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EFFECT OF ISCHEMIC CHANGES IN RENAL TISSUES ON APELIN LEVEL

Medicine

Ebru Canakci* Ordu University, School of Medicine, Training and Research Hospital, Department of Anaesthesia and Reanimation, Ordu, Turkey* (Corresponding Author)

Ahmet Karatas Ordu University, Faculty of Medicine, Department of Internal Medicine, Division of Nephrology, Ordu, Turkey

Havva Erdem Ordu University, School of Medicine, Training and Research Hospital, Department of Medical Pathology, Ordu, Turkey

Muruvvet Akcay Celik Ordu University, School of Medicine, Training and Research Hospital, Department of Medical Pathology, Ordu, Turkey

Abdullah Cirakoglu Ordu University, School of Medicine, Research and Training Hospital, Department of Urological Surgery , Ordu, Turkey

Zubeyir Cebeci Ordu University, School of Medicine, Training and Research Hospital, Department of Anaesthesia and Reanimation, Ordu, Turkey

ABSTRACT

Introduction and Objective: The main cause of acute kidney injury is reported to be renal ischemia.The present study aimed to histopathologically investigate the effects of experimental ischemia-reperfusion injury on the protective role of apelin in the oxidative damage occurred in rat kidneys compared to the group in which ischemic damage was not induced.

Materials and Methods: The experimental subjects were divided into two groups each of which included seven rats. The blood flow of the renal vessels (arteries and veins) of the rats in the ischemia/reperfusion (IR) experimentally group (=control group) was interrupted using a non-traumatic microvascular clamp for 60 minutes and ischemia was produced. In order to replace the indispensable fluid loss occurred while the abdomen was open, sterile saline weighing 5% of the body weight at 37°C was administered intra-abdominally during the experiment. The rats in both groups were kept alive in metabolic cages for 24 hours after the closing abdomen. After the reperfusion period ended, the left kidneys were removed and the rats were sacrificed.

Results: Statistically significant results were obtained by the Chi-Square test which was conducted to determine the difference between the staining intensity of the sham group and control group ($p=0.015$, $p<0.001$, $p<0.001$, respectively).The Mann-Whitney U test was used to determine the staining intensity. The medulla stained better than the cortex ($p=0.006$).

Conclusion: It is obvious that apelin has a protective role in experimental renal IR injury. This conclusion needs to be supported by clinical studies. Today, surgical procedures and organ transplantations have become very common. We suggest that long-term, large population clinical trials are needed to prevent and treat renal I/R injury that may occur perioperatively, and we believe that this study of ours will shed light on future clinical trials.

KEYWORDS

Experimental renal ischemia reperfusion, apelin, rat, staining intensity

INTRODUCTION:

Acute kidney injury (AKI) is a syndrome characterized by a sudden decrease in renal functions, impaired fluid and electrolyte as well as acid and base balance resulting in decreased urine output, accumulation of nitrogenous (creatinine and urea) and non-nitrogenous waste products [1,2].

Acute kidney injury requires long-term treatment in the hospital and has a high mortality rate. Renal ischemia/reperfusion injury is frequently encountered in many clinical conditions such as renal transplantation, partial nephrectomy, renal artery angioplasty, cardiopulmonary bypass, aortic bypass surgery, accidental or iatrogenic trauma, sepsis, hydronephrosis and elective urological operations [3].

The leading cause of AKI which is preceded by a series of complicated and interrelated events, causes damage and results in renal cell death is reported to be renal ischemia [4]. The pathophysiology of ischemia/reperfusion injury is associated with reactive oxygen species (ROS), reactive nitrogen species (RNS), purine metabolites, neutrophil accumulation, some vasoactive substances (endothelin, angiotensin II) and subsequently released lytic enzymes[5]. Reperfusion was stated to be necessary for the survival of ischemic kidney tissue. Reperfusion itself was reported to cause cell damage secondary to increased production of ROS, depletion of ATP, neutrophil infiltration, phospholipase activation, membrane lipid injury, loss of cytoskeletal function and intracellular Ca+2 accumulation [6].

Adipose tissue, a special type of connective tissue, is formed through poor chemical interactions of lipid-laden cells known as adipocytes in adult mammals. Although fat tissue is known to be the largest source of energy in the organism, the storage and release of energy from fat cells are controlled by hormonal signals (insulin, catecholamines, glucocorticoids, etc.) [7]. The proteins released from the adipocytes and connective tissue cells among adipocytes (adipokines) were identified to have autocrine, paracrine and endocrine effects [8]. The fact that adipose tissue is not only an energy store, but rather it functions as an active endocrine organ is widely accepted [9,10].

