotinin on ischemia/reperfusion (IR) injury on the rat kidney after infrarenal aortic occlusion.

MATERIALS AND METHODS

The study was performed at the Experimental Animal Research Laboratory. All rats received humane care in compliance with the EuropeanConvention on Animal Care.

ANIMALS AND GROUPING

Thirty female Wistar Albino rats, weighing between 300-350 g, were employed in this study. The animals were randomly divided into 3 groups: sham group (n=10), aorta exposed but was not clamped; IR group (n=10), infrarenal abdominal aorta was clamped for 3 hours, followed by 2 hours of reperfusion; aprotinin group (n=10), 30000 KIU/kg aprotinin (Trasylol; Bayer Pharmaceuticals, Turkey) administered before the aorta was clamped.

Experimental design

Animals were allowed free access to standard rat chow and water. At the room temperature (200C) anesthesia was administered by intramuscular injection of kethamine hydrochlorur (Ketalar, Pfizer, Turkey) of 30 mg/kg and xylosine hydrochlorur (Rompun, Bayer, Turkey) of 6 mg/kg to the left anterior foot. During the surgical procedures, anesthesia was maintained with IM kethamine at every 30-45 minutes and body temperature was maintained with a water-filled heating pad. Carotid arterial catheter was inserted for arterial blood sample and a jugular venous line was established for intravenous fluid infusion through the same 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 under aseptic conditions 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. 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 abdomen was resutured for the period of reperfusion.

At the end of the experiment, the left kidneys were removed and fixed with a 10 % formaldehyde solution. The tissues were embedded in parafin, sectioned in 5Ìm thick slices, and stained with routine hematoxylin and eosin. The specimens were histopathologically exam-

ined using light microscopy and evaluated by the same pathologist who was blinded to the study. A quantitative renal injury scoring system was used previously described by Klausner.5 The renal injury was rated according to acute tubular necrosis with a scoring system between 0-4. Grade 0: normal kidney; grade 1: minimal ATN.<5%; grade 2: mild ATN, 5-25%; grade 3: moderate ATN, 25-75 %; grade4: severe ATN,>75 %. Cell swelling, vacuolization, nuclear pyknosis, inflammatory infiltrate, and medullary congestion were also evaluated.

STATISTICAL ANALYSIS

Student's t test (two-tailed) was used to compare data between the groups. Values are given as means ± standart error (SE). Results were considered significant if the P value was less than 0.05.

RESULTS

Figure 1: Normal glomerular and tubular structure. (H&E, original magnification, x20)

All animals have completed the study and there was no any mortality. In hematoxylin and eosin stained sections, All groups had no ATN higher than 25%. But there were manifest inflammatory infiltration and ATN in the IR group and the renal injury scores were significantly higher than in the sham group (respectively, mean 1.2±0.7, 0.2±0.4, $p<0.05$). The renal injury score was 0.7±0.6 in the aprotinin group and when compare with the sham group there was no statistically difference ($p>0.05$). The IR group had higher injury score than the aprotinin group without statistically significant difference ($p>0.05$). Figure 1 shows normal glomeruler and tubuler structure. However figure 2 shows ATN.

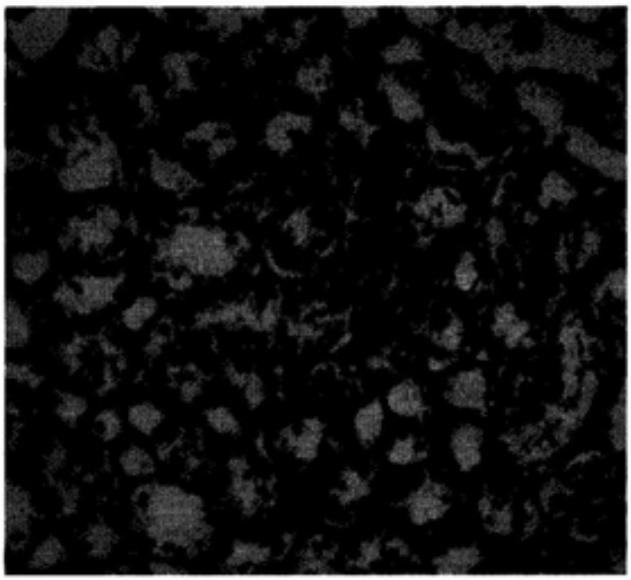

Figure 1: Hydropic degeneration and necrosis of individual cells in acute tubular necrosis. (H&E, original magnification, x20)

DISCUSSION

The skeletal muscle represents about 42% of entire body weight and 76% of the mass of the lower extremities. During reconsructive vascular surgery, ischemia occurs due to occlusion of aorta, iliac or femoral artery on the muscles of lower extremities. 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.6

A devastating consequence of tissue reperfusion is the damage in organs uninvolved in the initial ischemic insult.7 Sometimes reperfusion injury leads to life-threatening metabolic abnormalities and high mortality and morbidity rates.8 In some cases, acute renal and respiratory failure, cardiac dysfonction and even death can occur as a result of systemic toxic effects of reperfusion products, metabolic acidosis, myoglobinuria, electrolytic and enzymatic changes known as "myonephropathic-metabolic syndrome".1 Wahlberg et al showed that azotemia occurs in 23% of patients after abdominal aortic surgery.9

Beyond its antiproteolytic effects as a serine protease inhibitor, aprotinin was 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.3, 10

Aprotinin appears to have hemostatic and anti-inflammatory effects when the drug is at a kallikrein-inhibiting concentration.11, 12 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.13

Aprotinin is metabolized in the kidney and potentially nephrotoxic at high concentrations.14 Aprotinin is potantially a antigenic agent and can cause allergic reactions.15 In addition, aprotinin can lead to graft trombosis after coronary bypass operations.16

The model of abdominal aortic oclusion in rats, is a safe method to evaluate organ injury and protective effects of different agents after IR of lower extremities. In this study, there was a significant difference between sham and IR groups according to their renal injury scores, whereas no significant difference existed between sham and aprotinin groups. Renal injury scores were higher in IR group as compared with aprotinin group.

In conclusion, we have shown that IR of the lower extremities cause renal injury. Although the exact mechanism or mechanisms remain unclear, aprotinin pretreatment appears to be moderate useful to decrease renal injury in elective peripheral vascular and aortic aneurysm surgery. But the clinical application should be studied further.

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