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The comparative clinical efficacy of intravenous insulin regular, dexamethasone and flunixin meglumine on ovine experimental endotoxemia

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Abstract

The lack of an effective treatment for endotoxemia causes high mortality rates in affected animals. Forty clinically healthy 1-year old Iranian fat-tailed ewes were assigned randomly into 8 experimental equal (n = 5) groups, comprising Insln 1.5, Insln 3, Insln 6, Insln 9, Insln 20, Dexa, Flnx and Control. Lipopolysaccharide from *E. coli* serotype O55:B5 was used to induce endotoxemia in ewes at 20 µg/kg as bolus intravenous administration. All forty ewes were evaluated clinically before and 1, 2, 3, 4, 5, 6 and 24 h 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. Insulin regular, flunixine meglumine and dexamethasone were used at 180 min after LPS injection along with the fluid over 60 min. Insulin regular was infused at 1.5, 3, 6, 9 and 20 IU/kg in Insln 1.5, Insln 3, Insln 6, Insln 9 and Insln 20 groups, respectively. Flunixin meglumine and dexamethasone were used at 2.2 mg/kg and 1 mg/kg intravenously in Flnx and Dexa groups, respectively. In Insln 3 group, at 5th and 6th hours, the heart rate was significantly lower than other experimental groups. Respiratory rate of all studied animals at 5th and 6th hours in Insln 3, Flnx and Dexa were statistically similar together and lower than other groups, significantly. In conclusion, the anti endotoxic effects of insulin regular at 3 IU/kg were statistically similar to dexamethasone (1 mg/kg) and flunixin meglumine (2.2 mg/kg).

Keywords: Endotoxemia, Treatment, Insulin regular, Flunixin meglumine, Dexamethasone

Introduction

The presence of lipopolysaccharide (LPS) cell wall components of Gram-negative bacteria in the blood is defined as endotoxemia. When the term is used clinically, it implies only the presence of clinical signs typically caused by circulating endotoxins.

The most common form of toxemia in large animals is endotoxemia and is characterized clinically by abnormalities in many systems of the body (Radostits *et al.*, 2007). The endotoxins of several species of Gram-negative bacteria are a major cause of morbidity and mortality in farm animals, including sheep (Chalmeh *et al.*, 2013a). Patients subject to endotoxins exhibit an acute phase inflammatory response. This is characterized clinically by fever, drowsiness, and anorexia (Jaffer *et al.*, 2010). Despite recent advances in the understanding of the molecular cascade of the systemic inflammatory response syndrome and diagnosis and supportive treatments of endotoxemia, the lack of an effective treatment still remains a clinical problem and endotoxemia acts as

a common cause of high mortality in large animal practice (Radostits *et al.,* 2007). There are several therapeutic regimens for the treatment of endotoxemia. The basic approaches to endotoxemia treatment are prevention of movement of endotoxin into the bloodstream, neutralization of endotoxins, prevention of pro-inflammatory and inflammatory pathways and general supportive care with intravenous fluids, colloids, anti-inflammatory and inotropic agents (Moore and Barton, 2003).

Insulin is a hormone that is central in regulating carbohydrate and fat metabolism in the body. It facilitates the entry of glucose into muscle, adipose and several other tissues and stores 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 the proinflammatory and increasing the 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 restoring homeostasis in thermally injured and endotoxemic animals. In addition, insulin prevented liver damage and preserved liver function in these animals (Jeschke *et al.,* 2004).

The treatment of endotoxemia is generally performed in large animals by using non-steroidal anti-inflammatory drugs (NSAIDs) because of their analgesic, anti-inflammatory and antipyretic properties. Flunixin meglumine is an NSAID and is one type of drug used for the treatment of endotoxemia. Flunixin meglumine potently inhibits cyclooxygenase and the synthesis of eicosanoids, and also modulates the acute hemodynamic changes during endotoxemia. Although this drug is the most widely used NSAID in endotoxemia treatment, there is little experimental evidence demonstrating its efficacy over other NSAIDs in sheep (Chalmeh *et al.,* 2013b).

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).

Despite advances in diagnosis and supportive treatments, endotoxemia in farm animals is associated with high morbidity and mortality (Rivers *et al.,* 2001), and thus its control could potentially improve clinical outcomes. Anti-endotoxic and anti-inflammatory effects of insulin have been reviewed by several different researchers in medical sciences (Dandona *et al.,* 2005; Leffler *et al.,* 2007; Iwasaki *et al.,* 2009), but to the best of the author's knowledge, the investigations into the potential therapeutic characteristics of insulin for treatment of endotoxemia in large animal medicine are questionable.

