



THERAPEUTIC REGIMENS OF ENDOTOXAEMIA IN SHEEP

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Summary

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Endotoxaemia is an inflammatory condition which happens due to the presence of outer cell wall layer of Gram-negative bacteria in blood circulation, containing lipopolysaccharide commonly known as endotoxin. This condition causes high mortality in affected animals and sheep are highly susceptible in this regard. Several researchers have emphasised the therapeutic regimens of endotoxaemia and its sequels in sheep. Furthermore, sheep are among the most commonly used animal species in experimental studies on endotoxaemia, and for the past five decades, ovine models have been employed to evaluate different aspects of endotoxaemia. Currently, there are several studies on experimentally induced endotoxaemia in sheep, and information regarding novel therapeutic protocols in this species contributes to better understanding and treating the condition. This review aims to specifically introduce various treatment methods of endotoxaemia in sheep.

Key words: endotoxaemia, Gram-negative bacteria, inflammation, treatment, sheep

INTRODUCTION

Outer cell wall layer of Gram-negative bacteria contains lipopolysaccharide (LPS) commonly known as endotoxin, which is a potent inflammatory substance stimulating many specific and non-specific inflammatory reactions in mammals. As an inflammatory condition reflecting the presence of LPS in the bloodstream, endotoxaemia has been considered as a model to evaluate the inflammatory responses in animals and human beings.

For the past few decades, ovine models have been used to study the therapeutic aspects of endotoxaemia, with the results used and generalised in other large

animals. So far, many models of ovine endotoxaemia have been defined and developed, and various aspects of this condition have been evaluated. These models are in two general categories; first, live organisms or their LPSs are administered exogenously (Chalmeh *et al.*, 2014b;c), and second, the endogenous microbial flora is released to the bloodstream by, for instance, caecal ligation and puncture (also a model of peritonitis) (Fink, 2014). However, endotoxaemia induction is commonly performed by the injection of different doses of live Gram-negative bacteria or their cell wall component.

Various animal models and human volunteers have been used to study endotoxaemia. In older studies, small laboratory animals such as mice and rabbits have been utilised for the immunity aspects of endotoxaemia, and larger animals such as sheep have been subject to haemodynamic and cardiovascular investigations. However, in recent researches, ovine endotoxaemia is introduced as a reliable model to evaluate the effects of endotoxaemia on different therapeutic aspects. During the early 1960s, Halmagyi *et al.* (1963) evaluated the effects of endotoxin on sheep to determine their usefulness as a model for evaluating sepsis and its treatment. Various endotoxaemia treatments have been further proposed in ovine models. Given the importance of endotoxaemia treatment, several therapeutic regimes of ovine endotoxaemia models have been discussed. The main purpose was to provide a comprehensive review of the recent therapeutic regimens of endotoxaemia according to the studies on sheep for generalisation to other large animals.

PATHOPHYSIOLOGY OF ENDOTOXAEMIA

Once the LPS is released to the blood, it is removed by the mononuclear phagocyte system, and the response of these phagocytes to the LPS determines severity of the clinical illness (Chalmeh *et al.*, 2013a,c,d). The effects of endotoxins on host cells are not direct but they enhance the cells, e. g. endothelial and smooth muscle cells, polymorphonuclear granulocytes, platelets, thrombocytes, and cells of monocyte/macrophage lineage to produce soluble and cell bound mediators (Hattori *et al.*, 2017). Subsequently, these cells release the inflammatory mediators in-

