TÜBİTAK

Original Article

Turk J Med Sci
2011; 41 (2): 251-258
© TÜBİTAK
E-mail: medsci@tubitak.gov.tr
doi:10.3906/sag-1009-1109

Immunomodulatory role of leptin treatment in experimental sepsis caused by gram negative bacteria

Cemile KOCA[1], Havva ŞAHİN KAVAKLI[2], Özlem ALICI[3]

Aim: To investigate the effect of leptin treatment on circulating inflammatory cytokines and on tissue damage in experimental rat model of gram-negative sepsis.

Materials and methods: Adult male Wistar rats, 28 in total, were randomly divided into 4 groups (n = 7): sham, leptin, sepsis, and sepsis group treated with leptin (sepsis+leptin). Sepsis was induced by intraperitoneal (ip) injection of 2×10^{10} CFU of *Escherichia coli* ATCC 25922. Leptin and sepsis+leptin groups received a single dose ip 0.1 mg/kg leptin, while sham group received 1 mL of ip saline. Rats were sacrificed 24 h after the induction of sepsis. Blood samples, lung, and kidney tissues were collected for analysis.

Results: The sepsis group had significantly higher serum TNF-α, IL-6, and endothelin levels than the sham group ($P = 0.05$, $P < 0.001$, $P = 0.003$, respectively). The sepsis+leptin group had significantly lower IL-6 and endothelin levels ($P = 0.001$ and $P = 0.020$, respectively), and higher lung and kidney tissue myeloperoxidase activities when compared with the sepsis group ($P = 0.039$, $P = 0.033$, respectively).

Conclusion: According to our results, leptin has a profound influence on sepsis, and to some extent it restricts the inflammatory events in sepsis.

Key words: Cytokines, leptin, myeloperoxidase, rat, sepsis

Leptin tedavisinin deneysel sepsis modelinde immunmodulator rolü

Amaç: Deneysel gram negatif sepsis oluşturulmuş ratlarda leptin tedavisinin inflamatuar sitokinler ve doku hasarı üzerindeki etkilerini araştırılmak.

Yöntem ve gereç: 28 adet erkek Wistar cinsi rat randomize olarak 4 eşit gruba ayrıldı; sham, leptin, sepsis ve leptin tedavisi almış sepsis (sepsis+leptin) grupları. Sepsis, 2×10^{10} CFU ATCC 25922 *Escherichia coli* suşunun intraperitonel (ip) verilmesi ile oluşturuldu. Leptin ve sepsis+leptin gruplarına tek doz 0,1 mg/kg ip leptin, sham grubuna eş zamanlı 1 ml ip salin uygulandı. Sepsis indüklendikten 24 saat sonra ratlar sakrifiye edildi. Kan örnekleri leptin, endotelin, TNF-α, IL-6 ve IL-10 düzeylerinin tespiti için ayrıldı. Akciğer ve böbrek dokuları myeloperoksidaz (MPO) aktivite tayini için çıkarıldı.

Bulgular: Sepsis grubu sham grubuna göre anlamlı olarak yüksek TNF-α, IL-6 ve endotelin düzeylerine sahipti (sırasıyla, $P = 0,05$, $P < 0,001$, $P = 0,003$). Sepsis+leptin grubunda IL-6 ve endotelin düzeylerinde sepsis grubuna göre anlamlı azalma (sırasıyla $P = 0,001$ ve $P = 0,02$), akciğer ve böbrek dokusu MPO aktivitelerinde anlamlı artış (sırasıyla $P = 0,039$, $P = 0,033$) görüldü.

Sonuç: Çalışmamızın sonuçları doğrultusunda, leptin ve sepsis arasında kompleks bir ilişki olduğu, leptin uygulamasının sepsiste görülen inflamatuar olayları bir dereceye kadar baskılayabileceği düşünülmektedir.

Anahtar sözcükler: Sitokin, leptin, myeloperoksidaz, sıçan, sepsis

Received: 07.09.2010 – Accepted: 26.10.2010

[1] Department of Biochemistry, Faculty of Medicine, Fatih University, Ankara - TURKEY
[2] Department of Emergency Medicine, Ankara Atatürk Training and Research Hospital, Ankara - TURKEY
[3] Department of Infectious Diseases and Clinical Bacteriology, Medical Park Hospital, İstanbul - TURKEY

Correspondence: *Cemile KOCA, Department of Biochemistry, Faculty of Medicine, Fatih University, Alparslan Türkeş Cad. No: 57, Beştepe, Ankara - TURKEY*
E-mail: cemile_md@yahoo.com

Introduction

Severe sepsis is an acute inflammatory reaction that arises due to the host's response to infection, resulting in systemic inflammation and organ failure. Sepsis is characterized by the redundant release of multiple inflammatory mediators, including cytokines, into the blood stream (1).

