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Short communication

# Attenuating the endotoxin induced acute phase response by pentoxifylline in comparison with dexamethasone and ketoprofen in sheep

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ABSTRACT

Treating the endotoxemia in animals is necessary to prevent high mortality rates. Despite the suggestion of different endotoxemia therapeutic regimens, the lack of an effective treatment still remains a clinical problem in sheep. Hence, the present study was performed to clarify the antiendotoxic effects of pentoxifylline in comparison with dexamethasone and ketoprofen in experimentally induced endotoxemia in sheep. Thirty clinically healthy 1-year old Iranian fat-tailed ewes were randomly divided into 6 equal experimental ($n = 5$) groups, comprising negative and positive control, ketoprofen (3 mg/kg), dexamethasone (1 mg/kg) and pentoxifylline (30 and 60 mg/kg). Lipopolysaccharide from *Escherichia coli* serotype O55:B5 was infused at 2 μg/kg intravenously. Ketoprofen, dexamethasone and pentoxifylline were injected to related groups, at 90 min after endotoxemia induction over 60 min along with intravenous fluids. Blood samples were collected from all ewes prior and 1.5, 3, 4.5, 6, 24 and 48 h after lipopolysaccharide injection and sera and plasmas were separated, subsequently. Haptoglobin, serum amyloid A, tumor necrosis factor-alpha, interferon-gamma, superoxide dismutase and glutathione peroxidase were measured in all samples. Serum levels of haptoglobin, serum amyloid A, tumor necrosis factor-alpha, interferon-gamma in both pentoxifylline groups were significantly lower and higher than dexamethasone and ketoprofen ones, respectively. In conclusion, the efficacy of pentoxifylline was significantly higher and lower than dexamethasone and ketoprofen, respectively. Furthermore, its anti- and pro-inflammatory effects at 30 and 60 mg/kg were statistically similar together.



## 1. Introduction

Recent researches revealed the pathophysiological effects of endotoxemia and maintained the overwhelming production of inflammatory mediators takes place during the molecular cascade of the systemic inflammatory response syndrome (Chalmeh et al., 2013a). But, despite the suggestion of different endotoxemia therapeutic regimens (Chalmeh et al., 2013b,c,d), 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).

Pentoxifylline, a methylxanthine derivative and a non-specific phosphodiesterase inhibitor is a widely prescribed drug for the management of vascular disorders characterized by defective regional microcirculation (Tjon and Riemann, 2001). This drug also possesses other important pharmacological actions such as anti-inflammatory activities in toxin-induced inflammatory problems (Abdel-Salam et al., 2003) and it is stated that pentoxifylline may be beneficial in endotoxemia (Krysztopik et al., 2000).

Antiendotoxic and anti-inflammatory effects of NSAIDs and corticosteroids have been evaluated in sheep (Chalmeh et al., 2013b,c), but there are no investigations on potential therapeutic characteristics of different doses of pentoxifylline for treatment of endotoxemia in ovine models. Hence, the present experiment was conducted to evaluate and compare anti-inflammatory effects of pentoxifylline at two different doses, ketoprofen and dexamethasone on acute inflammatory status due to experimental endotoxemia in sheep. The results of the present study may reveal

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the potential effects of pentoxifylline on systemic inflammatory responses in small ruminant medicine in comparison with ketoprofen and dexamethasone.

## 2. Materials and methods

### 2.1. Animals

The present experiment was performed after being approved by the Ethics Committee of School of Veterinary Medicine, Shiraz University. Thirty clinically healthy 1-year old Iranian fat-tailed ewes ($35 \pm 1.5$ kg, body weight) were randomly selected for the project in April 2014 from Laboratory Teaching Barn of Agricultural College of Shiraz University. Four weeks before commencing experiments, each sheep received antiparasitic drugs similar to Chalmeh et al. (2013d).

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 6 experimental equal ($n = 5$) groups, comprising negative and positive controls, ketoprofen, dexamethasone, pentoxifylline 1 and pentoxifylline 2.

### 2.2. Chemicals and drugs

Phenol extracted LPS from *Escherichia coli* (*E. coli*) serotype O55:B5 (Sigma–Aldrich®; product no. L2880) was used to induce endotoxemia in ewes at 2 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) and divided into 30 equal doses each containing 70 μg endotoxin and stored at −80 °C until endotoxemia induction.