cluding cytokines, platelet activating factor, thromboxane A₂, prostaglandins, leukotrienes, proteinases, toxic oxygen metabolites and vasoactive amines to the bloodstream. The released cytokines from the pulmonary intravascular macrophages are responsible for many of the pathophysiological events of endotoxaemia and these cells are the most important producers of cytokines in sheep (Naylor *et al.*, 2020). A protein named LPS binding protein (LBP) is present in the bloodstream and circulating LPS binds to this protein. This complex is rapidly cleared by pulmonary and hepatic macrophages. It binds to a membrane-bound receptor (mCD14) on mononuclear cells by a protein (MD-2) and then attaches to toll-like receptor-4 (TLR-4) on the mononuclear cell membrane. The resulted complex (LPS–LBP–mCD14–MD-2 complex) is then internalised and LPS is destroyed during the process. This process activates the intracellular signaling pathway via nuclear factor κB (NF-κB), which translocates to the nucleus and causes transcription of many cytokine genes and release of proinflammatory cytokines, such as TNF-α, IL-1 and IL-6. Some of the activated genes are related to produce cyclooxygenase 2, nitric oxide, endothelial adhesion molecules and chemokines. Some of the CD14s termed soluble CD14 receptors (sCD14) are shed into the bloodstream and play an important role in the pathogenesis of endotoxaemia. Increased serum concentrations of sCD14 are responsible for the severity of some endotoxaemia clinical signs (Constable *et al.*, 2017).

The high circulating levels of the eicosanoids during endotoxaemia such as arachidonic acid metabolites, thromboxane A₂ and prostacyclin are responsible for the haemodynamic abnormalities (Hattori *et al.*, 2017). Endotoxin activates phospholi-

pase A₂, a cell-membrane enzyme, which leads to the hydrolysis of membrane-bound phospholipids. Subsequently, arachidonic acid is released from this damaged cell membrane and cyclooxygenase converts arachidonic acid into intermediate endoperoxides, which are substrates for the formation of prostaglandins, thromboxane, and prostacyclin. The products of cyclooxygenase activities induce multiple organ dysfunctions, shock and disseminated coagulopathy which lead to death (Constable *et al.*, 2017).

THERAPEUTIC REGIMENS

Antimicrobial therapy

Bactericidal Gram-negative antimicrobial agents are always indicated for treatment, whenever there is evidence of septicæmia or a localised infection causing endotoxaemia. The choice and route of administration depends on the pathogens suspected of causing the infection and endotoxaemia and the site of infection. The speed of killing Gram-negative bacteria may be an important clinical issue because antimicrobial agents with a rapid kill can produce a bolus release of endotoxin into the bloodstream by punching multiple holes in the bacteria. This causes a rapid explosion of the bacteria through osmotic fluid shifts and bolus release of endotoxin (Constable *et al.*, 2017).

Patel *et al.* (2012) investigated the pharmacokinetics of levofloxacin, a third-generation fluoroquinolone, at 3 mg/kg in experimentally induced endotoxaemia model of sheep. They stated that pharmacokinetic characteristics and absence of adverse reactions in levofloxacin revealed that this antibiotic may be a potentially useful drug to treat bacterial diseases in sheep with acute phase reactions. In one study, fever was induced by intravenous

administration of *E. coli* serovar O126:B8 LPS in Chotanagpuri sheep, and plasma ceftriaxone concentration was estimated following administration. The results indicated that ceftriaxone had greater distribution in the peripheral compartment during pyrexia. The suitable dosage regimen of ceftriaxone in febrile sheep by the intravenous route was calculated to be 15 mg/kg body weight at over 5 h interval (Ranjan *et al.*, 2011).

Polymyxin B has the capacity to bind with the lipid A portion of LPS and neutralise its biological activity, resulting in the restriction of the proinflammatory cytokine cascade (Morresey & Mackay, 2006). Hajimihammadi *et al.* (2018) evaluated the clinical and certain circulating inflammatory profiles of endotoxic sheep following treatment with polymyxin B. They revealed that polymyxin B did not induce its effects in a dose-dependent manner, and its anti- and pro-inflammatory effects at 6,000 and 12,000 IU/kg doses were statistically similar. Effects of intravenous polymyxin E (colistin) administration on ovine experimental endotoxaemia were evaluated by Setayesh *et al.* (2018). They used polymyxin E at 12,000 and 24,000 IU/kg following endotoxaemia induction, revealing that both doses could be used as an anti-endotoxic drug against the *E. coli* induced endotoxaemia in sheep.

