Effects of leptin on p53 expression and apoptosis in ischemia reperfusion induced renal injury

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KEY WORDS

kidney ◗ ischemia/reperfusion ◗ renal injury ◗ leptin, p53, apoptosis

ABSTRACT

Introduction. Ischemia due to arterial occlusion, shock, or organ transplantation is a common cause of cell death and organ failure. This study was designed to assess the possible protective effects of leptin in ischemia/reperfusion (I/R) injury of renal tissue.

Material and methods. Twenty-four male Sprague-Dawley rats were used. The animals were divided into 3 groups. Control, I/R, and I/R+Leptin group. Rats were subjected to renal ischemia for 60 min. The I/R+Leptin group was pretreated with leptin (10 µl, i.p), which was administered 2 hours prior to the ischemia process. Then the clamps were removed and reperfusion was applied for 60 min. After reperfusion the kidneys were removed. Samples were collected and stained with hematoxylin and eosin (H&E) as well as immunohistochemically with p53. Apoptosis kits were used and positive cells were counted with an image analysis program and analyzed statistically. Also, in routine staining, the degree of edema, vascularization, and infiltrative cell migration was semiquantitatively evaluated.

Results. The number of p53 positive and apoptotic cells was smaller in the leptin treated group than the I/R group (respectively p=0.004 and p=0.016). At the same time, the scores obtained from the I/R+Leptin group were smaller than from the I/R group (for edema p=0.002, for vascularization p=0.004, and for infiltration p=0.009).

Conclusions. These results suggest that leptin reduces renal oxidative injury and facilitates repair. Leptin may have a role as a renoprotective agent by inhibiting p53 positive cells and apoptosis.

INTRODUCTION

Leptin, a 167-amino acid peptide hormone, is the product of the obese (*ob*) gene and acts on central receptors for leptin (OB receptor) that control food intake and energy expenditure. It is specifically secreted by adipose tissue in mice, rats, and humans [1, 2]. It can influence hematopoiesis, thermogenesis, reproduction, angiogenesis, and immune homeostasis [1]. Leptin also has a protective effect on some tissues from I/R-induced organ failure and tissue damage [2].

Ischemia results in injury of tissue when the blood flow is interrupted, but a more severe tissue injury comes when blood flow is restored [3, 4]. Ischemia/reperfusion (I/R) injury often occurs in clinical practice and is associated with high morbidity and mortality. It is important to improve the ability of organs, including the heart, brain, gastrointestinal tract, and kidney, to tolerate ischemic injury [3, 4]. Although several mechanisms have been proposed to explain the pathogenesis of I/R injury, most of the attention has focused on the role of apoptosis.

The kidney plays a central role in the regulation of body salt and water balance. Dysregulation of transporters in the kidney is responsible for altered salt and water balance in severe pathophysiological states, including nephrotic syndrome, nephrogenic diabetes insipidus, hypertension, and acute renal failure [5]. I/R injury of the kidney is characterized by a series of events, including changes in vascular tone, enhanced vascular permeability to plasma proteins, and structural alterations in the renal tubule [6, 7]. However, gene expression and cell death mechanisms in I/R induced renal tissue were not sufficiently evaluated.

When tissue is exposed to destructive agents it immediately expresses different kinds of tumor suppressor genes to protect its genomic stability. P53, named as the "guardian of genome", is one of the most important tumor suppressor genes. If DNA is damaged by agents, the cell expresses the p53 gene and repairs the damaged DNA [8]. However, if DNA is not repaired successfully, p53 stimulates apoptotic cascades to prevent malignant cell formation. On the other hand, during normal fetal development, p53 expression may clearly be detected in developing tissues [9].

Apoptosis, also termed "physiological cell death," is a type of cell death. It is characterized by absence of cellular lyses and inflammation and represents a crucial mechanism of I/R injury [10]. The apoptotic mechanism is the most important process shaping organs during the intraembryonic period [11] and sustains the physiological balance of the number of cells during life [12]. At the same time, if the cells damaged by chemical or radioactive agents in organs are not repaired by anti-oncogenes, the apoptotic process is stimulated by such mediators as p53 (a guardian of genome), bcl-2, and caspases when damaged cells are eliminated to prevent malignancy. If some tissue is exposed to stress, the apoptotic index increases because of the increase in the number of injured cells. This reaction is beneficial for the organism.

