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Research Article

Anti-Inflammatory Effects of Insulin and Dexamethasone on Experimentally *Escherichia coli* Serotype O55:B5 Induced Endotoxemia in Iranian Fat-Tailed Sheep

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ABSTRACT

The present experiment was performed in order to compare the effect of insulin and dexamethasone on treating endotoxemia following the intravenous infusion of endotoxin of *Escherichia coli* serotype O55:B5 in the Iranian fat-tailed sheep. Twenty clinically healthy one-year old Iranian fat-tailed ewes were randomly assigned into four equal (n=5) experimental groups, comprising of Control, Dexa, Insln 1.5 and Insln 3 groups. Lipopolysaccharide from *Escherichia coli* serotype O55:B5 was administered intravenously at 20 µg/kg. All experimental groups undergone intravenous fluid therapy for 120 minutes after lipopolysaccharide injection. 180 minutes after lipopolysaccharide injection, dexamethasone (in Dexa group at 1 mg/kg) and insulin regular (in Insln 1.5 and 3 at 1.5 and 3 IU/kg, respectively) were injected along with the intravenous fluid for 60 minutes. The Control group just received lipopolysaccharide and was treated only with intravenous fluid without using any drug. The blood samples were collected from all ewes and assayed separated sera for serum acute phase proteins (serum amyloid A and haptoglobin), inflammatory cytokines (tumor necrosis factor-alpha and interferon-gamma) and oxidative stress biomarkers (super oxide dismutase and glutathione peroxidase). In all experimental groups a rapid increase was seen in the amount of acute phase proteins and inflammatory cytokines and a decrease was seen in oxidative stress biomarkers after endotoxemia induction ($P<0.05$). The results of the present study showed that insulin regular at 3 IU/kg controlled acute phase response following endotoxemia induction. The potency of insulin regular at 3 IU/kg was significantly higher than 1.5 IU/kg and dexamethasone at 1 mg/kg ($P<0.05$) in order to treat endotoxemia due to *Escherichia coli* serotype O55:B5 in Iranian fat-tailed sheep.

Keywords: Endotoxemia, treatment, insulin regular, dexamethasone, Iranian fat-tailed sheep

ÖZET

YAĞLI KUYRUKLU İRAN KOYUNLARINDA DENEYSEL ESCHERICHIA COLI SEROTİP O55:B5'E BAĞLI ENDOTOKSEMİDE İNSÜLİN VE DEKSAMETAZONUN ANTİ İNFLAMATUAR ETKİLERİ

Bu deneyde yağlı kuyruklu İran koyunlarında *Escherichia coli* serotip O55:B5'e ait endotoksinlerin intravenöz infüzyonunu takiben insulin ve deksametazonun endotoksemi tedavisindeki etkileri karşılaştırıldı. Yirmi adet klinik olarak sağlıklı bir yaşındaki yağlı kuyruklu İran koyunların tesadüfi seçimiyle Kontrol, Dexa, Insln 1,5 ve Insln 3'ten oluşan dört (n=5) adet deney grubu oluşturuldu. *Escherichia coli* serotip O55:B5'in lipopolisakkaridi 20 µg/kg dozda intravenöz olarak uygulandı. Tüm deney gruplarına lipopolisakkarit enjeksiyonu sonrası 120 dakika süreyle intravenöz

sıvı tedavisi uygulanmıştır. Lipopolisakkarid enjeksiyonundan 180 dakika sonra, deksametazon (Dexa grubuna 1 mg / kg) ve insülin (Insln 1,5 ve 3 gruplarına sırasıyla, 1,5 ve 3 IU / kg) 60 dakika süreyle damar içi sıvı ile birlikte enjekte edildi. Kontrol grubuna sadece lipopolisakkaritten oluşan ve herhangi bir ilacın kullanılmadığı intravenöz sıvı tedavi uygulandı.Tüm koyunlardan kan örnekleri toplanarak elde edilen serumlardan akut faz proteinleri (serum amiloid A ve haptoglobin), inflamatuar sitokinler (tümör nekroz faktörü-alfa ve interferon-gamma) ve oksidatif stres belirteçleri (süper oksit dismutaz ve glutatyon peroksidaz) değerleri ölçülmüştür. Bütün deney grupları içinde endotoksemi indüksiyondan sonra (P<0,05), akut faz proteinleri ve inflamatuar sitokin miktarlarında hızlı bir artış görülürken oksidatif stres belirteçlerinde düşüş görülmüştür. Bu çalışmanın sonucunda endotoksemi indüksiyonundan sonra 3 IU / kg dozda insulinin akut faz yanıtını kontrol ettiği görülmüştür. Yağlı kuyruklu İran koyunlarda *Escherichia coli* serotip O55:B5'e bağlı endotokseminin tedavisinde 3 IU/kg insülinin etkisi 1,5 IU/kg doz insülinden ve 1 mg/kg deksametazonun etkisinden anlamlı derecede yüksek olduğu (P<0,05) belirlenmiştir.