Apelin is an adipokine discovered by "reverse pharmacology". First, its receptor was identified in 1993, and then the apelin molecule was isolated as the endogenous ligand for this receptor in 1998 [11]. The apelin hormone recently included in these adipokines has many effects on energy metabolism, cardiovascular functions, insulin sensitivity and vascular responses through its local and systemic effects. However, it is not clear how these actions occur through which mechanisms. Current literature knowledge on the physiological roles of the peptide is limited, and studies for elucidating its physiological mechanisms need to be increased [12].

The present study aimed to histopathologically investigate the effects of experimental ischemia-reperfusion injury on the protective role apelin in the oxidative damage caused renal injury in the rat kidneys

compared to the group in which ischemic damage was not induced.

MATERIALS AND METHODS:

The approval was obtained from the Experimental Animals Ethics Committee of Ordu University School of Medicine (Date: 30.01.2018, Issue: 01). Fourteen female Wistar-Albino rats weighing 200-250 g reared in the Experimental Animal Production and Research Laboratory of Samsun (HADYEK) 19 Mayis University were used in our study. The rats taken from Samsun were brought to the Experimental Animal Breeding, Practice and Research Center of Ordu University. The rats were fed with standard rat diet and tap water ad libitum (as desired). The laboratory conditions were kept at the standard (22 ± 1°C and 12-hour light / dark cycle) conditions.

The experimental subjects were divided into two groups each of which included seven rats. Ten mg/kg xylazine (Rompun, Bayer, Turkey) and 90 mg/kg ketamine (Ketasol, Richterphar AG, Wels-Austria) were administered to the rats for anesthesia via the intramuscular route. Their abdominal regions were shaved on the test bench heated to 37oC degrees and betadine was used for skin antisepsis. A median incision was opened on the midline and abdominal organs were placed on a sterile gauze [13]. The blood flow of the renal vessels (arteries and veins) of the rats in the ischemia/reperfusion (IR)- experimentally group (=control group) was interrupted using a non-traumatic microvascular clamp (FST, 85 g, America) for 60 minutes and ischemia was produced[14]. In order to replace the indispensable fluid loss occurred while the abdomen was open, sterile saline weighing 5% of the body weight at 37°C was administered intra-abdominally during the experiment. At the end of the 60-minute ischemia, the clamps were taken off and the color change in the kidneys was observed after the blood flow was allowed for two minutes. The incision was closed and sterilized with betadine. The same procedure was applied to the rats in the sham group, however, a sham surgery was performed by not clamping the renal vessels and closing the incision at the end of 60 minutes. The rats in both groups were kept alive in metabolic cages for 24 hours after the closing abdomen. After the reperfusion periods ended, the rats were sacrificed and their left kidneys were removed under the anesthesia induced with 10 mg/kg xylazine and 50 mg/kg ketamine via the intramuscular route [15,16]. The removed kidneys were placed on an ice bar and their capsules were removed with the help of a scalpel. The kidneys were divided into two longitudinally. The left kidneys were placed in 10% formalin solution for pathological examination.

Histopathological Investigations

The kidneys of the rats were sampled in the sagittal plane and then blocks were created. Five-µ thick sections were taken from the paraffin blocks over the polysine slides. They were prepared for immunohistochemical examination. A Leica Bond-Max IHK staining device (Vision Biosystems, Melbourne, Australia) was used for the immunohistochemical analysis.

The sections were kept at 60 degrees Celsius for 30 minutes. They were kept at 72 degrees in the Bond Devax solution for deparaffinization. After washing with alcohol three times, they were washed with the Bond Wash solution for three times. They were awaited in the previously described pretreatment solution of the antibody at 100 degrees for 10 minutes. They were washed with the Bond Wash solution for three more times.Peroxide blockage was applied for 10 minutes. They were washed with the Bond Wash solution for three more times. They were incubated with apelin (Genetex GTX37465) Polyclonal(1:300). They were washed with the Bond Wash solution for three more times. They were treated with the post primer for seven minutes. They were again washed with the Bond Wash washing solution for three times and then treated with the polymer for seven minutes. They were washed with the Bond Wash solution again for two times and then washed with distilled water. They were incubated within the Bond Devax solution for seven minutes and washed with distilled water for three times. At the end of these procedures, apelin was assessed according to the rating procedure of Berta et al. (17). Thereby, the proportion of the cells with cytoplasmic positivity is taken into account. Accordingly, they were described as follows; 0 staining, no staining; 1-10% staining 1 + staining; 2 + staining 11-50% staining; 3 + staining, and >50% staining category (Figure 3-5) [17].

Statistical Analysis

The normality assessment of the data was performed using the single sample Kolmogorov-Smirnov test. The Chi-Square test was used to determine the difference between the sham group underwent sham surgery and IR-treated control group. The Mann-Whitney U test was used for detecting the difference between the localization and intensity of staining. The level of statistical significance limit was considered <0.05. The SPSS v 21.0 software package was used for statistical analysis.