For each experiment, each dose was thawed and infused intravenously as described below. Ketoprofen (Keptofen® 10%, Razak Pharmaceutical Co., Tehran, Iran, at 3 mg/kg), dexamethasone (Vetacoid® 0.2%, Aburaihan Pharmaceutical Co., Tehran, Iran, at 1 mg/kg) and pentoxifylline (Sigma–Aldrich Chemical Co., St. Louis, MO, USA, at 30 and 60 mg/kg) were intravenously injected to ketoprofen, dexamethasone and pentoxifylline experimental groups according to experimental design, respectively. The intravenous fluid used in the present experiment was dextrose 5% plus sodium chloride 0.45% (Shahid Ghazi Pharmaceutical Co., Tabriz, Iran).

### 2.3. Experimental procedures

#### 2.3.1. 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 thirty ewes were evaluated clinically before and 1.5, 3, 4.5, 6, 24 and 48 h after LPS injection. Clinical parameters monitored during experiments included rectal temperature, heart and respiratory rates, cardiac tonicity, mucous membrane color and capillary refill time. 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 90 min after LPS injection by dextrose 5% plus sodium chloride 0.45% at the rate of 20 mL/kg/h. Drugs (ketoprofen, dexamethasone and pentoxifylline) were used at along with fluid therapy at 90 min after LPS injection over 60 min. Pentoxifylline for each animal was diluted in used fluids over 60 min before administration. Positive control group received LPS and was treated only by intravenous fluid without any drugs and negative control group only received intravenous fluids.

#### 2.3.2. Blood sampling and biochemical assays

Blood samples were collected from all ewes through the fixed catheter prior and 1.5, 3, 4.5, 6, 24 and 48 h after LPS injection in plain and EDTA coated tubes. Immediately after collections, sera and plasmas were separated by centrifugation (for 10 min at $3000 \times g$) and stored at −22 °C until assayed. Haptoglobin (Hp), serum amyloid A (SAA), tumor necrosis factor-alpha (TNF-α), interferon-gamma (IFN-γ), superoxide dismutase (SOD) and glutathione peroxidase (GPx) were measured similar to Chalmeh et al. (2013a,b,c,d).

### 2.4. Statistical analyses

Data were expressed as mean ± standard deviation (SD). Statistical analysis was performed using one-way ANOVA with LSD post hoc test to compare mean concentrations of different serological factors and quantitative clinical parameters within similar hours between different experimental groups. Repeated measures ANOVA was used to detect significant changing patterns of serological factors during all experiments. The data of qualitative clinical parameters were presented as median (min–max) and Kruskal–Wallis test was used for comparison of these parameters between all 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 $P < 0.05$.

## 3. Results

Serum concentrations of SAA and Hp in different times of experimental groups are presented in Tables 1 and 2. SAA and Hp elevated rapidly after endotoxemia induction in all experimental groups except that negative control group. There was no significant changing pattern in negative control group. These rapid elevations were different between the hour zero and 1st time after endotoxin infusion ($P < 0.05$). Serum concentrations of SAA and Hp in ketoprofen group were lower than other experimental groups after hour 3 (Tables 1 and 2). Serum levels of Hp and SAA in both pentoxifylline groups were significantly lower than dexamethasone one.

Serum concentrations of TNF-α and IFN-γ in different hours in each experimental group are presented in Tables 3 and 4. Rapid elevation of serum TNF-α and IFN-γ was detected after endotoxemia induction in all experimental groups except than negative control one (Tables 3 and 4). There were significant differences between TNF-α at 1st time after endotoxin infusion and its base line levels at hour zero ($P < 0.05$). The same pattern was also observed for IFN-γ. The lowest and highest concentrations of both TNF-α and IFN-γ were detected in ketoprofen and dexamethasone groups, respectively. There were no significant differences between pentoxifylline 30 and 60 mg/kg groups.

Serum concentrations of SOD and GPx at 1st time after endotoxemia induction were significantly lower than base line levels at hour zero, in all experimental groups ($P < 0.05$, Tables 5 and 6), except than negative control one. SOD and GPx concentrations in ketoprofen group were significantly higher than other experimental groups after drugs administrations.

Rectal temperature in all received LPS groups was increased significantly after endotoxin infusion. The rectal temperature of ketoprofen group at hours 4.5 and 6 was significantly lower than other endotoxin received groups ($P < 0.05$). Heart rate of all experimental groups was increased significantly after endotoxin infusion. The rate of heart beat in ketoprofen group at hour 4.5 was lower than other LPS received group, significantly ($P < 0.05$). The increasing pattern of respiratory rate was detected after intravenous LPS infusion. The respiratory rate of ketoprofen group was significantly