In this study, we decided to evaluate the effects of leptin on p53 gene expression and apoptosis in I/R induced renal injury.

MATERIALS AND METHODS

Twenty-four Spraque-Dawley rats weighing 300-350 g of either sex were used in the experiments. Rats were supplied from the Eskisehir Osmangazi University Experimental Research Center. They were group housed in polycarbonate cages (45 × 24 × 21 cm), in a temperature (21 ±1°C) and humidity (45-55%) controlled room that was maintained on a 12/12 reversed light cycle (lights off at 8:00 am).

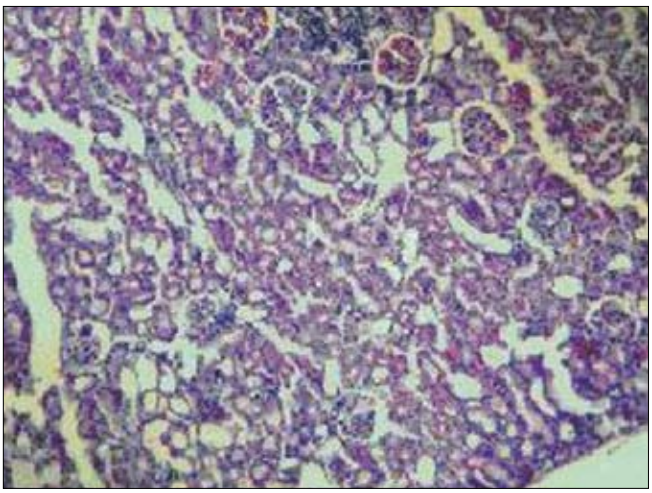

Fig. 1. Histologic views of rat kidney in the leptin+I/R group (H&E, x100).

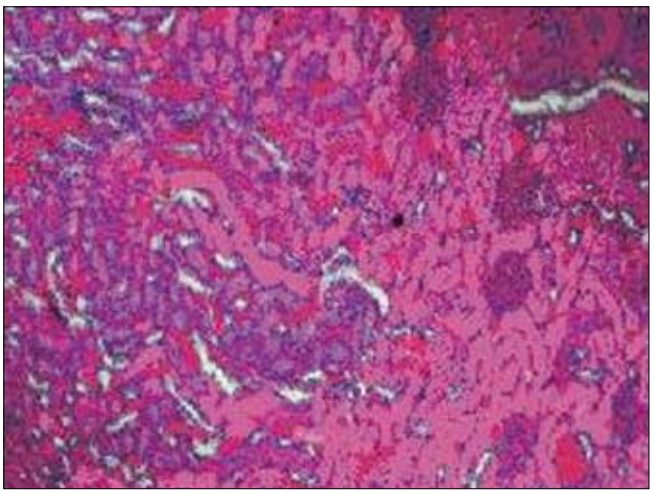

Fig. 2. Histologic views of rat kidney in the I/R group (H&E, x100).

They were fed with a standard rat chow (Oguzlar Yem, Eskisehir, Turkey) and allowed to drink water *ad libitum*. All experimental protocols were approved by the Eskişehir Osmangazi University Intuitional Local Animal Care and Use Committee.

Animals were randomly divided into three groups, each consisting of 8 animals; a control group, an ischemia-reperfusion (I/R) group, and a leptin, and I/R (leptin+I/R) group.

Leptin (10 µg/kg, i.p) was administered 2 hours prior to the ischemia process. The rats were anesthetized during all surgical procedures with ketamine (50 mg/kg, i.p) and rompun (20 mg/kg, i.p) and placed on a temperature regulated table (37 °C +0.5°C) to maintain body temperature. The abdominal region was shaved with a safety razor and sterilized with povidone iodine solution. A middle incision was made and a non-traumatic vascular clamp was applied to the left renal pedicle for 60 minutes followed by reperfusion for 60 minutes. None of the animals died during the I/R period. At the end of the reperfusion period, rats were killed and the left kidneys were removed and stained with H&E as well as immunohistochemically with p53 and apoptosis kits. Positive cells were counted with an image analysis program and analyzed statistically. On the other hand, after routine staining, the degree of edema, vascularization, and infiltrative cell migration was semi-quantitatively evaluated.