Intravenous fluid therapy

Hypotension is one of the defining characteristics of septic shock, and secondary hypoperfusion is deemed to be critical in the development of multiple organ dysfunctions (Rivers *et al.*, 2008). This has been the logic behind employing fluid resuscitation as the first therapeutic intervention in septic shock in order to reverse perfusion deficits and prevent progression

to organ dysfunction (Rivers *et al.*, 2008). Experimentally, this has been supported by numerous models of endotoxaemic shock with improved outcomes when cardiac output was increased with fluid resuscitation (Parker, 2017). While animal literature demonstrates consistent benefit with fluid resuscitation, the clinical evidence remains equivocal. Observational studies of fluid resuscitation have suggested both benefit (Garland *et al.*, 2010) and harm (Sadaka *et al.*, 2014) regarding the only randomised control trial of fluid resuscitation, demonstrating increased mortality (Maitland *et al.*, 2011). This discrepancy in effect may be due to the differences in the type of shock between clinical sepsis and commonly used experimental models. When administered to humans, endotoxin produces the characteristic hyperdynamic response seen in clinical sepsis (Bonanno, 2018). However, in both small and large animal models of endotoxaemia, endotoxin frequently produces hypodynamic shock with rapid and severe reductions in cardiac output (Kingsley & Bhat, 2016). The models used to demonstrate the benefits of fluid resuscitation are all notable for being hypodynamic models of shock (Rahal *et al.*, 2009). It is conceivable that the therapies aimed at increasing cardiac output such as fluid resuscitation may have different effects in hypo- and hyper-dynamic shock. Similarly, regional hypoperfusion and impaired oxidative metabolism have been proposed as central mechanisms in the development of organ dysfunction in septic shock (Rivers *et al.* 2008). This has been experimentally supported by a number of animal models, demonstrating reduced regional blood flow across the vital organs. These observations were reported in hypodynamic models of sepsis and endotoxaemia (Kingsley & Bhat, 2016).

Byrne *et al.* (2018) developed an ovine model of endotoxaemia capable of producing hyperdynamic shock. They induced hyperdynamic shock as hypotension below a mean arterial pressure of 60 mmHg with normal or increased cardiac output, leading to impaired oxidative metabolism and development of multiple organ dysfunctions. In their study, the endotoxin infusion was successful in producing distributive shock, but cardiac index remained within the normal range. Lactate/pyruvate ratios were not significantly abnormal in the heart, brain, kidney or arterial circulation, and liver microdialysis samples demonstrated persistently high lactate/pyruvate ratios. They concluded that impaired oxidative metabolism occurred in the liver, suggesting impaired splanchnic perfusion which may be a modifiable factor in the progression of multiple organ dysfunction and death.

Rapid resolution of tissue hypoxia is a critical point in endotoxaemia treatment. Sheep that spontaneously developed high oxygen transport had better outcomes (Pittet *et al.*, 2000). Metabolic acidosis is among the important sequels of hypoxic situation, and volume expansion has often failed to reverse intramucosal acidosis (Lagoa *et al.*, 2004). Dubin *et al.* (2006) evaluated the effects of supranormal elevations of blood flow on oxygen transport and tissue oxygenation in a sheep model of endotoxaemia. Their main finding was that increased blood flow prevented the development of intramucosal acidosis. However, anion-gap metabolic acidosis was larger in hyperresuscitated animals. Dubin *et al.* (2008) revealed that persistent villi hypoperfusion following endotoxaemia caused intramucosal acidosis in sheep, unable to restore following fluid resuscitation.

Table 1. A brief description and some examples of therapeutic regimens of endotoxaemia in sheep