Circulating LPS enhances the production and release of inflammatory mediators and the clinical signs of endotoxemia are commonly related to these mediators. These factors interfere with hemodynamic functions and cause hyperdynamic phase of endotoxemia such as fever, gastrointestinal dysfunction, changes in blood pressure, respiratory disorders, etc. (Danek and Żurek, 2014). There are many behavioral and clinical changes in sheep during the endotoxemia related to the dose LPS and the sensitivity to endotoxin. These alterations are a result of multiple organ dysfunctions such as cardiovascular, respiratory and digestive systems (Radostits *et al.,* 2007). Fever takes place in the sheep exposed to endotoxins, but its extent is dependent on the dose of LPS (Heidari *et al.,* 2016). Sokkar *et al.* (2003) showed that the second administration of LPS did not result in a substantial increase in rectal temperature and it may be due to endotoxin tolerance. Several researchers evaluated the clinical signs of endotoxic sheep before and after different therapeutic regimens (Sokkar *et al.*, 2003; Heidari *et al.,* 2016; Hajimohammadi *et al.*, 2018). They revealed that clinical characteristics of these patients improve following treatment, hence, it may be suggested that clinical alterations of endotoxic sheep are related to acute phase reactions during endotoxemia.

The present experiment was conducted to clinical evaluation of 5 different doses of insulin regular in comparison with standard anti endotoxic doses of flunixin meglumine and dexamethasone in the treatment of endotoxemia induced by *E. coli* serotype O55:B5 in Iranian fat-tailed sheep based on assessing the clinical parameters.

Materials and Methods

Animals

The present experiment was performed after being approved by the Ethics Committee of School of Veterinary Medicine, Shiraz University. Forty 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, 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 both 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 8 experimental equal (n = 5) groups, comprising Insln 1.5, Insln 3, Insln 6, Insln 9, Insln 20, Dexa, Flnx and Control.

Chemicals and drugs

Phenol extracted LPS from *E. coli* serotype O55:B5 (Sigma–Aldrich®; product NO. L2880) was used to induce endotoxemia in ewes at 20 µg/kg as bolus intravenous administration. In our experiment, each sheep received only one dose of the LPS and no

further administration was allowed. So, LPS tolerance phenomenon was prevented (Radostits *et al.,* 2007). This endotoxin was diluted in sterile phosphate-buffered saline (PBS), divided into forty 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 the experimental design. Flunixin meglumine (Meganix®5%, Erfan Pharmaceutical Co., Tehran, Iran) and dexamethasone (Vetacoid®0.2%, Aburaihan Pharmaceutical Co., Tehran, Iran) were infused 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

A 16 gauge 5.1 cm catheter was secured in the left jugular vein and used for blood samplings, endotoxin and drugs infusions. All forty ewes were evaluated clinically before and 1, 2, 3, 4, 5, 6 and 24 h after LPS injection. Clinical parameters were monitored during experiments included rectal temperature, heart and respiratory rates, mucous membrane color, capillary refill time, appetite and fecal consistency. In the present study, we presented the appetite and fecal consistency as normal (grade 1) and weak (grade 2). The normal mucous membranes color was graded as 1 and congestion of these membranes presented as grade 2. Furthermore, blood glucose was monitored in all animals, using a rapid response glucose meter device (Accucheck Active®, Roche, Germany).

Thawed LPS was diluted in 250 ml of normal saline and infused intravenously at the rate of 10 ml/kg/h. Fluid therapy was performed in all experimental groups over 120 min after LPS injection by dextrose 5% plus sodium chloride 0.45% at the rate of 20 ml/kg/h. Insulin regular, flunixine meglumine and dexamethasone were used at 180 min after LPS injection along with the fluid over 60 min. Insulin regular was infused at 1.5, 3, 6, 9 and 20 IU/kg in Insln 1.5, Insln 3, Insln 6, Insln 9 and Insln 20 groups, respectively. Flunixin meglumine and dexamethasone were used at 2.2 mg/kg and 1 mg/kg intravenously in Flnx and Dexa groups, respectively. The control group received LPS and was treated only by intravenous fluid without any drugs.