Although there is an enormous amount of research, the pathophysiology of sepsis is still poorly understood. The main pathophysiological feature of sepsis is the uncontrollable inflammatory response resulting in an increase in both pro- and anti-inflammatory mediators. Increased expression of pro-inflammatory cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, and anti-inflammatory cytokines such as IL-10 in the circulation are strongly associated with death (2). Increases in the incidences of sepsis, high sepsis mortality rates, complex pathophysiology, and overall difficulties in treatment make sepsis and sepsis-associated multi-organ failure a challenge for scientists and clinicians.

Leptin is the 16-kDa protein mainly secreted by adipose tissue, and is expressed by the *obese* (*ob*) gene, which was named for the obese phenotype of leptin-deficient *ob/ob* mutant mice (3). Leptin signals through the leptin receptor (Ob-R), which is encoded by the *diabetes* (*db)* gene. Primarily, leptin has a role in suppression of appetite, regulating body weight, and stimulation of energy expenditure. In addition, leptin has been shown to involve the regulation of several physiological processes such as reproduction, fetal development, angiogenesis, immunity, hematopoiesis, and bone remodeling (4). Besides its hormonal function, leptin can be considered as a cytokine that belongs to the family of long-chain helical cytokines, and has structural similarity with IL-6, prolactin, growth hormone, IL-12, IL-15, and granulocyte colony-stimulating factor, and its receptor is related to the class I cytokine receptors. Apart from adipose tissue, leptin receptor is expressed in other tissues such as bone marrow, spleen, liver, pancreas, lung, kidney, and on peripheral monocytes, lymphocytes, and endothelial cells. Because of its nature as a cytokine, growing evidence shows that leptin has an important role in the modulation of the immune response and inflammation. Leptin exerts proliferative and antiapoptotic activities in a variety of cell types, and affects cytokine production, the activation of monocytes/macrophages, wound healing, angiogenesis, and hematopoiesis. Moreover, leptin production is acutely increased during infection and inflammation (5).

The pathology behind the septic condition in the patient/animal is not simply about inflammation; thus, much more is needed to clarify the mechanism of sepsis, and to optimize present clinical therapies or develop novel approaches. As at the moment current data about sepsis and leptin interactions somehow are contradictory, we think this subject needs more attention. Under the light of some studies in which high leptin levels are positively correlated with survival in septic patients, and anti-inflammatory/anti-apoptotic actions of leptin would suggest that leptin may be considered a new potential therapeutic target for sepsis and sepsis modulated organ failure (6,7). The aim of the present study was to investigate the role of leptin in inflammation during *Escherichia coli*-induced sepsis, and to examine the potential association between leptin and various inflammatory mediators in sepsis.

Materials and methods

Twenty-eight adult male Wistar rats (weight range: 250 to 300 g) were used for the experiment. All animals had free access to food and water ad libitum throughout the study. The rats were divided randomly into 4 groups, each consisting of 7 animals: the sham group, the leptin group, the sepsis group, and the sepsis group treated with leptin (sepsis+leptin group). The study was approved by the animal research ethics committee of Fatih University, School of Medicine.

The sham group received a 1 mL intraperitoneal (ip) injection of 0.9% saline solution. The leptin group and the sepsis+leptin group underwent a single dose leptin administration. The leptin (L5037) was purchased from Sigma Chemical Co. (St. Louis, MO, USA). It was dissolved according to instructions and then stored at –20 °C. The leptin was diluted in phosphate-buffered saline (PBS) (pH 7.4) as 250 μg in 1 mL before use; then leptin administered groups were treated with 0.1 mg/kg leptin (ip) within 30 min of the induction of sepsis (8,9). Sepsis was induced by ip injection of 2×10^{10} CFU of *E. coli* ATCC25922. *E.*

coli ATCC25922 was grown in a brain-heart infusion broth. In the logarithmic phase of the growth, the suspension was centrifuged at 1000 × *g* for 15 min, the supernatant was discarded, and the bacteria were resuspended and diluted into sterile saline. The rats received an ip inoculum of 1 mL of saline containing 2 × 10^{10} CFU of *E. coli* ATCC 25922 (10). All injections were performed between 0800 and 1000 hours.