Anahtar Kelimeler: Endotoksemi, tedavi, insulin, deksametazon, yağlı kuyruklu İran koyunu

Introduction

Endotoxemia is the most common form of toxemia in large animals, including the sheep, caused by the presence of lipopolysaccharide (LPS) cell wall components of Gram-negative bacteria in the blood, and characterized clinically by abnormalities in many systems of the body (Radostits et al., 2007). The endotoxins of several species of Gram-negative bacteria such as *Escherichia coli* are major cause of morbidity and mortality in farm animals. Endotoxins of Gram-negative bacteria are the reason of many diseases in ruminants such as mastitis, peritonitis, pneumonia and pleuritis, pericarditis, septic metritis, myositis, meningoencephalitis and some enteritides. There are five therapeutic approaches that should be considered in the treatment of endotoxemia: (1) prevention of endotoxin circulation; (2) neutralization of endotoxin before it interacts with inflammatory cells; (3) prevention of the synthesis, release, or action of inflammatory mediators; (4) prevention of endotoxin-induced cellular activation; and (5) general supportive care with intravenous fluids or colloids and inotropic agents (Moore and Barton, 2003).

The corticosteroids are commonly used in treatment of endotoxemia and dexamethasone is most routinely used in endotoxic animals. Corticosteroids improve capillary endothelial integrity and tissue perfusion, decrease activation of complement and clotting cascade, decrease neutrophil aggregation, stabilize lysosomal membranes, protect against hepatic injury and improve survival rate (Radostits et al., 2007).

Insulin is a hormone that is central in regulation carbohydrate and fat metabolism in the body. Insulin causes cells in the liver, muscle, and fat tissue to take up glucose from the blood, storing it as glycogen in the liver and muscle. Insulin decreases the incidence of sepsis and improves the mortality of critically ill patients (Dandona et al., 2005). In endotoxemic as well as in thermally injured rats, insulin attenuates the systemic inflammatory response by decreasing pro-inflammatory and increasing anti-inflammatory cascade (Leffler et al., 2007). Recently, Van den Berghe et al. (2006) showed that intensive insulin therapy decreases morbidity and mortality in critically ill patients. Recent studies suggested an anti-inflammatory effect of insulin by increasing the anti- and decreasing the pro-inflammatory cascade and thereby homeostasis restoration in endotoxemic animals. In addition, insulin prevented liver damage and preserved liver function in these animals (Jeschke et al., 2004).

Despite advances in the diagnosis and supportive treatments, endotoxemia is associated with high morbidity and mortality (Rivers et al., 2001), thus, its control could potentially improve clinical outcomes. Anti-endotoxic and anti-inflammatory effects of insulin were reviewed in several and different researches in medical sciences (Dandona et al., 2005; Iwasaki et al., 2009; Leffler et al., 2007), but there is a lack in investigations on potential therapeutic characteristics of insulin for treatment of endotoxemia in large animal medicine. The present experiment was conducted to evaluate the anti-inflammatory

effects of insulin and dexamethasone on treatment of *Escherichia coli* serotype O55:B5 induced endotoxemia in Iranian fat-tailed sheep based on measurement of serum acute phase proteins (serum amyloid A and haptoglobin), inflammatory cytokines (tumor necrosis factor-alpha and interferon-gamma) and oxidative stress biomarkers (superoxide dismutase and glutathione peroxidase). The results of the present study may reveal the potential for a new therapeutic regimen for endotoxemia in small ruminant medicine.

Materials and Methods

Animals

The present experiment was performed after being approved by the Ethics Committee of School of Veterinary Medicine, Shiraz University. Twenty clinically healthy 1-year old Iranian fat-tailed ewes (25±1.5 kg, bodyweight) were randomly selected for the project in April 2011. All animals were maintained in Laboratory Teaching Barn of Agricultural College of Shiraz University, Badjgah region (latitude of 29° 32' N and longitude 52° 35' E, 1810 m above sea level), south of Iran. Four weeks before commencing experiments, each sheep received albendazole (15 mg/kg, orally; Dieverm®600, Razak Pharmaceutical Co, Tehran, Iran) and ivermectin (0.2 mg/kg, subcutaneously; Erfamectin®1%; Erfan Pharmaceutical CO, Tehran, Iran) to control internal and external probable parasites. All ewes were maintained in open-shed barns with free access to water and shade. The ration included mainly alfalfa hay, corn silage, corn and barley. Subsequently, ewes were assigned randomly into 4 experimental equal (n=5) groups, comprising Insln 1.5, Insln 3, Dexa and Control.