Statistical analysis

Data were expressed as mean ± standard error of mean (SEM) for quantitative clinical parameters. Statistical analysis was performed using one-way ANOVA with LSD post hoc test to compare mean concentrations of different quantitative clinical parameters within similar hours between different experimental groups. The data of qualitative clinical parameters were presented as median (min–max) and Kruskal–Wallis test was used for comparison of these parameters among all

groups using SPSS software (SPSS for Windows, version 11.5, SPSS Inc., Chicago, Illinois).

studied animals at 5th and 6th hours in Insln 3, Flnx and Dexa were statistically similar together and

Table 1. Effect of insulin regular (at 1.5, 3, 6, 9 and 20 IU/kg), flunixin meglumine (at 2.2 mg/kg) and dexamethasone (at 1 mg/kg) on rectal temperature (ºC, mean ± SEM) at different times following induction

Groups	Times (h)							
	0	1	2	3	4	5	6	24
Control	39.4±0.1[a]	39.9±0.2[a]	39.9±0.1[a]	40.0±0.1[a]	40.2±0.1[a]	40.1±0.0[a]	40.2±0.1[a]	39.6±0.2[a]
Insln 1.5	39.6±0.2[a]	39.9±0.3[a]	40.0±0.2[a]	40.0±0.1[a]	40.0±0.2[a]	40.1±0.1[a]	40.0±0.0[a]	39.5±0.1[a]
Insln 3	39.5±0.1[a]	39.9±0.1[a]	39.8±0.4[a]	39.9±0.2[b]	39.9±0.1[b]	39.8±0.1[b]	39.6±0.0[b]	39.4±0.2[a]
Insln 6	39.5±0.2[a]	39.9±0.3[a]	39.9±0.2[a]	40.0±0.1[a]	40.1±0.0[a]	40.0±0.0[a]	39.8±0.1[b]	39.5±0.1[a]
Insln 9	39.5±0.2[a]	39.8±0.2[a]	39.9±0.1[a]	40.0±0.1[a]	39.9±0.0[b]	40.0±0.1[a]	40.0±0.0[a]	39.6±0.2[a]
Insln 20	39.5±0.1[a]	39.9±0.4[a]	40.0±0.1[a]	40.0±0.1[a]	40.1±0.1[a]	40.0±0.0[a]	39.9±0.0[b]	39.5±0.2[a]
Flnx	39.4±0.1[a]	39.9±0.2[a]	40.0±0.1[a]	40.0±0.1[a]	39.9±0.1[b]	39.9±0.1[b]	39.9±0.1[b]	39.7±0.2[a]
Dexa	39.5±0.1[a]	39.8±0.1[a]	39.9±0.1[a]	40.0±0.1[a]	39.9±0.1[b]	39.9±0.1[b]	39.9±0.1[b]	39.6±0.1[a]

Different letters indicated significant differences between experimental groups at similar hours (P<0.05).

The level of significance was set at $P < 0.05$.

Results

The results of clinical parameter monitoring are presented in Tables 1 to 5. In all experimental groups there were no significant differences between rectal temperatures, capillary refill time, appetite and mucous membrane color at all examined hours. In Insln 3 group, at 5th and 6th hours, the heart rate was significantly lower than other experimental groups. Respiratory rate of all

lower than other groups, significantly. Fecal consistency was normal in all animals at all times. All sheep were considered permanent survivors, alive and healthy after the experiments.

Table 2. Effect of insulin regular (at 1.5, 3, 6, 9 and 20 IU/kg), flunixin meglumine (at 2.2 mg/kg) and dexamethasone (at 1 mg/kg) on heart rate (beats/min, mean ± SEM) at different times following induction and treatment of endotoxemia in Iranian fat-tailed sheep.