All animals were sacrificed using ketamine and cardiac puncture, 24 h after bacterial challenge. Blood samples were collected for the determination of leptin, endothelin, TNF-α, IL-6, and IL-10 levels, and then were centrifuged at 3000 × *g* for 10 min. Serum samples were stored at -80 °C until they were assayed. Lung and kidney tissue samples were immediately removed and stored at -80 °C for the determination of tissue associated myeloperoxidase (MPO) activity, as indirect evidence of tissue neutrophil infiltration.

The quantitative determination of serum cytokines, leptin and endothelin levels, Rat serum TNF-α (Biovendor GmbH, Germany), IL-6 (BioSource TM, CA, USA), IL-10 (Bender Medsystems, Vienna, Austria), cytokines levels, and serum levels of rat leptin (BioSourceTM, CA, USA) and rat endothelin (Assay Designs, MI, USA), were measured using commercially available enzyme linked immunosorbent assay (ELISA) kits according to the manufacturer's instructions.

MPO activity such as lung and kidney tissue MPO activity (MPO1 and MPO2, respectively) was determined by the method of Koike et al. (11). Tissue samples were homogenized in 20 mM potassium phosphate buffer (pH 7.4), and centrifuged at 20,000 × *g* at 4 °C for 20 min. The pellet was resuspended in 50 mM potassium phosphate buffer (pH 6.0) containing 0.5 g/dL hexadecyltrimethyl ammonium bromide, and frozen at −80 °C. Before assay, batched samples were thawed, sonicated for 90 s, incubated for 2 h in a water bath (60 °C), and then centrifuged at 10,000 × *g* for 5 min. The supernatants were used for MPO assay. MPO activity was assessed by measuring the H_2O_2-dependent oxidation of O-dianisidin. MPO activity, as 1 unit, was defined as the change in absorbance at 410 nm in 1 min per 1 g tissue. MPO activity was expressed as U/g tissue.

Statistical analysis

Statistical analyses were performed by use of SPSS 13.0. The compliance with normal distribution of our data is examined by Shapiro-Wilk test. Differences between the groups were statistically analyzed by 2-way analysis of variance (ANOVA). Post-hoc tests were performed using a multiple comparison procedure (Tukey test or Tamhane test where appropriate). Data are expressed as mean ± standard deviation (SD). A P value of ≤ 0.05 was regarded as statistically significant.

Results

All the data recorded during our experiment are shown in the Table.

Intraperitoneal injection of 2 × 10^{10} CFU of *E. coli* ATCC 25922 resulted in an inflammatory stage in the animals. This was confirmed by an increase in serum TNF-α and IL-6 levels (P = 0.05, P < 0.001 vs. the sham group, respectively). The serum endothelin level was significantly higher in the sepsis group than in the saline administered rats (P = 0.003). Additionally, the serum IL-10 level, an anti-inflammatory cytokine, was decreased, but lung and kidney MPO activities (MPO1 and MPO2, respectively) were increased in the sepsis group when compared with the sham group. However, these differences were not statistically significant. The leptin treatment did not show any statistically significant effects on plasma leptin levels in either the leptin or the sepsis groups when compared with the sham group. IL-6 levels were significantly higher in the sepsis group when compared to the leptin group (P < 0.001). Lung tissue MPO activity was significantly higher in sepsis+leptin group when compared with the sepsis (P = 0.039), leptin (P = 0.010), and sham (P = 0.001) groups (Table). MPO activity in kidney tissue was significantly higher in the sepsis+leptin group when compared with the sham (P = 0.003) and sepsis (P = 0.033) groups (Table). TNF-α levels increased in the leptin group, and even more in sepsis groups when compared to the sham group. It decreased in the sepsis+leptin group; however, this decrease was not significant. Exogenous leptin treatment significantly reduced IL-6 levels in the sepsis+leptin group (P = 0.001 vs. the sepsis group) (Table). In the sepsis group, IL-10 level was lower than the sham group, and leptin treatment in the sepsis group caused IL-

Table. Biochemical parameters in the blood, lung, and kidney tissues in groups (n = 7).