Therapeutic regimens	How it works against endotoxaemia?	Some examples of drugs
Antimicrobial therapy	Killing Gram-negative bacteria; Binding with the lipid A portion of LPS	Levofloxacin; ceftriaxone; Polymyxin B and E
Intravenous fluid therapy	Reversing perfusion deficits; Preventing progression to organ dysfunction; Rapid resolution of tissue hypoxia	Crystalloids
Corticosteroids	Organelle and cell-membrane stabilisation; Improving cellular metabolism and gluconeogenesis; Improving microcirculation; Decreasing production of endogenous toxins; Decreasing leukocyte activation and degranulation; Reducing reticuloendothelial depression and histologic organ damage; Improving capillary endothelial integrity and tissue perfusion; Decreasing activation of complement and clotting cascade; Decreasing neutrophil aggregation; Stabilising lysosomal membranes; Protecting against hepatic injury; Improving survival rate	Methylprednisolone; Betamethasone; Dexamethasone
NSAIDs	Analgesic, antiinflammatory and antipyretic properties; Inhibiting the cyclooxygenase; Suppressing the production of thromboxane and prostaglandins; Reducing the acute haemodynamic response to endotoxaemia	Indomethacin; meclofenamate; Ibuprofen; ketoprofen; Flunixin meglumine
Drugs affecting cardiovascular system	Maintaining adequate organ perfusion pressure; Contracting vascular smooth muscle; Increasing systemic arterial blood pressure; Evaluating vasoconstrictive activity; Improving oxygen transport and preventing the intramucosal acidosis	Norepinephrine; vasopressin; Dopexamine; terlipressin; Levosimendan; dobutamine; Adrenomedullin; nicotinamide; Glipizide
Detergents	Inhibiting the release of cytokines by LPS-stimulated macrophages; Decreasing hepatic acute phase proteins production	Triton X-100; tyloxapol
Endothelin receptor antagonists	Slowing the progression of pulmonary hypertension; Attenuating lung injuries	BMS-182874; Tezosentan

Table 1 (cont'd). A brief description and some examples of therapeutic regimens of endotoxaemia in sheep

Therapeutic regimens	How it works against endotoxemia?	Some examples of drugs
Thromboxane synthesis inhibitors	Inhibiting thromboxane A ₂ synthesis; Abolishing the rise in pulmonary artery pressure; Decreasing the rise of airway pressure	UK-38485; BM-13.177; OKY-046
Antioxidants	Minimizing oxidative stress and scavenging free radicals; Protecting the tissues against attack by oxidants; Cytoprotectant and preventing the lung injuries	Taurine; N-acetyl-cysteine; Dimethyl sulphoxide
Prostaglandins	Inhibiting platelet aggregation; vasodilation; Inhibiting the release of lysosomal enzyme products from leukocytes; Decreasing the pulmonary hypertension and the interstitial edema	Prostaglandin E; Prostaacyclin
Xanthine derivatives	Suppressing endotoxin-induced cytokines production; Stimulating respiratory center and bronchodilator	Pentoxifyline
Insulin therapy	Decreasing the proinflammatory and increasing the anti-inflammatory responses; preventing liver damage; Reducing of reactive oxygen species generation; Antiapoptotic action	Insulin regular
Immunotherapy	LPS neutralisation	Anti-ovine interleukin-1 β monoclonal antibody
Chemotherapy	Similar function to lipid A at lesser degrees	Lipid X
TLRs inhibitors	Reducing the release of cytokines and stimulation of inflammatory cells	TAK-242
Nitric oxide	Pulmonary vasodilator effect	Nitric oxide
Protein C	Inhibiting translocation of NF- κ B; Reducing migration and accumulation of pulmonary leukocytes; Anticoagulant, anti-inflammatory, fibrinolytic and antiapoptotic effects	Recombinant human activated protein C
Fructose-1,6-diphosphate	Inhibiting respiratory burst and oxyradical generation of activated neutrophils; Immunosuppressive properties	Fructose-1,6-diphosphate
Methylene blue	Attenuating the increment in lung fluid filtration and improving gas exchange; Reducing pulmonary capillary pressure and permeability-surface area product; Improving cardiovascular functions; Inhibiting the cyclo-oxygenase products of arachidonic acid, preventing fever	Methylene blue

Passmore *et al.* (2019) used intravenous 0.9% saline bolus (40 mL/kg over 60 min) to experimentally induced ovine endotoxaemia model. They finally concluded that endotoxaemia impairs secondary haemostasis and induces changes in the intrinsic, extrinsic and anti-coagulant pathways. These changes to haemostasis are exacerbated following resuscitation with 0.9% saline, a commonly used crystalloid in clinical settings.