Chemicals and drugs

Phenol extracted LPS from *Escherichia coli* serotype O55:B5 (Sigma-Aldrich®; product NO. L2880) was used to induce endotoxemia in ewes at 20 µg/kg. This endotoxin was diluted in sterile phosphate-buffered saline (PBS) and divided into twenty equal doses each containing 500 µg endotoxin and stored at -80°C until endotoxemia induction. For each experiment, each dose was thawed and infused intravenously as described below. Insulin regular (Lansulin®R, Exir Pharmaceutical CO., Boroojerd, Iran) was intravenously injected to Insln experimental groups according to experimental design. Dexamethasone (Vetacoid®0.2%, Aburaihan Pharmaceutical CO., Tehran, Iran) was infused to Dexa group intravenously as described in the experimental procedures, below. The intravenous fluid used in the present experiment was dextrose 5% plus sodium chloride 0.45% (Shahid Ghazi Pharmaceutical CO., Tabriz, Iran).

Experimental procedures

Induction and treatment of endotoxemia

The schematic diagram of the present experimental design is represented in figure 1. A 16 gauge 5.1 cm catheter was secured in the left jugular vein and used for blood samplings, endotoxin and drugs infusions. All twenty ewes were evaluated clinically before and 1, 2, 3, 4, 5, 6 and 24 hours after LPS injection. Clinical parameters monitored during experiments, included rectal temperature, heart and respiratory rates, mucous membrane color, capillary refill time, appetite and fecal consistency. Thawed LPS was diluted in 250 milliliter of normal saline and infused intravenously at the rate of 10 ml/kg/hour. Fluid therapy was performed in all experimental groups over 120 minutes after LPS injection by dextrose 5% plus sodium chloride 0.45% at the rate of 20 ml/kg/hour. Drugs (insulin regular and dexamethasone) were used at 180 minutes after LPS injection along with the fluid over 60 minutes. Insulin regular was infused at 1.5 and 3 IU/kg in Insln 1.5 and Insln 3 groups, respectively. Dexamethasone was used at 1 mg/kg intravenously in Dexa group. Control group received LPS and treated only by intravenous fluid without any drugs. Blood glucose was monitored in all animals, using a rapid response glucose meter device (Accucheck Active®, Roche, Germany) to prevent hypoglycemia.

Blood sampling and serological assays

Blood samples were collected from all ewes through the fixed catheter prior and 1, 2, 3, 4, 5, 6 and 24 hours after LPS injection in plain tubes. Immediately after collections, sera were separated by centrifugation (for 10 minutes at 3,000×g) and stored at −22°C until assayed.

Figure 1 Schematic diagram of the present experimental design. Lipopolysaccharide (LPS) was injected at zero hour and intravenous fluid therapy was commenced 2 hours later in Iranian fat tailed sheep. Dexamethasone and insulin regular were infused via fluid between 3rd to 4th hrs, over 60 minutes in Dexamethasone, Insulin 1.5 and Insulin 3 groups. Venous blood sampling was performed in all demonstrated hours

Şekil 1. Deneysel çalışmanın şematik diyagramı. Yağlı kuyruklu İran koyunlarına lipopolisakkarid (LPS) sıfırıncı saatte enjekte edildi ve intravenöz sıvı tedavisi 2 saat sonra başlandı. Deksametazon ve insülin 3. ve 4. saatlerde 60 dakika boyunca sıvı yoluyla Deksametazon, İnsülin 1,5 ve İnsülin 3 gruplarına infüze edildi. Venöz kan örneklemeleri belirtilen saatlerde gerçekleştirildi.

Blood sampling and serological assays

Blood samples were collected from all ewes through the fixed catheter prior and 1, 2, 3, 4, 5, 6 and 24 hours after LPS injection in plain tubes. Immediately after collections, sera were separated by centrifugation (for 10 minutes at 3,000×g) and stored at −22°C until assayed.

Haptoglobin (Hp) was measured according to prevention of the peroxidase activity of hemoglobin, which is directly proportional to the amount of Hp (Tridelta Development Plc, Wicklow, Ireland). Serum amyloid A (SAA) was measured by a solid phase sandwich ELISA test kit (Tridelta Development Plc, Wicklow, Ireland). Tumor necrosis factor-alpha (TNF-α) and interferon-gamma (IFN-γ) were measured by a solid phase sandwich ELISA test kit (AbC 606 and AbC 607, respectively; Votre fournisseur AbCys S.A. Paris, France). The superoxide dismutase (SOD) activity was measured by a modified method of iodophenyl nitrophenol phenyltetrazolium chloride (Feldman et al., 2000), (RANSOD Kit, Randox Com, UK). The glutathione peroxidase (GPx) activity was measured by the method of Paglia and Valentine (1967), (RANSEL kit, Randox Com, UK).