Parameters	Sham	Leptin	Sepsis	Sepsis+Leptin
MPO 1 (U/g tissue)	0.52 ± 0.13	0.98 ± 0.27[b]	1.24 ± 0.49[b]	2.30 ± 0.80[a]
MPO2 (U/g tissue)	0.51 ± 0.09	0.75 ± 0.29	0.64 ± 0.08[b]	0.91 ± 0.18[a]
Leptin (pg/mL)	384.76 ± 117.39	338.77 ± 115.76[b]	465.42 ± 193.92[b]	918.51 ± 336.01[a]
TNF-α (pg/mL)	250.61 ± 77.93	349.49 ± 133.56	423.77 ± 132.76[a]	319.34 ± 97.29
IL-6 (pg/mL)	59.63 ± 5.40	61.54 ± 3.85[c]	85.58 ± 7.78[a,b]	70.42 ± 7.66[a]
IL-10 (pg/mL)	212.48 ± 66.98	172.66 ± 35.41	156.21 ± 47.19	195.88 ± 59.98
Endothelin (pg/mL)	0.77 ± 0.32	0.69 ± 0.24	1.63 ± 0.62[a,b]	0.96 ± 0.34

MPO1: lung myeloperoxidase activity; MPO2: kidney myeloperoxidase activity; TNF-α: tumor necrosis factor-α; IL-6: interleukin-6; IL-10: interleukin-10.
Values are given as (mean ± SD)
[a] Significant difference compared to sham group ($P \leq 0.05$)
[b] Significant difference compared to sepsis+leptin group ($P \leq 0.05$)
[c] Significant difference compared to sepsis group ($P \leq 0.05$)

10 levels to increase, although these changes were not statistically significant. Serum endothelin levels increased significantly in septic rats when compared with the rats that received saline. Leptin treatment significantly reduced this increase in the sepsis+leptin group ($P = 0.020$ vs. sepsis group) (Table).

Discussion

Leptin is a multifunctional hormone cytokine that is involved in the metabolism and immune response (4,5). The aim of the present study was to investigate the role of leptin treatment in the regulation of inflammation during sepsis induced by bacterial challenge. We have demonstrated that the exogenous leptin treatment attenuated some of the inflammatory changes in rats with the bacterial challenge induced sepsis model. Even though we generally observed improvements, to some extent, in septic conditions in leptin treated rats, it is not easy to evaluate the results of leptin treatment in inflammation because of the dual role of leptin both as a pro- and anti-inflammatory effector in the inflammation. Several studies have demonstrated an increase in leptin production that occurs during infection and inflammation, suggesting that leptin is a part of the cytokine network which governs the inflammatory-immune response and the host defense mechanisms (4). Many studies have shown that leptin can stimulate various immune cells, and regulate the production of several pro- and anti-inflammatory cytokines through its receptors. In turn, these proinflammatory cytokines can increase systemic leptin concentrations in vivo. Leptin mRNA expression, as well as circulating leptin levels, were increased by many acute phase factors such as TNF-α, IL-1 IL-6, during bacterial infection, or lipopolysaccharide challenge in various studies (12). Similarly, in our *E. coli* induced sepsis model, the sepsis group without exogenous leptin treatment showed an increase in leptin level; however, this increase was not statistically significant. Interestingly, leptin levels were decreased in the leptin group when compared to the sham group, but the difference was statistically insignificant. The slight decrease observed in leptin levels may be related to the pharmacokinetics of leptin. In the literature, different plasma leptin levels after exogenous leptin administration at different time courses were reported (13). Exogenous leptin treatment may cause up-regulation of membrane bound leptin receptors, increase the intracellular uptake of leptin, and decrease circulating leptin levels. For instance, Cano et al. showed that serum leptin levels in 0.1 mg/kg single dose leptin treated

rats decreased to the same levels as the control group 6 h after injection (13). Similar to our finding, Gulen et al. found lower serum leptin levels in the leptin treated group than in the sham group after 5 day leptin treatment (9). A significant increase in serum leptin levels was observed in the sepsis group treated with leptin. This significant increase could be an additive effect of both leptin treatment and inflammation induced leptin synthesis in septic animals.