Corticosteroids

Corticosteroids have been extensively used in the past for the treatment of endotoxaemia and shock. Some reasons for the use of corticosteroids include organelle, cell-membrane and lysosomal membrane stabilisation, improvement in cellular metabolism, gluconeogenesis, microcirculation, capillary endothelial integrity, tissue perfusion, and survival rate, protection against hepatic injury, and reduction in the following: production of endogenous toxins such as myocardial depressant factors, leukocyte activation and degranulation, reticuloendothelial depression and histologic organ damage, activation of complement and clotting cascade and neutrophil aggregation (Constable *et al.*, 2017).

Methylprednisolone has been used in some research as pretreatment of experimentally induced ovine endotoxaemia models. When this corticosteroid is administered at 30 min prior to endotoxaemia induction, the initial pulmonary hypertension is less, and the increase in the late phase of lung vascular permeability is prevented. Methylprednisolone begins to act during the initial pulmonary hypertensive response to endotoxin, further preventing the late phase increase in lung vascular permeability; however the drug has no effects once vascular permeability

is increased. Large doses of methylprednisolone given before or soon after endotoxaemia prevent the increase in lung vascular permeability caused by endotoxin, but do not reverse the abnormality once it occurs (Poli-de-Figueiredo *et al.*, 2008). Ertmer *et al.* (2007) reported that methylprednisolone reversed vasopressin hyporesponsiveness in ovine endotoxaemia.

Newnham *et al.* (2003) evaluated the effects of betamethasone on chorioamnionitis induced by intra-amniotic endotoxin in sheep. Their findings confirmed that betamethasone treatment was able to suppress the initial inflammation in the amnion-chorion; on the other hand, inflammatory cells and cytokines in amniotic fluid were not suppressed following betamethasone treatment, presumably due to the slow clearance of bioactive endotoxin from the amniotic fluid. In another study, betamethasone was used at 1 mg/kg following intravenous endotoxin infusion in sheep (Heidari *et al.*, 2016), and betamethasone reduced the circulating levels of acute phase proteins and inflammatory cytokines at this dose. Chalmeh *et al.* (2014a,b) utilised dexamethasone at 1 mg/kg 60 minutes following endotoxaemia induction in sheep. They reported that this drug, unlike the control group, combated the acute phase response following endotoxaemia.

Non-steroidal anti-inflammatory drugs

Non-steroidal anti-inflammatory drugs (NSAIDs) have been generally used for the treatment of endotoxaemia due to their analgesic, antiinflammatory and antipyretic properties. They suppress the production of thromboxane and prostaglandins and reduce the acute haemodynamic response to endotoxaemia (Constable *et al.*, 2017).

Administering indomethacin before endotoxin infusion prevents or markedly reduces pulmonary vasoconstriction in the sheep. Meclofenamate and ibuprofen attenuate the hypoxaemia and early pulmonary hypertension caused by endotoxaemia, but have no effects on the late increase in lung fluid and solute exchange. Ibuprofen reverses the early pulmonary hypertensive changes and early haemodynamic alterations associated with endotoxaemia (Byrne *et al.*, 2018). Flunixin meglumine was administered to endotoxic sheep at 2.2 mg/kg, dexamethasone was injected at 1 mg/kg (Chalmeh *et al.*, 2013a), betamethasone at 1 mg/kg (Heidari *et al.*, 2016) and insulin regular at 3 IU/kg (Chalmeh *et al.*, 2013b); the ability of flunixin meglumine to reduce acute phase response was similar to both dexamethasone and betamethasone, but lower than that of insulin regular. Flunixin meglumine is a potent inhibitor of cyclooxygenase, and its ability to inhibit the synthesis of eicosanoids such as prostaglandin E₂ may explain the anti-inflammatory action of the drug. Flunixin meglumine also modulates the acute haemodynamic changes and hyper L-lactataemia commonly observed during endotoxaemia, which may increase the survival rate. Endotoxin-stimulated production of thromboxane B₂ and prostaglandin F_{1 α} are blocked by flunixin meglumine (Constable *et al.*, 2017). Rahmani Shahraki *et al.* (2016) showed that ketoprofen, another commonly used NSAIDs in sheep, attenuated the acute phase response at 3 mg/kg following ovine experimental endotoxaemia induction.