Groups	Times (h)							
	0	1	2	3	4	5	6	24
Control	87.6±6.2[a]	110.2±10.5[a]	119.5±12.4[a]	115.4±11.9[a]	123.7±10.5[a]	120.6±8.6[a]	118.4±11.7[a]	90.5±8.7[a]
Insln 1.5	80.5±4.8[a]	105.8±8.6[a]	108.9±10.5[a]	115.9±11.8[a]	118.8±8.9[a]	115.8±10.2[a]	120.5±7.4[a]	83.4±7.5[a]
Insln 3	83.3±3.2[a]	115.0±12.6[a]	112.9±8.9[a]	120.3±12.5[a]	110.6±8.9[b]	101.5±9.5[b]	90.8±8.3[b]	85.9±6.2[a]
Insln 6	88.2±5.2[a]	109.5±10.8[a]	115.5±9.6[a]	115.4±8.6[a]	110.7±7.5[b]	112.5±10.2[a]	105.8±9.6[a]	83.7±8.8[a]
Insln 9	89.1±7.1[a]	111.2±8.7[a]	120.8±7.9[a]	122.5±7.7[a]	118.8±5.6[a]	112.6±11.2[a]	100.7±8.6[c]	85.6±5.9[a]
Insln 20	85.4±9.5[a]	110.6±7.9[a]	115.6±8.9[a]	118.6±8.7[a]	115.7±8.6[a]	118.6±8.6[a]	112.3±8.6[a]	80.3±5.6[a]
Flnx	89.1±5.1[a]	109.1±6.7[a]	119.5±4.1[a]	121.7±3.2[a]	111.1±6.8[b]	106.5±4.2[c]	100.1±6.4[c]	82.4±4.7[a]
Dexa	88.2±4.2[a]	110.7±5.4[a]	121.7±5.1[a]	122.5±4.2[a]	110.5±5.7[b]	107.1±3.4[c]	99.7±4.9[c]	84.3±6.3[a]

Different letters indicated significant differences between experimental groups at similar hours (P<0.05).

Table 3. Effect of insulin regular (at 1.5, 3, 6, 9 and 20 IU/kg), flunixin meglumine (at 2.2 mg/kg) and dexamethasone (at 1 mg/kg) respiratory rate (times/min, mean ± SEM) at different times following induction and treatment of endotoxemia in Iranian fat-tailed sheep.

Groups	Times (h)							
	0	1	2	3	4	5	6	24
Control	25.2±2.6[a]	35.5±2.8[a]	34.7±3.4[a]	56.7±4.3[a]	58.7±3.4[a]	61.8±2.4[a]	57.3±2.8[a]	28.7±4.7[a]
Insln 1.5	24.6±3.4[a]	38.8±3.7[a]	35.7±4.2[a]	45.5±5.3[a]	52.2±2.1[a]	57.5±4.1[a]	58.6±4.4[a]	25.9±1.2[a]
Insln 3	22.8±2.5[a]	33.7±4.3[a]	32.1±2.8[a]	52.6±3.3[a]	40.2±4.1[b]	32.2±3.1[b]	33.8±2.1[b]	24.7±2.4[a]
Insln 6	23.5±2.4[a]	36.7±4.2[a]	36.4±4.1[a]	48.8±6.4[a]	42.6±3.4[b]	44.7±4.3[c]	46.7±1.2[c]	28.4±2.4[a]
Insln 9	25.4±2.8[a]	35.5±2.2[a]	38.8±2.4[a]	51.8±1.3[a]	48.6±2.5[a]	47.7±5.3[c]	44.7±2.3[c]	22.6±1.8[a]
Insln 20	28.6±1.8[a]	37.6±3.3[a]	33.7±2.7[a]	47.5±3.9[a]	42.2±2.8[b]	43.9±4.7[c]	46.2±2.4[c]	24.5±2.7[a]
Flnx	27.2±1.7[a]	36.5±2.7[a]	35.4±3.1[a]	46.1±3.4[a]	40.6±3.1[b]	34.5±2.1[b]	35.1±1.1[b]	26.1±1.7[a]
Dexa	26.1±2.0[a]	34.1±3.4[a]	36.2±2.1[a]	49.1±2.9[a]	41.7±1.0[b]	33.7±3.4[b]	36.5±1.7[b]	25.6±2.4[a]

Different letters indicated significant differences between experimental groups at similar hours (P<0.05).

Discussion

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 acute phase response 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 acute phase proteins 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, requirement to maintain normal serum albumin levels was significantly decreased in the insulin group when compared with the control group (Jeschke *et al.*, 2004).

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 leads 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

Table 4. Effect of insulin regular (at 1.5, 3, 6, 9 and 20 IU/kg), flunixin meglumine (at 2.2 mg/kg) and dexamethasone (at 1 mg/kg) on capillary refill time (sec, mean ± SEM) at different times following induction and treatment of endotoxemia in Iranian fat-tailed sheep.