Histological determinations

All the specimens were fixed in 10% neutral formalin, dehydrated in increasing alcohol series, cleared in xylene, and embedded in paraffin. Several 5 micron sections obtained from these specimens were mounted on poly-L-lysine coated slides.

DNA Nick End-Labeling of Tissue Sections

Dead cells that exhibited DNA fragmentation were determined by the TDT-mediated dUTP-biotin nick end-labeling (TUNEL) technique using a commercial kit (Tdt-Fragel DNA Fragmentation Detection kit QIA33 Calbiochem, Darmstadt). After the sections were deparaffinized and rehydrated, they were washed in tris-buffered saline (TBS) for 5 minutes. Two mg/ml Proteinase K 1:100 in 10 mM Tris pH was used for permeabilization of tissues at room temperature for 20 minutes and then rinsed with TBS. Ten percent H_2O_2 in methanol was used for inactivation of endogenous peroxidases at room temperature for 5 minutes and then rinsed with TBS. After equilibration, a buffer 1:5 in deionized water was applied and all sections were covered with 3 µl TdT enzyme plus 57 µl TdT Labeling Reaction mix and covered with a piece of parafilm and then incubated at 37°C for 1.5 hours in a humidified chamber. At the end of incubation, the sections were washed with TBS and the labeling reaction was ended with Stop Buffer at room temperature for 5 minutes and then washed with TBS again. For detection, all sections were covered with 100 µl of Blocking Buffer at room temperature for 10 minutes and conjugate in Blocking Buffer was applied in a humidified chamber at room temperature for 30 minutes. After the sections were washed with TBS, 100 µl solution prepared with one tablet of diaminobenzidine (DAB) plus an H_2O_2/urea tablet in tap water was applied at room temperature for 15 minutes and then rinsed with deionized water. One hundred µl of methyl green counterstain solution was used for counterstaining of sections at room temperature for 3 minutes. Then the slides were dipped 2 times into absolute ethanol and xylene, respectively, and mounted with Entellan (Sigma, St Louis, MO).

Statistical Analysis

Data were expressed as mean ± SEM and analyzed with the Mann-Whitney U test. A value of $p < 0.05$ was considered statistically significant. For statistical analysis the program SPSS 10.0 for Windows was used.

RESULTS

To examine the effects of leptin on an I/R injured kidney, rats were treated with leptin prior to I/R injury.

Microscopic determinations

Edema, vascularization, and infiltration fields were seen in the I/R group relative to the control and leptin treated group in H&E tissue determinations. However there were some inflammatory cells and edema in the leptin treated group, but these fields were smaller and weaker than in the I/R group. In the control group, all the structures of the kidney were normal in appearance. H&E stained sections of rat kidneys are shown in figs 1 and 2.

Apoptosis

After 60 min of ischemia and 60 min of reperfusion, the number of apoptotic cells significantly decreased in the leptin+I/R group (Fig. 3) when compared to the I/R group (Fig. 4), (p=0.004) . There were no apoptotic cells in the control group.

P53 expression

P53 (+) cells in the leptin+I/R group (Fig. 5) decreased relative to the I/R group (Fig. 6) and this difference between the groups was significant (p: 0.016). In the control group, the glomerular structure and tubules in the cortex and medulla were normal in appearance.