Drugs affecting the cardiovascular system

In endotoxic sheep, cardiocirculatory support with exogenous catecholamines is often required to maintain adequate organ

perfusion pressure. Norepinephrine is frequently employed in this setting owing to its strong α -adrenergic properties resulting in vascular smooth muscle contraction and, ultimately, an increase in systemic arterial blood pressure (Rivers *et al.*, 2001). Although high norepinephrine doses are often required to re-establish a sufficient perfusion pressure of vital organs, they may contribute to clinically relevant side effects, such as reduction in microvascular blood flow and development of multiple organ dysfunctions (Krejci *et al.*, 2006). Lange *et al.* (2007a) evaluated the haemodynamic effects of titrated norepinephrine in healthy versus endotoxic sheep. Their findings confirmed that endotoxaemia led to an early hyporesponsiveness against norepinephrine caused by a drug-independent mechanism rather than a tachyphylaxis due to long-term administration of norepinephrine. Also, the effects of titrated arginine vasopressin alone or in combination with norepinephrine on haemodynamics and oxygen transport in healthy and endotoxaemic sheep were evaluated by other researchers (Westphal *et al.*, 2003). They reported that during ovine endotoxaemia, arginine vasopressin reduced cardiac index, compromised oxygen delivery and increased pulmonary vascular resistance index, side effects possibly limiting its use as a sole vasopressor during sepsis. Potentially, a simultaneous infusion of arginine vasopressin and norepinephrine could represent a useful therapeutic option. Westphal *et al.* (2004) observed that dopexamine, a synthetic analogue of dopamine, reversed the vasopressin-associated impairment in tissue oxygen supply, yet reduced systemic blood pressure in ovine endotoxaemia.

Vasopressin is an endogenous peptide hormone synthesised in the hypothalamus.

Although it induces vasodilation via V₂-receptors by increasing adenosine 3',5'-cyclic monophosphate in smooth muscles, it exerts a much stronger effects on V₁-receptors. V₁-receptors are coupled with phospholipase C, increasing intracellular Ca²⁺ concentration. Thus, the activation of V₁-receptors leads to the contraction of vascular smooth muscles, hence vasoconstriction (Silverstein & Beer, 2015). Silverstein & Beer (2011) evaluated the vasoconstrictive activity of terlipressin, the synthetic analog of vasopressin, at 15 µg/kg on ovine endotoxaemia. They found that during endotoxaemia, terlipressin increased pulmonary vascular resistance index. This was accompanied by a significant decrease in cardiac index, whereas mean pulmonary arterial pressure did not change after the application of terlipressin. In another study, researchers found out that during ovine endotoxaemia, titrated terlipressin reversed hypotension but impaired the pulmonary circulation (Westphal *et al.*, 2002). Lange *et al.* (2001) compared the continuous versus bolus infusion of terlipressin in ovine endotoxaemia model. They concluded that the continuous infusion of terlipressin stabilised haemodynamics and improved myocardial performance in endotoxaemic ewes without obvious side effects. Continuous low-dose terlipressin infusion may represent a useful alternative treatment for arterial hypotension related to sepsis and systemic inflammatory response syndrome.

Levosimendan, a new calcium-sensitising inotropic drug, improves cardiac function and survival in patients with congestive heart failure and acts as a vasodilator by stimulating ATP-sensitive potassium channels in vascular smooth muscle cells (Dubin *et al.*, 2007). Dubin *et al.* (2006) reported that high doses of

levosimendan improved oxygen transport and prevented the development of intramucosal acidosis in a normodynamic sheep model of endotoxaemia. In another study, Dubin *et al.* (2007) compared the oxygen transport and haemodynamic responses of an ovine experimental model of septic shock infused with levosimendan or dobutamine. They revealed that levosimendan, in comparison with dobutamine, increased intestinal blood flow and diminished intramucosal acidosis. Some studies have revealed that dobutamine has favourable effects on ovine endotoxaemia, but the occurrence of tachycardia and arterial hypotension may limit its therapeutic use under septic conditions (Bröking *et al.*, 2008).