The role of leptin in inflammation remains incompletely understood. Leptin's action as a modulator of inflammatory/immune response is likely to be complex. In various animal and human experimental studies, both pro- and anti-inflammatory effects and controversial results have been described for leptin. Leptin may contribute to the development and progression of autoimmune responses, and leptin deficiency is associated with reduced inflammation in models of autoimmune disease, such as experimental allergic encephalomyelitis, collagen-induced arthritis, and autoimmune hepatitis (14). On the other hand, leptin appears to exert anti-inflammatory properties in models, such as the administration of endotoxin and TNF-α, and bacterial and viral infections. In these models, leptin deficiency is associated with increased susceptibility to the toxicity of proinflammatory stimuli (15,16). The increased sensitivity to endotoxin and TNF-α in *ob/ob* mice and normal mice can be reversed by leptin administration. Cytokine production from T lymphocytes is suppressed in leptin-deficient children and restored by leptin administration. The general consensus is that leptin exerts a proinflammatory role, while at the same time protecting against infections. It has a pivotal role as an immuno-modulating agent (5,17).

Leptin protects T lymphocytes from apoptosis and regulates T-cell proliferation and activation mainly with T-helper cells activity in the cellular immune response. This anti-apoptotic role of leptin is especially important in sepsis where apoptosis of lymphocytes results in decreased septic survival (4,18). In addition to its effects on T lymphocytes, leptin also influences monocyte activation, phagocytosis, expression of adhesion molecules, and cytokine production. It participates in and crosstalks with some of the main intracellular signaling pathways (4). In neutrophils, leptin exerts anti-apoptotic effects and stimulates complement-mediated phagocytosis, chemotaxis, and the release of oxygen radicals (4,19). Leptin stimulates growth hormones, which may be important in immune homeostasis, given the fact that this cytokine-like hormone has marked influences on immune responses by controlling the survival and proliferation of immune cells (4). Leptin also affects natural killer cell differentiation, proliferation, and activation (20). It stimulates production of proinflammatory cytokines such as TNF-α, IL-2, and IL-6 (17). *Ob/ob* mice (leptin deficient) are characterized by an impaired antioxidant defense, as evidenced by a reduced activity of catalase, glutathione peroxidase, and glutathione reductase (21). Leptin therapy corrects these abnormalities. On the other hand, leptin may increase oxidative stress through multiple mechanisms, especially in hyperleptinemic states, such as obesity (22).

In our sepsis model, we found decreased IL-10 levels, and significantly increased TNF-α and IL-6 levels in the sepsis group when compared to the sham group. High proinflammatory cytokine levels and low anti-inflammatory levels were exerted by leptin administration in the sepsis group, indicating the improving effects of leptin in sepsis. Similarly, in a cerulein-induced acute pancreatitis model, serum TNF-α and IL-1β levels were found to decrease significantly 24 h after single-dose exogenous leptin administration (7). Our understanding of the role of leptin in inflammation is incomplete. Leptin has structural similarity with cytokines such as IL-6, and its receptor is related to the class I cytokine receptors. Overstimulation of Ob-R by exogenous leptin may result in a decrease in cytokine levels. In contrast, there are studies reporting that exogenously administered leptin increased mortality in different sepsis models, and was associated with an increased expression of adhesion and coagulation molecules, cytokine secretion, macrophage infiltration into the liver and kidney, and endothelial barrier dysfunction (23).

Endothelin is a vasoactive mediator produced primarily by endothelial cells; also, it is a potent vasoconstrictor. Piechota et al. demonstrated that endothelial dysfunction causes increased endothelin concentrations in sepsis and its level correlates with the degree of sepsis severity (24). Endothelin acts

as one of the many important essential regulators of muscular tonus of capillary vessels during severe sepsis associated with increased vascular resistance. Leptin is closely associated with endothelin (25). Functional leptin receptors are present on endothelial cells, so leptin may facilitate endothelin production in endothelial cells; furthermore, endothelin stimulates leptin production in adipocytes. However, the actions of leptin to modulate endothelial function remain controversial. For instance, some studies suggest that leptin contributes to endothelial dysfunction or damage in some pathological states (22). By contrast, no correlation between leptin and endothelial function was observed in healthy adolescents and hypertensive patients (22).

In our study, we found the highest serum endothelin levels among the sepsis group. This increase declined significantly in the sepsis group treated with leptin. High endothelin levels in sepsis indicates poor prognosis of sepsis. Somehow decreased endothelin levels in the sepsis group treated with leptin, comparing to the sepsis group, may improve the detrimental effects of endothelin in sepsis.