Statistical analyses

Data were expressed as mean ± standard error of mean (SEM). Statistical analysis was performed using one-way ANOVA with LSD post-hoc test to compare mean concentrations of different serological factors within similar hours between different experimental groups. Paired samples t-test was used to determine differences between two different times in each experimental group using SPSS software (SPSS for Windows, version 11.5, SPSS Inc, Chicago, Illinois). The level of significance was set at $P<0.05$.

Results

Serum concentrations of SAA and Hp in different times of experimental groups are presented in Figure 2. SAA and Hp elevated rapidly after endotoxemia induction in all experimental groups (Figure 2). These rapid elevations were different between the zero and 3rd hours (after which intravenous fluid therapy commenced; $P<0.05$). Serum concentrations of SAA and Hp in Insln 3 group were lower than other experimental groups after 3rd hour (Figure 2). Concentrations of SAA in Insln 3 group were significantly lower than other experimental groups, in hour 6 but there were no significant differences between treatment groups in hour 24 ($P<0.05$, Figure 2A). Hp concentrations in Insln 3 group were significantly lower than Insln 1.5 and Dexa groups in hours 5, 6, 24 and hours 5 and 6, respectively ($P<0.05$, Figure 2B).

Serum concentrations of TNF-α and IFN-γ in different hours in each experimental group are presented in Figure 3. Rapid elevation of

serum TNF-α and IFN-γ was detected after endotoxemia induction in all experimental groups (Figure 3). There were significant differences between elevation of TNF-α at 3rd hour and their base line levels at hour zero ($P<0.05$, Figure 3A). The same pattern was observed for IFN-γ, also. Serum concentrations of TNF-α and IFN-γ in Insln 3 group were lower than other experimental groups after 3rd hour (Figure 3). The results of the one-way ANOVA showed that concentrations of TNF-α in Insln 1.5 and Insln 3 groups, respectively, were significantly higher and lower than Dexa group in hours 4, 6 and 24 ($P<0.05$; Figure 3A). Serum concentrations of IFN-γ in Insln 3 group were significantly lower than Insln 1.5 group in hours 4, 5, 6 and 24 and Dexa group in hours 4, 6 and 24, respectively ($P<0.05$, Figure 3B).

Serum concentrations of SOD and GPx at 3rd hour (at which intravenous fluid therapy commenced) were significantly lower than base line levels at hour zero ($P<0.05$, Figure 4). Levels of SOD and GPx in Control and Insln 1.5 groups were not significant different at all hours. SOD and GPx concentrations in Insln 3 group were significantly higher than Insln 1.5 group at 4th, 5th, 6th and 24th hours ($P<0.05$). Concentration of SOD in Dexa group was significantly lower than Insln 3 group at 4th and 5th hours. Serum levels of SOD in Dexa group were higher than Insln 1.5 group at 4th hours and all hours after it, significantly ($P<0.05$, Figure 4A). Serum levels of SOD were not significantly different between zero (base line level) and 24th hours in Insln 3 and Dexa groups. Concentrations of GPx in Dexa and Insln 3 groups were not significant different at all hours ($P>0.05$, Figure 4B). Significant differences were seen in serum GPx concentrations between Insln 1.5 and Insln 3 groups at 4th, 5th, 6th and 24th hours. Concentrations of GPx were not significantly different between zero (base line level) and 24th hours in Insln 3 group ($P=0.23$).

All sheep were considered permanent survivors, alive and healthy after all experiments.

Discussion

The acute phase response (APR) involves the induction of an inflammatory mediator cascade which is characterized by both local vascular effects and systemic, multiorgan effects. The latter include biosynthetic changes, systemic responses to an acute inflammatory stimulus is the alteration in the hepatic biosynthetic profile of acute phase proteins (APPs) (Gabay and Kushner, 1999). APPs and their changes due to various inflammatory and non-inflammatory conditions have been studied particularly pronounced in the liver, which modify the profile of circulating plasma proteins. One of the most intensively studied intensively in many animal species (Murata et al., 2004; Murata, 2007; Petersen et al., 2004). The evaluation of APPs can assist the veterinarians to diagnose the disease along with the hematological and clinical tests. Haematological factors are very variable in different stages of inflammatory diseases, and clinical tests diagnose the disease when it has been developed. However, APPs increase during the development of disease and decrease in the recovery stage and can diagnose the disease in the early stages (Nazifi et al., 2008).