Adrenomedullin is a vasodilatory peptide hormone, playing a key role in the regulation of cardiovascular homeostasis. In view of the circulatory failure in sepsis, it is still debated whether the occurrence of vascular hyporeactivity against adrenomedullin plays a causative or protective role (Karpnich *et al.*, 2011). Westphal *et al.* (2003) evaluated the haemodynamic response following a titrating infusion of human adrenomedullin in healthy and endotoxaemic sheep. Their findings corroborated the fact that adrenomedullin produces a hyperdynamic circulation in the presence and absence of systemic inflammation.

The nuclear enzyme Poly (ADP-Ribose)-Polymerase (PARP) has been hypothesised as playing a major role in various forms of inflammation. PARP activation is induced by DNA strand breakage and can lead to intracellular energy depletion and, ultimately, cell death. It is further thought to influence cardiovascular function and organ failure in endotoxaemia (Morales *et al.*, 2014). Scharfe *et al.* (2003) evaluated the effect of

PARP inhibitor nicotinamide on cardiovascular function in healthy and chronically endotoxaemic sheep. They revealed that treatment with nicotinamide resulted in a significantly higher systemic vascular resistance index and lower cardiac index in endotoxaemic sheep, but not in controls.

Lange *et al.* (2007b) intravenously infused glipizide, an adenosine triphosphate-sensitive potassium channel inhibitor, in endotoxaemic sheep. They found that continuous (Lange *et al.*, 2007b) and short-term (Lange *et al.*, 2006) glipizide infusion may represent a useful therapeutic alternative for the treatment of arterial hypotension associated with sepsis and systemic inflammatory response syndrome. Hessler *et al.*, (2019) mentioned that glipizide infusion increased systemic vascular resistance index and decreased cardiac index and heart rate in endotoxin sheep compared to control group.

Detergents

Detergents are known to be enzyme inhibitors and nonspecific blockers of many receptor-mediated processes. Nonionic detergents block the endocytosis of LPS by endothelial cells in culture. The release of cytokines by LPS-stimulated macrophages can be inhibited by detergents which can also block macrophage activation in intact animals (Jacobson, 2015). For therapeutic use, a suitable detergent should be biodegradable and have a high safety margin, requirements met by certain detergents of the Triton family. The archetype detergent Triton X-100 is a nonionic polyoxyethylene-phenol detergent. Unfortunately, Triton is heavily toxic to cells and animals if inserted into the cell membrane, and insufficient concentrations leads to the formation of pores that cause cell lysis. The main biological use of Triton is cell disruption. Triton WR-1339

(tyloxapol) is a mixture of linear polymers of Triton X-100 ranging between 7 and 10 Triton repeats. Tyloxapol is much less toxic than Triton X-100 (Staub *et al.*, 2001). Staub *et al.* (2001) infused intravenously 1 mg/kg *E. coli* endotoxin in 10 instrumented sheep with chronic lung lymph fistulas. One week later, they administered intravenously 40 mmol/kg tyloxapol 1–4 h before infusing the same dose of endotoxin. In those paired studies, they compared pulmonary haemodynamics, lung lymph dynamics, body temperature, circulating leukocyte concentrations and circulating TNF for 6 h. In all 10 sheep, tyloxapol blocked 80–90% of the pulmonary responses and 70–90% of the systemic responses. They concluded that tyloxapol was safe, inexpensive, easy to use and immediately effective. In another research, phenol LPS extracted from *E. coli* serotype O55:B5 was intravenously infused at 2 µg/kg in clinically healthy 1-year-old Iranian fat-tailed ewes. Tyloxapol was intravenously injected to endotoxaemic sheep at two different doses containing 200 and 400 mg/kg at 90 min following endotoxaemia induction (Heidari *et al.*, 2016). Serum concentrations of haptoglobin, serum amyloid A, TNF-α and IFN-γ in treatment groups were significantly lower than in positive controls. However, there were no significant differences among the doses of tyloxapol. They concluded that tyloxapol acts as an anti-inflammatory mediator through reducing pro-inflammatory cytokines and hepatic acute phase proteins after endotoxaemia induction in sheep. Heidari *et al.* (2016) reported that the efficacy of tyloxapol at 200 and 400 mg/kg was significantly higher and lower than betamethasone at 1 mg/kg and flunixin meglumine at 2.2 mg/kg, respectively. They further revealed that tyloxapol did not induce its effects in

a dose-dependent manner, and its anti- and pro-inflammatory effects at 200 and 400 mg/kg were statistically similar.