Tissue MPO activity is frequently utilized to estimate tissue polymorphonuclear leukocytes accumulation in inflamed tissues as an evidence of host defense and inflammation. During bacterial infection, the main functions of neutrophils are to kill off invading pathogens and the resolution of associated inflammation. Myeloperoxidase (MPO), a heme-containing peroxidase, is considered to contribute substantially to the microbicidal activity of neutrophils and monocytes through the generation of a reactive oxidant, mainly hypochlorous acid. Hypochlorous acid is responsible for the oxygen-dependent microbicidal activity of phagocytes. On the other hand, excessive generation of MPO-derived oxidants has been linked to tissue damage in many diseases, especially those characterized by acute or chronic inflammation (26). As a protection, organisms develop defense mechanisms against these generated oxidants. Proteins such as serum albumin, as well as a number of low molecular weight-reducing agents, react rapidly with the highly reactive products of the MPO system, and prevent them from reaching a sensitive target of biological importance. We are thus protected from indiscriminant damage by phagocyte-produced oxidants. By definition, the more reactive the product of the MPO system, the more likely it will be scavenged (27). Overall, we should stress that neutrophils are the first cells to be activated in the host immune response to infection or injury. They are critical cellular effectors in both humoral and innate immunity, and a MPO system that is required for the microbicidal activity of neutrophils. In the study by Hirche et al., they demonstrated that mice deficient in MPO were more likely to die from infection than wild-type mice; so MPO plays an important role in host antibacterial defense, ensuring the antimicrobial resistance of the body (28).

Orlova and Shirshev demonstrated that when monocytes of the peripheral blood of healthy young women were incubated with leptin, it stimulated the activity of MPO secreted by monocytes in cell culture supernatants (29). Among leukocytes, mononuclear phagocytes have the highest density of Ob-R expression, and leptin exerts its effect by binding its receptor on cell surfaces. During the activation of monocytes/macrophages, the amount of Ob-R on their membrane increases considerably (29).

In our study, we observed elevated MPO activities in lung and kidney tissues in sepsis, and the further increase of MPO activity in leptin treated sepsis group means leptin enhances neutrophil activation and plays a role in combating infections. The significant high tissue MPO activities in sepsis may be due to stimulatory effects of inflammation. Increases in MPO activity after leptin treatment in the sepsis group may indicate that leptin alleviates sepsis induced tissue injuries by interfering with MPO activity. Lin et al. showed that, in the mice sepsis model, sepsis decreased the ability of the lung, liver, and kidney to exclude free radicals and bacteria by suppressing their MPO activity. Leptin treatment increased MPO activities and reversed the alterations at the histological level 12 h after injury (30). Yan et al. reported that exogenous leptin recruitment protects organ functions and recovers tissue MPO activity in the mice sepsis model (31). In their sepsis model, made with cecal ligation and perforation, it was found that 12 h after injury sepsis suppressed MPO activity in lung and intestine tissues, indicating decreased neutrophilic activity of these tissues. Leptin

and indomethacin treated groups in the experiment reversed this alteration by increasing tissue MPO activity at a level similar with sham. Leptin stimulated MPO to catalyze free radicals into hydroperoxide in neutrophils through binding with specific leptin receptors on the cells, and induced a subsequent recovery of tissue MPO activity to eliminate excess irritants released by septic injury (32). MPO response to sepsis was different between our group and Yan et al. This difference may come from different sepsis models. On the other hand, contradictory results were seen in the literature about tissue MPO activity in leptin treated sepsis models. Gultekin et al. reported decreased pancreatic and lung MPO activity after leptin treatment in rats with cerulein-induced acute pancreatitis (7). In general, overall results suggest that leptin is required for normal neutrophil complement-mediated phagocytosis of bacteria.

Leptin levels are known to increase in critically ill septic patients, and high levels of leptin are positively correlated with survival. In one study, plasma leptin levels were 3-fold higher in patients who survived the septic episode than in nonsurvivors (32). Recently, it was demonstrated that leptin deficiency in *ob/ob* mice leads to higher mortality and more severe organ damage in sepsis. Moreover, systemic leptin replacement improved the immune response to sepsis. They reported that leptin signaling in the brain increases survival during sepsis in the leptin-deficient, as well as in wild-type mice; in addition, endogenous CNS leptin action is required for an adequate systemic immune response, which suggests a possible therapeutic potential for leptin analogs in infectious disease (6).