Groups	Times (h)							
	0	1	2	3	4	5	6	24
Control	2.3±0.2^{a}	3.0±0.1^{a}	3.2±0.2^{a}	2.7±0.1^{a}	2.5±0.1^{a}	2.2±0.1^{a}	2.2±0.1^{a}	2.1±0.1^{a}
Insln 1.5	2.5±0.1^{a}	2.7±0.1^{a}	3.5±0.3^{a}	2.6±0.2^{a}	2.2±0.2^{a}	2.1±0.0^{a}	2.1±0.1^{a}	2.0±0.1^{a}
Insln 3	2.0±0.0^{a}	2.7±0.1^{a}	2.9±0.2^{a}	2.7±0.1^{a}	2.4±0.1^{a}	2.0±0.1^{a}	2.0±0.0^{a}	2.0±0.0^{a}
Insln 6	2.2±0.1^{a}	2.8±0.2^{a}	3.1±0.2^{a}	2.5±0.1^{a}	2.1±0.0^{a}	2.1±0.1^{a}	2.3±0.0^{a}	2.1±0.2^{a}
Insln 9	2.3±0.2^{a}	2.9±0.2^{a}	3.2±0.3^{a}	2.5±0.2^{a}	2.4±0.1^{a}	2.3±0.1^{a}	2.1±0.1^{a}	2.1±0.0^{a}
Insln 20	2.0±0.0^{a}	3.0±0.0^{a}	3.4±0.1^{a}	2.7±0.1^{a}	2.1±0.0^{a}	2.0±0.0^{a}	2.0±0.0^{a}	2.0±0.0^{a}
Flnx	2.2±0.1^{a}	2.9±0.1^{a}	3.2±0.2^{a}	2.6±0.2^{a}	2.3±0.1^{a}	2.1±0.1^{a}	2.0±0.1^{a}	2.1±0.1^{a}
Dexa	2.3±0.1^{a}	3.0±0.2^{a}	3.3±0.2^{a}	2.7±0.1^{a}	2.4±0.1^{a}	2.0±0.1^{a}	2.1±0.1^{a}	2.0±0.1^{a}

Different letters indicated significant differences between experimental groups at similar hours (P<0.05).

Jeschke *et al.* (2004) showed that insulin alters the

response through the signal transcription factors.

Table 5. Effect of insulin regular (at 1.5, 3, 6, 9 and 20 IU/kg), flunixin meglumine (at 2.2 mg/kg) and dexamethasone (at 1 mg/kg) on appetite and mucous membrane color at different times following induction and treatment of endotoxemia in Iranian fat-tailed sheep.

Groups	Times (h)							
	0	1	2	3	4	5	6	24
Control	1 (1-1)^{a}	1 (1-2)^{a}	2 (1-2)^{a}	2 (1-2)^{a}	2 (1-2)^{a}	1 (1-2)^{a}	1 (1-2)^{a}	1 (1-1)^{a}
Insln 1.5	1 (1-1)^{a}	2 (1-2)^{a}	2 (1-2)^{a}	2 (1-2)^{a}	2 (1-2)^{a}	1 (1-2)^{a}	1 (1-2)^{a}	1 (1-1)^{a}
Insln 3	1 (1-1)^{a}	1 (1-2)^{a}	2 (1-2)^{a}	2 (1-2)^{a}	1 (1-2)^{a}	1 (1-2)^{a}	1 (1-2)^{a}	1 (1-1)^{a}
Insln 6	1 (1-1)^{a}	2 (1-2)^{a}	2 (1-2)^{a}	2 (1-2)^{a}	2 (1-2)^{a}	1 (1-2)^{a}	1 (1-2)^{a}	1 (1-1)^{a}
Insln 9	1 (1-1)^{a}	1 (1-2)^{a}	2 (1-2)^{a}	2 (1-2)^{a}	2 (1-2)^{a}	1 (1-2)^{a}	1 (1-2)^{a}	1 (1-1)^{a}
Insln 20	1 (1-1)^{a}	2 (1-2)^{a}	2 (1-2)^{a}	2 (1-2)^{a}	2 (1-2)^{a}	1 (1-2)^{a}	1 (1-2)^{a}	1 (1-1)^{a}
Flnx	1 (1-1)^{a}	1 (1-2)^{a}	2 (1-2)^{a}	2 (1-2)^{a}	1 (1-2)^{a}	1 (1-2)^{a}	1 (1-2)^{a}	1 (1-1)^{a}
Dexa	1 (1-1)^{a}	1 (1-2)^{a}	2 (1-2)^{a}	2 (1-2)^{a}	1 (1-2)^{a}	1 (1-2)^{a}	1 (1-2)^{a}	1 (1-1)^{a}

Data expressed as median (min-max). The grade of normal appetite and mucous membrane color is 1, and weak appetite and congested mucous membrane graded as 2. Different letters indicated significant differences between experimental groups at similar hours (P<0.05).