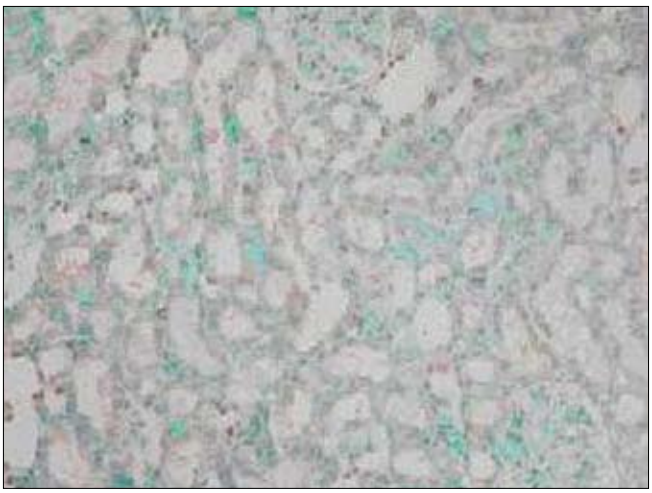

Fig. 3. Apoptotic cells of rat kidney in the leptin+I/R group (x200).

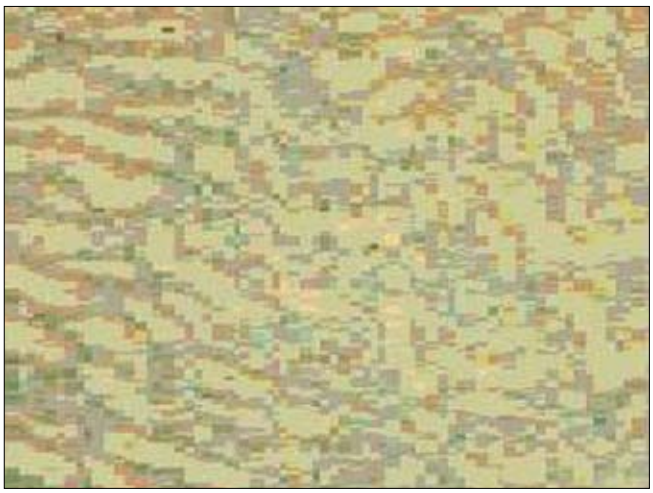

Fig. 4. Apoptotic cells of rat kidney in the I/R group (x200).

DISCUSSION

In this study the number of p53 expressing and apoptotic cells was significant in the I/R injured renal tissue, whereas the number of apoptotic cells was decreased in the leptin treated group. These results show that leptin plays an important role in the attenuation of I/R induced renal injury by decreasing the number of apoptotic cells and p53 expression.

Acute renal failure caused by ischemia is a complex syndrome involving renal vasoconstriction, extensive tubular damage, tubular cell necrosis, glomerular filtration failure, and glomerular injury [13]. The mechanisms underlying I/R damage to kidneys are most likely multi-factorial and interdependent, involving hypoxia, inflammatory responses, and free radical damage [6, 14]. A number of processes have been implicated in the pathogenesis of oxygen deprivation-induced cell injury. These include: disturbances of the cells calcium metabolism, activation of phospholipases with the production of toxic lipid metabolites, and loss of cell volume [15, 16].

Apoptosis (programmed cell death) is a physiologically controlled mechanism that occurs in several pathological situations [16]. It is increasingly recognized as a major mode of cell death after ischemic injury to the kidney. Ischemia/reperfusion (I/R) injury is accompanied by multiple changes in signaling molecules and metabolic effectors that can, independently or in concert, trigger cell death in various ways. These mechanisms include changes in intracellular pH, calcium, ceramide, and free radicals as well as adenosine triphosphate (ATP) depletion and hypoxia. While all of these factors are grossly deranged during abrupt necrotic cell death, they can also be specific effectors of apoptotic death under certain circumstances [17]. In some experimental studies researchers used many agents to induce apoptosis, but the mechanisms involved in leptin-induced I/R apoptosis of the kidney are not well known.

Some of the research found in literature was focused on apoptosis by using various agents. There is also some research related to leptin on different organs, but the mechanisms involved in leptin-induced I/R apoptosis on kidney are not well known.