RESULTS:

In our study, the experimental ischemic AKI model induced with 60-minute ischemia and 24-hour reperfusion application in the rats was conducted on a total of 14 rats, namely two groups including seven rats in each. The rats were anesthetized in the 24th hour of reperfusion and tissue specimens were collected. No loss occurred in the groups throughout the experiment. In our study, a grading ranging between 0 and 4 was carried out for the immunohistochemical assessment of the renal tissues (Table-I). In the light microscope assessment, the expression of apelin was observed to be more in the medulla than in the cortex. The expression of apelin was also noted to be more in the control group than in the sham group.

Statistically significant results were obtained by the Chi-Square test performed to determine the difference between the sham group and control group ($p=0.015$, $p<0.001$, $p<0.001$, respectively). The Mann-Whitney U test was used to determine the intensity of staining. The expression of apelin was observed to be more in the medulla compared to the cortex ($p=0.006$).

DISCUSSION:

Renal IR injury is frequently encountered in numerous clinical pictures such as renal transplantation, partial nephrectomy, renal artery angioplasty, cardiopulmonary bypass, aortic bypass surgery, accidental or iatrogenic trauma, sepsis, hydronephrosis, elective urological operations, contrast ınduced nephropathy, and shock after resuscitation in emergency and intensive care units [18,19]. Acute renal failure requires long-term hospitalization, high treatment costs, and has high mortality rate [20]. It was reported that about 20% of patients who died in hospital had an acute renal injury and this rate raises up to 50% in intensive care units [19]. Renal replacement therapy is required in approximately 10% of patients with acute renal failure. End-stage renal disease develops in 2-10% of intensive care patients in whom acute renal failure develops. End-stage renal disease requires long-term dialysis therapy [20].

In the renal IR model created by 60 minutes of ischemia and 24 hours of reperfusion in the rats, it was observed that histopathological renal injury occurred and that staining with apelin antibody was more prominent in the ischemic regions. These results indicate that renal IR injury developed and that experimental model was produced.

The outer cortex of the kidney has a high oxygen reserve and therefore if the duration of ischemia is short, the cells in this area are relatively preserved. The epithelial cells of the external medulla are the most vulnerable cells to hypoxia because they function at the anoxic border in normal kidney and have a high metabolic rate to be able to perform their reabsorption tasks [21]. In our study, the medulla was stained more intensely than the cortex.

In the experimental renal ischemia-reperfusion model developed by Bircan B. et al., the rats were divided into five groups. In addition to the sham and control groups, apelin was administered to the rats at the doses of 1, 10,100 mcg/kg intraperitoneally. Antioxidant enzyme activity levels were found high in the apelin-treated rat groups while simultaneous oxidant enzyme levels were found to be low. BUN and creatinine levels were found to be lower in the apelin-treated rats groups, more notably in the high dose groups. Bircan B. et al. stated that apelin had a protective effect on renal ischemia-reperfusion injury. The authors noted that apelin-13 may have been a novel protective agent against ischemia-reperfusion [22]. Apelin is also a peptide released from the adipose tissue. It is a peptide which plays a role in many physiological pathways such as energy metabolism, nutritional behavior, reproductive function [23-25]. The recent studies conducted recently demonstrated that apelin might have had a good antioxidant

effect [26, 27]. Similarly, in the studies carried out by Yang et al., they reported that apelin activated many protective mechanisms in ischemia and ischemia-related diseases, especially in cardiac, cerebral, renal and hepatic injuries[28]. Also, in our study, the preparations stained more intensely with the apelin antibody were in the control group (study group). The higher uptake of apelin antibody by ischemic cells may be related to the protective effect of apelin on ischemia.

In the study by Ozturk et al. in which they applied 60 minutes of ischemia following 60 minutes of ischemia on the left renal artery of the rats, they showed histopathologically that renal injury including significant necrosis, tubular dilatation, luminal congestion, and cyst accumulation increased significantly in the IR group [29]. Mousavi et al. reported that histopathologically, tubular dilatation, cast accumulation, inflammation, necrosis and cellular vacuolization were observed in the IR group in their study in which they induced renal IR injury using 60 minutes of ischemia and 24 hours of reperfusion [30]. Apelin is an endogenous ligand of the G-protein coupled (APJ) receptor and it shows its effects by binding to APJ [30]. The effects of apelin vary depending on its forms. The apelin which was composed of 13 and 17 amino acids was reported to have a stronger biological activity than the apelin form containing 36 amino acids[31]. Since apelin-13 has N-terminal pyroglutamate residues, its biological activity is higher compared to other apelin forms. Apelin-13 is claimed to be eight times more effective than apelin-17 and 60 times more effective than apelin-36 [31]. Since apelin-13 has high biological activity, research has focused on this form of apelin. Although apelin-13 was accepted as the most biologically active form, the binding affinity of apelin-36 to APJ was shown to be much higher than that of apelin-13 [32].