SAA and Hp as well as other APPs have been proposed as markers of stress in animals (Pineiro et al., 2007). SAA is an apolipoprotein of high-density lipoprotein and considered as one of the major APPs in vertebrate (Gruys et al., 1994). Determination and evaluation of SAA showed that this APP could be valuable factor in the diagnosis of infections (Alsemgeest et al., 1994). Lehtolainen et al. (2004) reported that during experimental endotoxin-induced mastitis in cattle, SAA concentrations increased both in serum and in milk. The results of the present experiment showed that SAA was elevated rapidly after endotoxemia induction in all experimental groups, significantly ($P<0.05$; Figure 2A).

SAA reflected the course of inflammation and its level correlated with the clinical severity of the inflammation. SAA has the greatest role in bacterial and pyogenic infections and increases in common infectious diseases (Nazifi

et al., 2008). At the beginning of inflammatory reactions or in injury, SAA concentrations increase rapidly. However, the underlying mechanism that causes increase in SAA has not been clearly defined (Nazifi et al., 2008).

Hp is an alpha2-globulin synthesized in the liver and is another major APP in numerous species of production and companion animals (Feldman et al., 2000). In ruminants, the level of circulating Hp is negligible in normal animals but increases over 100-fold with immune stimulation (Conner et al., 1989). Many studies have indicated the significance of Hp as a clinically useful parameter for the evaluation of the occurrence and severity of inflammatory diseases in sheep (Pfeffer and Rogers, 1989). Determinations and evaluations of serum Hp showed that this protein could be valuable in the diagnosis of infection and inflammatory conditions (Alsemgeest et al., 1994). In the present experiment, a rapid elevation in serum concentrations of Hp in all ewes were observed following endotoxin infusion, significantly (P<0.05; Figure 2B).

The results of the present study showed that intravenous infusion of insulin regular at 3 IU/kg was more effective than dexamethasone at 1 mg/kg in decreasing the serum concentrations of APPs (both SAA and Hp) in different hours, significantly (P<0.05; Figure 2).

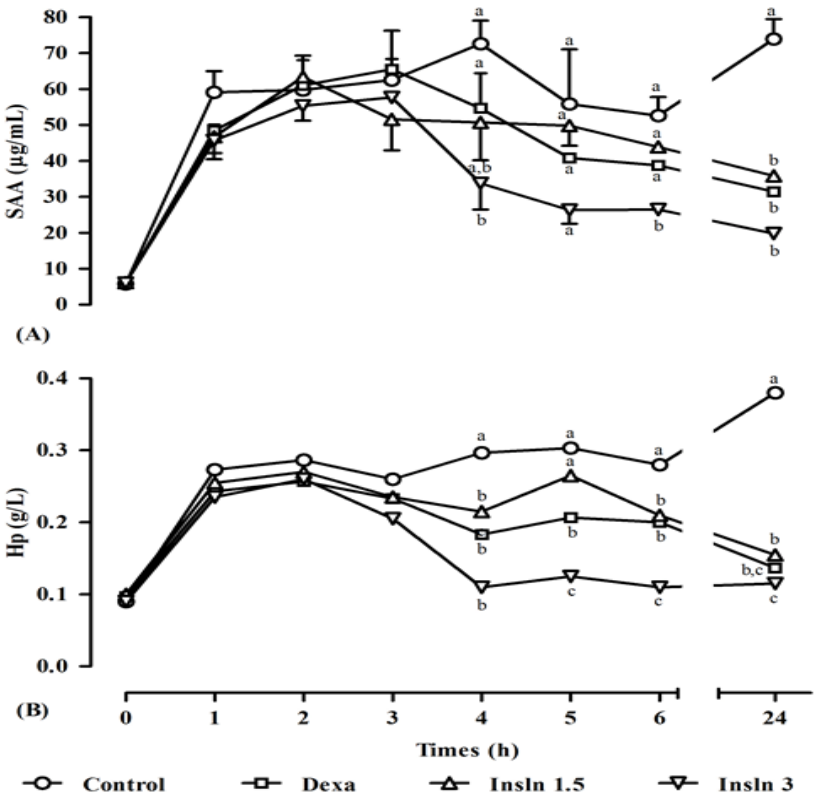

Figure 2. Effect of dexamethasone (1 mg/kg) and insulin regular (1.5 and 3 IU/kg) on serum concentrations of serum amyloid A (SAA, A) and haptoglobin (Hp, B) in different times following induction and treatment of endotoxemia in Iranian fat-tailed sheep. [a, b, c] different letters show significant differences in similar hours between groups (P<0.05).

Şekil 2. Yağlı kuyruklu İran koyunlarında endotoksemi indüksiyonu ve tedavisini takiben deksametazon (1 mg/kg) ve insülin (1,5 ve 3 IU/kg)'in serum amiloid A (SAA, A) ve haptoglobin (Hp, B) konsantrasyonuna etkisi. [a, b, c] farklı gruplar arasındaki aynı saat içindeki önemli farklılıkları P <0.05) göstermektedir.