In our previous study, leptin was shown to have a protective effect on gastric and renal tissue in rats when injured by I/R mechanisms [2, 18]. Some research reports that leptin inhibits apoptosis in the thymus and stellate cells as well as B and T lymphocytes [19-21]. Magarinos reported that the possible effects of leptin on trophoblastic cell proliferation, survival, and apoptosis in humans [22] as well as its effects through ovarian receptors can suppress mammalian ovarian cell apoptosis [23]. Leptin also stimulates proliferation and inhibits apoptosis in human colon cancer cells [24]. In light of these studies we chose to investigate the effects of leptin on apoptosis in kidney tissue and we showed that leptin significantly decreases the number of apoptotic and p53 (+) cells in I/R induced renal tissue injury. P53 expression during I/R induced renal injury indicates genomic damages. Leptin can repair these genomic damages in I/R induced renal tissue injury.

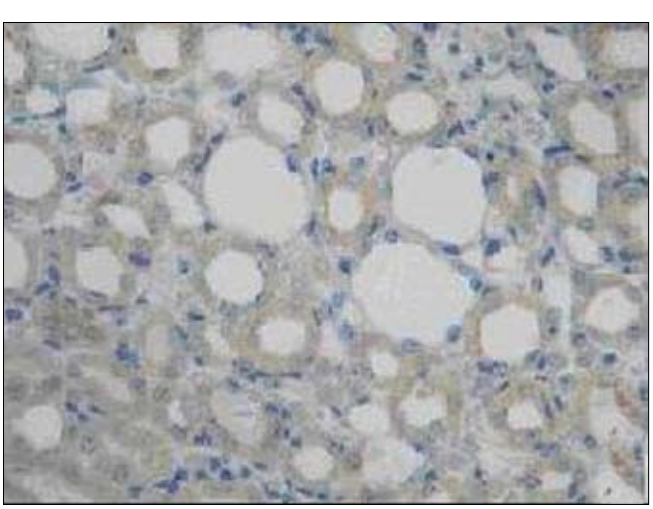

Fig. 5. P53 positive cells of rat kidney in the leptin+I/R group (x200).

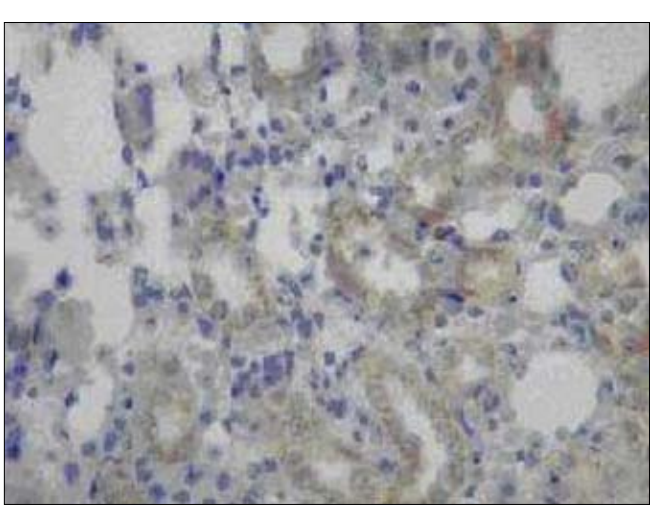

Fig. 6. P53 positive cells of rat kidney in the I/R group (x200).

CONCLUSION

Pretreatment with leptin seems to be effective in prevention of I/R induced renal tissue injury by decreasing the number of apoptotic and p53 (+) cells. These results revealed an important role of leptin in I/R induced apoptosis and suggest that leptin might be useful in protecting renal tissue against I/R associated injury.