In another study, it was demonstrated that leptin acts as an endogenous mediator of neuroprotection during cerebral ischemia via intracellular signaling pathways, and exogenous leptin administration protects against ischemic neuronal injury both in vitro and in vivo cerebral ischemia models (33). In a review, Signore et al. indicated the beneficial effects of leptin in a variety of neurodegenerative diseases, including Parkinson's and Alzheimer's disease, and suggested the possibility of leptin treatment in aging related neurodegenerative diseases (34).

Our data show that leptin is associated with inflammation and sepsis. However, due to leptin's pro- and anti-inflammatory effects on inflammation, the role of leptin in the sepsis mechanism and the use of leptin as a possible therapeutic agent are still debated. The different sepsis models in studies, the complexity of sepsis itself, different leptin dosages, and divergent working groups in experiments may be the reasons for the various results. Additionally, leptin's heterogeneous and opposing actions on inflammation and sepsis may be another reason for the conflicting results in publications.

Finally, based on our results, we think that leptin restricts the inflammatory events in sepsis to some extent; therefore, leptin may arise as a new immunotherapeutic target in sepsis.

Acknowledgements

This work was supported financially by Fatih University with the grant number P53010704.

References

1. Hotchkiss RS, Karl IE. Cytokine blockade in sepsis—Are two better than one? Crit Care Med 2001; 29: 671-2.
2. Kellum JA, Kong L, Fink MP, Weissfeld LA, Yealy DM, Pinsky MR et al. Understanding the inflammatory cytokine response in pneumonia and sepsis: results of the Genetic and Inflammatory Markers of Sepsis (GenIMS) Study. Arch Intern Med 2007; 167: 1655-63.
3. Zhang Y, Proenca R, Maffei M, Barone M, Leopold L, Friedman JM. Positional cloning of the mouse obese gene and its human homologue. Nature 1994; 372: 425-32.
4. Otero M, Lago R, Lago F, Casanueva FF, Dieguez C, Gómez-Reino JJ et al. Leptin, from fat to inflammation: old questions and new insights. FEBS Lett 2005; 579: 295-301.
5. Fantuzzi G, Faggioni R. Leptin in the regulation of immunity, inflammation, and hematopoiesis. J Leukoc Biol 2000; 68: 437-46.
6. Tschöp J, Nogueiras R, Haas-Lockie S, Kasten KR, Castañeda TR, Huber N et al. CNS leptin action modulates immune response and survival in sepsis. J Neurosci 2010; 30: 6036-47.