Table 6. Effect of insulin regular (at 1.5, 3, 6, 9 and 20 IU/kg), flunixin meglumine (at 2.2 mg/kg) and dexamethasone (at 1 mg/kg) on circulating glucose (mg/dL, mean ± SEM) at different times following induction and treatment of endotoxemia in Iranian fat-tailed sheep.

Groups	Times (h)							
	0	1	2	3	4	5	6	24
Control	90.2±11.5[a]	108.2±12.8[a]	126.1±15.9[a]	248.2±28.6[a]	532.0±32.5[a]	446.0±30.9[a]	324.0±31.2[a]	98.1±12.7[a]
Insln 1.5	98.1±10.1[a]	110.5±15.8[a]	127.8±12.0[a]	286.2±21.5[a]	322.0±30.1[b]	345.0±31.4[b]	278.5±24.0[b]	92.1±10.6[a]
Insln 3	86.5±9.5[a]	118.0±10.3[a]	129.3±11.1[a]	245.3±19.4[a]	239.0±22.5[c]	230.0±28.0[c]	200.0±19.5[c]	100.0±9.7[a]
Insln 6	87.0±8.4[a]	105.1±12.0[a]	132.2±10.0[a]	260.0±12.8[a]	200.0±34.4[d]	170.0±18.9[d]	150.0±12.1[d]	99.5±10.1[a]
Insln 9	83.1±7.3[a]	110.4±9.7[a]	128.3±9.7[a]	249.1±10.2[a]	170.0±25.1[e]	145.0±12.4[e]	140.0±13.8[d]	101.2±9.2[a]
Insln 20	85.0±8.2[a]	112.3±8.4[a]	133.0±8.3[a]	251.9±22.7[a]	120.0±14.2[f]	100.0±9.2[f]	90.0±10.6[e]	95.4±8.7[a]
Flnx	90.1±10.5[a]	110.2±9.0[a]	128.9±10.2[a]	249.8±27.2[a]	503.4±37.1[a]	411.8±37.1[a]	312.4±30.4[a]	94.3±9.1[a]
Dexa	89.2±9.8[a]	115.8±10.5[a]	133.7±11.8[a]	252.9±33.1[a]	633.7±46.5[g]	620.3±41.2[g]	576.8±38.9[f]	99.8±8.3[a]

Different letters indicated significant differences between experimental groups at similar hours (P<0.05).

Thus insulin may act as an anti-inflammatory molecule by two different pathways: by decreasing pro-inflammatory mediators and by increasing anti-inflammatory mediators (Jeschke *et al.,* 2004, Chalmeh *et al.,* 2013c). Intensive insulin therapy exerts an anti-inflammatory effect on patients by reducing levels of acute phase proteins, inflammatory cytokines, improving the systemic inflammatory response, and suppressing the hepatic acute phase proteins (Jeschke *et al.*, 2004).

Nonsteroidal anti-inflammatory drugs (NSAIDs) have been used in general for the treatment of endotoxemia, because of their analgesic, anti-inflammatory and antipyretic properties. NSAIDs overcome the production of cytokines and reduce the acute hemodynamic response to endotoxemia. Flunixin meglumine is the most commonly used type of NSAID in the treatment of endotoxemia in horses and cattle, and has remained the best choice for treatment of this condition. Flunixin meglumine also adjusts acute hemodynamic changes commonly seen during endotoxemia, which may increase survival rates (Radostits *et al.,* 2007). The results of the present experiment showed that intravenous administration of flunixin meglumine at 2.2 mg/kg improved the clinical signs of endotoxemia at similar manner to the insulin at 1.5 IU/kg and dexamethasone at 1 mg/kg.

Corticosteroids are used extensively for the treatment of endotoxemia and endotoxic shock.