REFERENCES

1. Lin J, Yan G-T, Wang LH et al: *Leptin fluctuates in intestinal ischemia-reperfusion injury as inflammatory cytokine*. Peptides 2004; 25: 2187-2193.
2. Erkasap N, Uzuner K, Serteser M et al: *Gastroprotective effect of leptin on gastric mucosal injury induced by ischemia-reperfusion is related to gastric histamine content in rats*. Peptides 2003; 24: 1181-1187.
3. Liu X, Chen H, Zhan B, et al: *Attenuation of reperfusion injury by renal ischemic postconditioning: The role of NO*. Biochemical and Biophysical Research Communications 2007; 359: 628-634.
4. Kahraman A, Erkasap N, Serteser M, Köken T: *Protective effect of quercetin on renal ischemia/reperfusion injury in rats*. J Nephrol 2003; 16: 219-224.
5. Dae GK, Eun JS, Mi KM, et al: *Yukmijihwang-tang ameliorates ischemia/reperfusion-induced renal injury in rats*. J Ethnopharmacol 2006; 104: 47-53.
6. Şener G, Şehirli Ö, Velioğlu-Öğünç A et al: *Montelukast protects against renal ischemia/reperfusion injury in rats*. Pharmacol Res 2006; 54: 65-71.
7. Chander V, Chopra K: *Renal protective effect of Molsidomine and L-Arginine in ischemia-reperfusion induced injury in rats*. J Surg Res 2005; 128: 132-139.
8. Levine AJ: *p53, the cellular gatekeeper for growth and division*. Cell 1997; 88: 323-331.
9. Tosun M, Tosun E, Kalkan S, Avunduk MC: *P53 expression between 13-17 weeks old human male fetus gonads*. J Mol Histol 2007; 38 (4): 271-274.
10. Kienle K, Rentsch M, Müler T, et all: *Expression of BCL-2 in liver grafts after adenoviral transfer improves survival following prolonged ischemia and reperfusion in rat liver transplantation*. Transpl Proc 2005; 37: 439-441.
11. Helal MA, Mehmet H, Thomas NSB, et all: *Ontogeny of human fetal testicular apoptosis during first, second, and third trimesters of pregnancy*. J Clin Endocrinol Metab 2002; 87: 1189-1193.
12. Tosun M, Kalkan S: *Apoptosis ve Önemi*. Sendrom 2002; 14 (6): 120-126.
13. Chander V, Chopra K: *Renal protective effect of Molsidomine and L-Arginine in ischemia-reperfusion induced injury in rats*. J Surg Res 2005; 128: 132-139.
14. Şener G, Tuğtepe H, Yüksel M et al: *Resveratrol improves ischemia/reperfusion-induced oxidative renal injury in rats*. Arch Med Res 2006; 37: 822-829.
15. Avlan D, Tamer L, Ayaz L et al: *Effects of trapidil on renal ischemia-reperfusion injury*. J Ped Surg 2006; 41: 1686-1693.
16. Ozben T: *Oxidative stress and apoptosis: impact on cancer therapy*. J Pharm Sci 2007; 96 (9): 2181-2196.
17. Dagher PC: *Apoptosis in ischemic renal injury: roles of GTP depletion and p53*. Kidney Int 2004; 66 (2): 506-509.
18. Erkasap S, Erkasap N, Koken T: *Effect of leptin on renal ischemia-reperfusion damage in rats*.J Physiol Biochem 2004; 60 (2): 79-84.
19. Mansour E, Pereira FG, Araujo EP et al: *Leptin inhibits apoptosis in thymus through a janus kinase-2-independent, insulin receptor substrate-1/phosphatidylinositol-3 kinase-dependent pathway*. Endocrinology 2006; 147 (11): 5470-5479.
20. Qamar A, Sheikh SZ, Masud A et al: *In vitro and in vivo protection of stellate cells from apoptosis by leptin*. Dig Dis Sci 2006; 51: 1697-1705.
21. Papathanassoglou E, El-Haschimi K, Li XC et al: *Leptin receptor expression and signaling in lymphocytes: kinetics during lymphocyte activation, role in lymphocyte survival and response to high fat diet in mice*. J Immunol 2006; 176: 7745-7752.
22. Magarinos MP, Sanchez-Margalet V, Kotler M et al: *Leptin promotes cell proliferation and survival of trophoblastic cells*. Biology of Reproduction 2007; 76: 203-210.
23. Sirotkin AV, Grossmann R: *Leptin directly controls proliferation, apoptosis and secretory activity of cultured chicken ovarian cells*. Comparative Biochemistry and Physiology 2007; 148: 422-429.
24. Ogunwobi OO, Beales ILP: *Cyclo-oxygenase-independent inhibition of apoptosis and stimulation of proliferation by leptin in human colon cancer cells*. Dig Dis Sci 2007; 52: 1934-1945.

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