7. Gultekin FA, Kerem M, Tatlicioglu E, Aricioglu A, Unsal C, Bukan N. Leptin treatment ameliorates acute lung injury in rats with cerulein-induced acute pancreatitis. World J Gastroenterol 2007; 13: 2932-38.
8. Hacioglu A, Algin C, Pasaoglu O, Pasaoglu E, Kanbak G. Protective effect of leptin against ischemia-reperfusion injury in the rat small intestine. BMC Gastroenterol 2005; 5: 37.
9. Gülen S, Dinçer S. Effects of leptin on oxidative stress in healthy and Streptozotocin-induced diabetic rats. Mol Cell Biochem 2007; 302: 59-65.
10. Giacometti A, Ghiselli R, Cirioni O, Mocchegiani F, D'Amato G, Orlando F et al. Therapeutic efficacy of the magainin analogue MSI-78 in different intra-abdominal sepsis rat models. J Antimicrob Chemother 2004; 54: 654-60.
11. Koike K, Moore EE, Moore FA, Read RA, Carl VS, Banerjee A. Gut ischemia/reperfusion produces lung injury independent of endotoxin. Crit Care Med 1994; 22: 1438-44.
12. Faggioni R, Feingold KR and Grunfeld C. Leptin regulation of the immune response and the immunodeficiency of malnutrition. FASEB J 2001; 14: 2565–71.
13. Cano V, Merino B, L Ezquerra L, Somoza B and Ruiz-Gayo M. A cholecystokinin-1 receptor agonist (CCK-8) mediates increased permeability of brain barriers to leptin British Journal of Pharmacology 2008; 154: 1009-15.
14. Siegmund B, Lear-Kaul KC, Faggioni R, Fantuzzi G. Leptin deficiency, not obesity, protects mice from Con A-induced hepatitis. Eur J Immunol 2002; 32: 552-60.
15. Faggioni R, Fantuzzi G, Gabay C, Moser A, Dinarello CA, Feingold KR et al. Leptin deficiency enhances sensitivity to endotoxin-induced lethality. Am J Physiol 1999; 276: 136-42.
16. Takahashi N, Waelput W, Guisez Y. Leptin is an endogenous protective protein against the toxicity exerted by tumor necrosis factor. J Exp Med 1999; 189: 207-12.
17. Fantuzzi G. Adipose tissue, adipokines, and inflammation. Allergy Clin Immunol 2005; 115: 911-19.
18. Wesche-Soldato DE, Swan RZ, Chung CS, Ayala A. The apoptotic pathway as a therapeutic target in sepsis. Curr Drug Targets 2007; 8: 493-500.
19. Bruno A, Conus S, Schmid I, Simon HU. Apoptotic pathways are inhibited by leptin receptor activation in neutrophils. J Immunol 2005; 174: 8090-96.
20. Tian Z, Sun R, Wei H, Gao B. Impaired natural killer (NK) cell activity in leptin receptor deficient mice: leptin as a critical regulator in NK cell development and activation. Biochem Biophys Res Commun 2002; 298: 297-302.
21. Watson AM, Poloyac SM, Howard G, Blouin RA. Effect of leptin on cytochrome P-450, conjugation, and antioxidant enzymes in the ob/ob mouse. Drug Metab Dispos 1999; 27: 695-700.
22. Koh KK, Park SM, Quon MJ. Leptin and cardiovascular disease: response to therapeutic interventions. Circulation 2008; 117: 3238-49.
23. Shapiro NI, Khankin EV, Van Meurs M, Shih SC, Lu S, Yano M et al. Leptin exacerbates sepsis-mediated morbidity and mortality. J Immunol 2010; 185: 517-24.
24. Piechota M, Banach M, Irzmanski R, Barylski M, Piechota-Urbanska M, Kowalski J et al. Plasma endothelin-1 levels in septic patients. J Intensive Care Med 2007; 22: 232-39.
25. Adiarto S, Emoto N, Iwasa N, Yokoyama M. Obesity-induced upregulation of myocardial endothelin-1 expression is mediated by leptin. Biochem Biophys Res Commun 2007; 353: 623-27.
26. van der Veen BS, de Winther MPJ, Heeringa P. Myeloperoxidase: Molecular mechanisms of action and their relevance to human health and disease. Antioxidants & Redox Signaling 2009; 11: 2899-2937.
27. Klebanoff SJ. Myeloperoxidase: friend and foe. J Leukoc Biol 2005; 77: 598-625.
28. Hirche TO, Gaut JP, Heinecke JW, Belaaouaj A. Myeloperoxidase plays critical roles in killing Klebsiella pneumoniae and inactivating neutrophil elastase: effects on host defense. J Immunol 2005; 174: 1557-65.
29. Orlova EG, Shirshev SV. The effect of leptin on the microbiocidal activity of monocytes in humans. Fiziol Cheloveka 2007; 33: 109-13.
30. Lin J, Yan GT, Xue H, Hao XH, Zhang K, Wang LH. Leptin protects vital organ functions after sepsis through recovering tissue myeloperoxidase activity: an anti-inflammatory role resonating with indomethacin. Peptides 2007; 28: 1553-60.
31. Yan GT, Lin J, Hao XH, Xue H, Zhang K, Wang LH. Heart-type fatty acid-binding protein is a useful marker for organ dysfunction and leptin alleviates sepsis-induced organ injuries by restraining its tissue levels. Eur J Pharmacol 2009; 616: 244-50.
32. Bornstein SR, Licinio J, Tauchnitz R, Engelmann L, Negrao AB, Gold P et al. Plasma leptin levels are increased in survivors of acute sepsis: associated loss of diurnal rhythm, in cortisol and leptin secretion. J Clin Endocrinol Metab 1998; 83: 280-83.
33. Valerio A, Dossena M, Bertolotti P, Boroni F, Sarnico I, Faraco G et al. Leptin is induced in the ischemic cerebral cortex and exerts neuroprotection through NF-kappaB/c-Rel-dependent transcription. Stroke 2009; 40: 610-17.
34. Signore AP, Zhang F, Weng Z, Gao Y, Chen J. Leptin neuroprotection in the CNS: mechanisms and therapeutic potentials. J Neurochem 2008; 106: 1977-90.