TNF-α is a cytokine involved in systemic inflammation and member of a group of cytokines that stimulate the APR. The primary role of TNF-α is in the regulation of immune cells.

Significant (P<0.05) and rapid elevation of serum TNF-α was observed before commencing intravenous fluid therapy in all experimental groups. In the present experimental endotoxemia induction, marked and significant depression of TNF-α was observed in Insln 3 group than other experimental groups in different hours after commencing fluid therapy (P<0.05; Figure 3A).

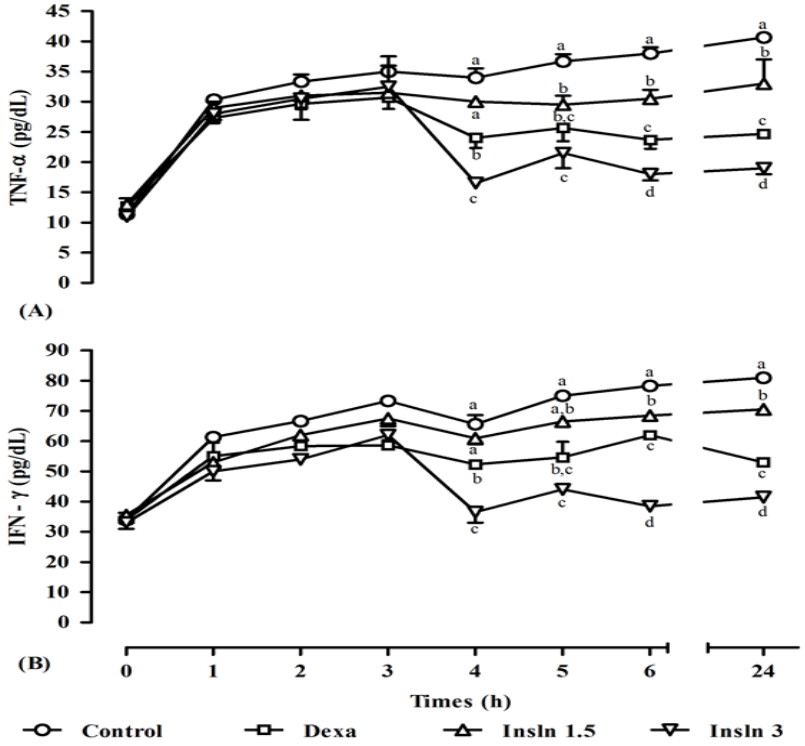

Figure 3. Effect of dexamethasone (1 mg/kg) and insulin regular (1.5 and 3 IU/kg) on serum concentrations of tumor necrosis factor-alpha (TNF-α, A) and interferon-gamma (IFN- γ, B) in different times following induction and treatment of endotoxemia in Iranian fat-tailed sheep. [a, b, c, d] different letters show significant differences in similar hours between groups (P<0.05)

Şekil 3. Yağlı kuyruklu İran koyunlarında endotoksemi indüksiyonu ve tedavisini takiben deksametazon (1 mg/kg) ve insülin (1,5 ve 3 IU/kg)'in serum tümör nekroz faktörü-alfa (TNF-α, A) ve interferon-gamma (IFN-γ, B) konsantrasyonuna etkisi. [a, b, c] farklı gruplar arasındaki aynı saat içindeki önemli farklılıkları (P <0.05) göstermektedir.

Satomi et al. (1985) showed that exogenous insulin injection inhibited TNF-α production in a dose-related manner in animals after LPS challenge. Iwasaki et al. (2009) showed that insulin had a mild but significant suppressive effect on TNF-α. Addition of insulin to cultures of peritoneal exudate cells from *Propionibacterium acnes* primed mice blocked TNF-α production, whereas in control animals it did not do the same effect (Das, 2002). Insulin suppressed TNF-α production by mononuclear cells (Boichot et al., 1999).