In their review, Huang Z. et al. suggested that apelin and APJ receptor system were a novel treatment option for renal diseases. The studies showed that apelin mRNA is highly expressed in the external medulla of the kidney, which plays an important role in the water and sodium balance process. Also, in our study, the medullary cells were stained more intensely with apelin. In addition, further research indicates that the apelin / APJ system exhibits a wide range of activity in the kidney. The authors, therefore concluded that the apelin/APJ system had a role in renal diseases such as renal fibrosis, renal ischemia/reperfusion injury, diabetic nephropathy, polycystic kidney disease and hemodialysis (33). The apelin/APJ system may improve renal interstitial fibrosis by reducing extracellular matrix accumulation. The apelin/APJ system was stated to significantly reduce renal ischemia/reperfusion injury by inhibiting renal cell death (33).Similarly, Chen H. et al. reported that apelin had a protective effect on acute renal injury in the experimental renal IR model and that this effect was exerted by inhibiting the TGF-β1 enzyme and reducing apoptosis. The authors noted that apelin-13 may be included in the treatment of acute kidney injury (34). Also, in our study, the result that all ischemic areas were stained more intensely with apelin might have been associated with this condition. Our results are consistent with the results of the study by Chen H. et al.

In the experimental study by Sagiroglu T. et al., renal IR was induced and the effects of leptin and apelin on renal function were investigated. Ischemic cells were observed to be significantly less in the apelin and leptin-treated groups of rats. Also, the levels of urea, creatinine were found to be higher in the ischemic groups to which apelin and leptin were not administered. The authors indicated that apelin and leptin had protective effects on renal ischemia-reperfusion injury histopathologically and functionally (35). Our study and the studies conducted showed that apelin had a protective effect on renal ischemia-reperfusion injury (22,28,34,35).

In conclusion, it is obvious that apelin has a protective role in experimental renal IR injury. This result should be supported by clinical studies. Today, surgical procedures and organ transplantations have become very common. All clinicians interested in clinical pharmacology should include apelin in their clinical studies. Thus, we suggest that long-term, large population clinical trials are needed to prevent and treat renal I/R injury that may occur perioperatively, and we believe that this study of ours will shed light on future clinical trials.

TABLE AND FIGURE LEGENDS:

Table I: Histopathological results demonstrating the staining degree of the groups

Group	Cortex glomerulus	Cortex tubulus	Medulla tubulus
Sham1	2	0	2
Sham2	1	1	2
Sham3	1	0	1
Sham4	1	0	1
Sham5	1	0	1
Sham6	1	0	2
Sham7	0	0	0
Control1	2	1	2
Control2	1	0	3
Control3	1	0	2
Control4	1	0	3
Control5	1	0	2
Control6	1	0	2
Control7	1	0	2

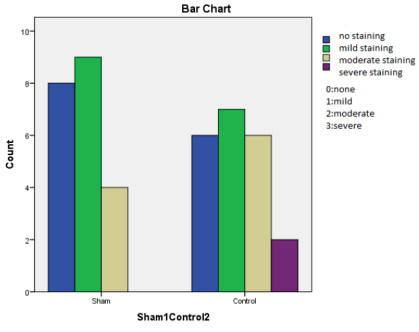

Figure 1: Differences in the expression of apelin between the sham group and control group

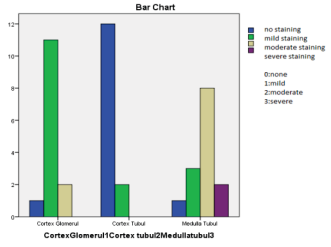

Figure 2: Differences in the expression of apelin between the sham group and control group

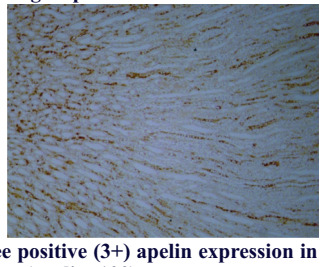

Figure 3: Three positive (3+) apelin expression in the medulla in the control group (apelinx100)

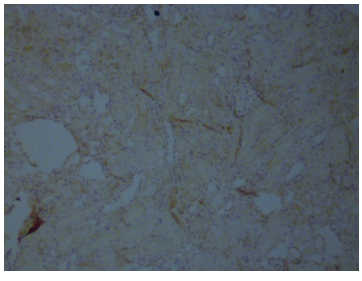

Figure 5. One positive (1+) apelin expression in the glomerulus in the sham group (apelinx200) glomerular staining

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