These drugs are less often administered to endotoxemic animals, as a result of a number of studies supporting the use of NSAIDs. Corticosteroids promote capillary endothelial integrity and tissue perfusion, decrease the activation of the complement and clotting cascade, decrease neutrophil aggregation, stabilize lysosomal membranes, protect against hepatic injury and improve survival rate. However, there are concerns about their use in septicemic animals, because they may cause immunosuppression (Radostits *et al.,* 2007). Dexamethasone is the most commonly used corticosteroid in endotoxemia. However, a review

of literature revealed that corticosteroids are the most helpful therapeutically when given as a pretreatment in experimental endotoxemia situations (Reynolds *et al.*, 2002). It is currently believed that corticosteroids, if they are to be clinically effective, must be given as early as possible to endotoxemic animals (Radostits *et al.*, 2007). Our findings revealed that intravenous administration of dexamethasone at 1 mg/kg, after endotoxemia induction by *E. coli* serotype O55:B5 and the appearance of acute phase response, was effective to improve clinical signs similar to flunixin meglumine at 2.2 mg/kg and insulin regular at 3 IU/kg.

It is a very old observation that the body temperature rises after administration of endotoxin (Centanni, 1894). This elevated body temperature is a true pyrogenic response, in contrast with hyperthermia. In common laboratory animals, small doses of endotoxin induce a monophasic fever response, while moderate to large doses may induce a biphasic fever response. The initial fever response, which is due to a direct effect on the thermoregulatory center in the hypothalamus, is believed to have a latency time of approximately one hour. If a second peak occurs, it will appear approximately four hours after the endotoxin administration (Lohuis *et al.* 1988). The orchestration of the cytokines induces fever during endotoxemia. In the current research, rectal temperature of endotoxic sheep decreased significantly in treatment groups, but there were no significant differences among Insln 3, Dexa and Flnx groups (Table 1).

Heart rate variations or beat-to-beat oscillations of the heart rate represent physiological phenomenon determined predominantly by the autonomic nervous system control (Zila *et al.*, 2015). Therefore, the measurement of the heart rate is a noninvasive technique that can be used to investigate the dynamic balance between sympathetic and vagal activity (Tonhajzerova *et al.*, 2012). It was shown that the analysis of heart rate is a sensitive method to study autonomic nervous system activity in animals and to detect discrete changes in sympathetic-vagal balance, particularly in vagal function (Mokra *et al.*, 2013). The autonomic nervous system and the inflammatory response are intimately linked (Kox *et al.*, 2011). The acute response to endotoxemia includes activation of innate immune mechanisms as well as changes in autonomic nervous activity (Tracey, 2002). Sympathetic and vagal nerves are thought to have anti-inflammation functions (Straub *et al.*, 2008). Heart rate assessment has been suggested to provide insights into the acute effect of sympathetic and cholinergic anti-inflammatory pathways. In the present study, heart rate increased following the induction of endotoxemia in all studied groups. But, this parameter in Insln 3 group was significantly lower that other groups at 4th to 6th hours. It may be suggested that, the decreasing pattern of heart rate in this group, may be related to combat acute phase response to endotoxemia and balance of autonomic nervous system, subsequently (Table 2).

Endotoxin has dramatic effects on the structure and function of the lungs in intact animals and also on isolated lung cells, and both the *in vivo*

and *in vitro* effects of endotoxin are complex. In all animals, endotoxin causes obvious and subtle effects on functions of both airways and the pulmonary circulation. These effects include diffuse lung inflammation and injury of pulmonary vascular endothelium. Endotoxin can also directly injure endothelial cells *in vitro* (Brigham and Meyrick, 1986). In this research, respiratory rate increased during endotoxemia and decreased following the therapeutic regimens. The lowest respiratory rate was recorded in Insln 3 group in comparison with other ones (Table 3).

Conclusion

In the present experiment, it has been shown that insulin acts as an anti-inflammatory mediator by improving the clinical signs of endotoxemia induced by *E. coli* serotype O55:B5 in the Iranian fat-tailed sheep. According to our findings, insulin induces its effects by dose dependent manner. Furthermore, the anti endotoxic effects of insulin regular at 3 IU/kg were statistically similar to dexamethasone (1 mg/kg) and flunixin meglumine (2.2 mg/kg). The results of the present experiment may suggest a new potential therapeutic regimen on endotoxemia in small ruminant medicine.

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