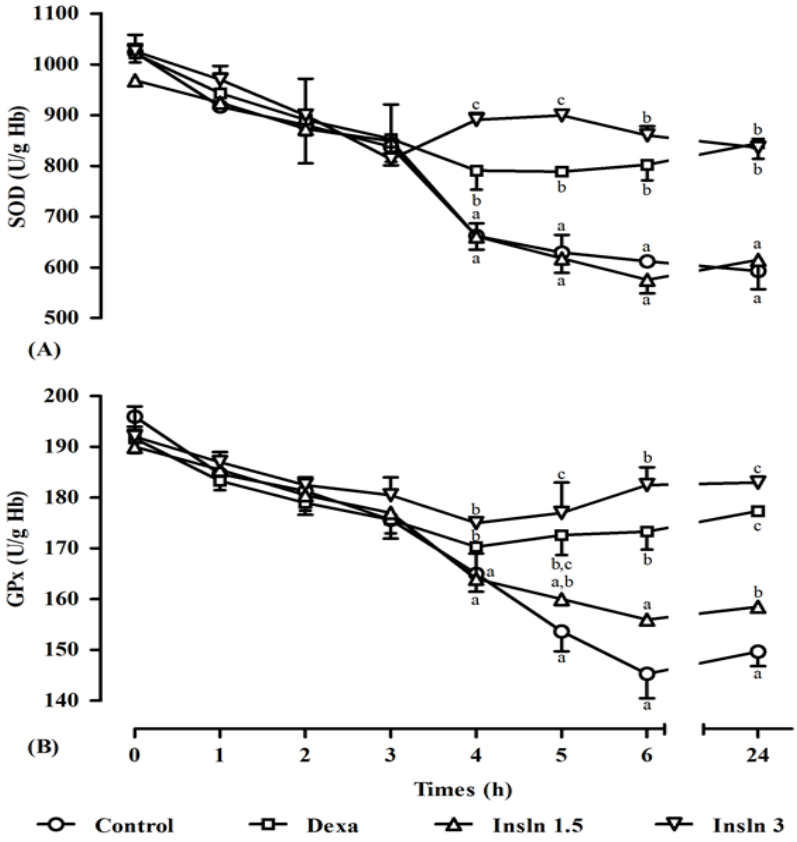

Figure 4. Effect of dexamethasone (1 mg/kg) and insulin regular (1.5 and 3 IU/kg) on serum concentrations of super oxide dismutase (SOD, A) and glutathione peroxidase (GPx, B) in different times following induction and treatment of endotoxemia in Iranian fat-tailed sheep. [a, b, c] different letters show significant differences in similar hours between groups (P<0.05).

Şekil 4. Yağlı kuyruklu İran koyunlarda endotoksemi indüksiyonu ve tedavisini takiben deksametazon (1 mg/kg) ve insülin (1,5 ve 3 IU/kg)'in serum süperoksit dismütaz (SOD, A) ve glutatyon peroksidaz (GPx, B) konsantrasyonuna etkisi. [a, b, c] farklı gruplar arasındaki aynı saat içindeki önemli farklılıkları (P <0.05) göstermektedir.

IFN-γ is a dimerized soluble cytokine that is the only member of the type II class of interferons. IFN-γ is a cytokine that is critical for innate and adaptive immunity against viral and intracellular bacterial infections. The importance of IFN-γ in the immune system stems in part its immunostimulatory and immunomodulatory effects. IFN-γ is produced predominantly by natural killer and natural killer T cells as part of the innate immune response. Endotoxin activates macrophage microbicidal effector functions and the production of proinflammatory cytokines, such as IFN-γ (Schroder et al., 2004).

The results of the IFN-γ assay showed that the serum concentrations of this inflammatory cytokine was lower significantly in Insln 3 group than other experimental groups in several hours after treatment (P<0.05; Figure 3B).

The pathology of endotoxemia is probably jointly mediated by multiple cytokines released during sepsis. Both exogenously administered and endogenously produced IFN-γ demonstrably contribute to endotoxic mortality as well (Heinzel, 1990). The ability of IFN-γ to increase macrophage TNF-α production by both transcriptional and translational mechanisms has been well described (Burchett et al., 1988).

SOD is the key antioxidant enzyme because superoxide is one of the main reactive oxygen species in the cell. SOD is responsible for the quenching of superoxide radicals which are released during the chemical reactions of the several metabolic pathways. Free radicals are formed by various ways. In the body, oxygen as well as hydroxyl radicals are generated during energy productions. When they formed, these free radicals initiate their own reactions by that exerting potentially harmful effects on the several systems of the body. Normally, the free radicals are converted to a less reactive form by the free radical quenching enzymes which are naturally found in the cell. By protecting the O_2 metabolizing cells against the harmful effects of the superoxide free radicals, this enzyme catalyzes the removal of the O_2 free radical. On the other hand, the enzymatic levels of SOD are altered to a considerable extent in various diseased states exhibiting either elevation or depletion in activity (Bauer and Bauer, 1999).

In the present experiment, serum concentrations of SOD were decreased after endotoxemia induction and these decreasing were continued in Control and Insln 1.5 groups until hour 24 (Figure 4A). Levels of SOD were increased after treatment and these levels were near to base line levels at 24th hour in Dexa and Insln 3 groups.

Wiryana (2009) revealed that insulin treatment of hyperglycemic patients can decrease over production of superoxide anions and concomitantly decrease SOD consumption and lead to increasing serum SOD levels.

GPx is an endogenous antioxidant which protects cells from free radicals (Pompella et al., 2003). GPx is found almost exclusively in its reduced form, since the enzyme that reverts it from its oxidized form, glutathione reductase, is constitutively active and inducible upon oxidative stress (Pastore et al., 2003). The activity of GPx is used as an indicator for oxidative stress and its activity increases in affected animals (Podil'chalk et al., 1996). According to our findings, serum GPx activity was depressed after intravenous LPS infusion and these depression were continued in Control and Insln 1.5 groups until hour 24 (Figure 4B). Levels of GPx were increased after treatment and these levels were near to base line levels at 24th hour in Dexa and Insln 3 groups.

In the Kang's et al. (2003) study, serum starvation induced reactive oxygen species accumulation, was suppressed by the addition of insulin so they have suggested that insulin can modulate reactive oxygen species generation. Kang et al. (2003) had demonstrated that the antiapoptotic action of insulin is paralleled by the reduction of reactive oxygen species generation. A recent study has suggested that generation of reactive oxygen species in response to insulin is integral to activation of the distal insulin signaling cascade (Mahadev et al., 2001).

The present experiment showed that the serum concentration of oxidative stress enzymes was near to base line levels in Insln 3 group. This finding may be explained as effectiveness of insulin on decreasing reactive oxygen species pathways; hence the consumption of oxidative stress enzymes is lower than other experimental groups.

Insulin is becoming more and more attractive as an agent to improve outcome of critically ill patients and attenuate the pro-inflammatory cascade (Leffler et al., 2007). Insulin exerts an anti-inflammatory effect on cellular mediators and hepatic APR after an inflammation. The systemic inflammatory response after inflammation leads to hypermetabolism and thus protein degradation and catabolism. As a consequence, the structure and function of essential organs, such as the liver are compromised and contribute to multi-organ failure and mortality (Takala et al., 1999). Pro-inflammatory mediators such as pro-inflammatory cytokines and APPs were thought to trigger and enhance this response and to mediate the catabolic effects (Frost et al., 1997). In an animal model, insulin had anti-inflammatory effects by decreasing pro-inflammatory signal transcription factors and pro-inflammatory cytokines, while increasing anti-inflammatory cytokines (Jeschke et al., 2002). In the present experimental study,

insulin at 3 IU/kg was significantly ($P<0.05$) decreased pro-inflammatory cytokine and hepatic acute phase protein concentrations, in comparison to insulin and dexamethasone at 1.5 IU/kg and 1 mg/kg, respectively (Figures 2 and 3). Insulin acts as an anti-inflammatory molecule through direct cellular effects rather than indirect effects. Insulin alters the intracellular signal cascade in the liver. Decreased APP expression was associated with increased synthesis of constitutive-hepatic-proteins, such as prealbumin and retinol binding protein. As a clinical relevant consequence, albumin substitution requirement to maintain normal serum albumin levels was significantly decreased in the insulin group when compared with the control group (Jeschke et al., 2004). Jeschke et al. (2004) shown that insulin alters the intracellular signal cascade in the liver. Insulin decreases some of the pro-inflammatory signal transcription factors (Jeschke et al., 2002). An upregulation of both transcription factors lead to impaired organ function and protein synthesis, such as albumin (Niehof et al., 2001). Therefore, it appears that insulin improves organ function and protein synthesis during the hypermetabolic response through the signal transcription factors. Thus insulin may act as an anti-inflammatory molecule by 2 different pathways: by decreasing pro-inflammatory mediators and by increasing anti-inflammatory mediators (Jeschke et al., 2004). Intensive insulin therapy exerts an anti-inflammatory effect on patients by reducing levels of APPs, inflammatory cytokines, improving the systemic inflammatory response, and suppressing the hepatic APR (Jeschke et al., 2004).

In conclusion, in the present experiment, it has been shown that insulin acts as an anti-inflammatory mediator by decreasing pro-inflammatory cytokines, pro-inflammatory hepatic APPs and modulating oxidative enzymes activity after *Escherichia coli* serotype O55:B5 endotoxemia induction in the Iranian fat-tailed sheep. According to our findings insulin induces its effects by dose dependent manner. Furthermore, the anti- and pro-inflammatory effects of insulin at 3 IU/kg are significantly higher than dexamethasone at anti-endotoxic shock dose (1 mg/kg) in treatment of endotoxemia in Iranian fat-tailed sheep. The results of the present experiment may suggest a new potential therapeutic regimen on endotoxemia in small ruminant medicine. Potentially, these results may be used in treatment of other infectious inflammatory conditions in small ruminants.

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