Endothelin receptor antagonists

Endothelin receptor antagonists are a type of targeted therapy used to treat patients with pulmonary hypertension. Targeted therapies slow the progression of pulmonary hypertension and may even reverse some of the damage to the heart and lungs (Kowalczyk *et al.*, 2015). Snapper *et al.* (1998) reported that BMS-182874, a selective, nonpeptide endothelin receptor antagonist, blocked the effects of exogenously administered endothelins in chronically instrumented awake sheep. A possible role was investigated for endothelin in endotoxin induced pulmonary hypertension in sheep by studying animals given intravenous endotoxin with and without pretreatment with BMS-182874. Administration of BMS-182874 alone caused a reduction in pulmonary artery pressure and systemic arterial pressure. Endotoxin alone caused an acute, nearly three-fold increase in pulmonary artery pressure, followed by a sustained but less severe increase in pulmonary artery pressure 2–5 h after endotoxin. These changes were accompanied by a three-fold increase in lung lymph flow and dramatic increases in the concentrations of plasma and lung lymph thromboxane B₂. Pretreatment with BMS-182874 significantly attenuated the early endotoxin-induced acute increase in pulmonary artery pressure and completely blocked the late sustained pulmonary hypertension, but had no effect on the increase in thromboxane levels. BMS-182874, in addition to functioning as an endothelium receptor antagonist, appears to counteract the activity of thromboxane at the receptor level. Tezosentan is a non-selective endothelin

receptor antagonist, reported by Kuklin *et al.* (2005) to attenuate lung injury in endotoxic sheep.

Thromboxane synthesis inhibitors

The pulmonary vasoconstriction in ovine endotoxaemia is mediated by a substantial pulmonary production of thromboxane A₂, a potent vasoconstrictor and platelet aggregant (Kowalczyk *et al.*, 2015). In this regard, some researchers have hypothesised that pre-treatment with a thromboxane synthetase inhibitor prior to endotoxin administration may prevent the increase in pulmonary artery pressure and inhibit thromboxane A₂ synthesis. Henry *et al.* (1991) reported that pulmonary vascular response to endotoxin infusion was attenuated by a thromboxane synthesis inhibitor (UK-38485) in unanesthetised sheep. Pre-treatment with BM-13.177, a thromboxane antagonist, (bolus 5 mg/kg, followed by 0.75 mg/kg/min intravenously) abolished the increase in pulmonary artery and airway pressure. These findings revealed that BM-13.177 specifically antagonised thromboxane A₂ on the putative receptor in pulmonary vascular and airway smooth muscle following endotoxaemia in sheep. OKY-046 is another selective thromboxane synthetase inhibitor utilised by Redl *et al.* (1991) as pre-treatment in ovine endotoxaemia. They reported that OKY-046 attenuated the endotoxin-induced increase in pulmonary arterial pressure and prevented the early decrease in right ventricular ejection fraction and cardiac output. However, thromboxane synthetase inhibition failed to prevent endotoxin-induced hypoxaemia. The marked increase in plasma thromboxane concentrations, usually observed following the administration of endotoxin, was prevented by pretreating the animals with OKY-046. On the other hand, increased

plasma prostacyclin concentrations were observed in the sheep treated with the thromboxane synthetase inhibitor.

Antioxidants

Endotoxaemia can be considered as an oxidative stress condition in sheep (Chalmeh, 2013), hence the use of antioxidant therapies in endotoxaemic sheep performed by several researchers. Taurine (2-amino-ethanesulfonic acid) is a sulphur-containing amino acid (β) and naturally-occurring antioxidant (Aydın *et al.*, 2016) which is not a constituent of mammalian proteins. One of its main roles is to protect tissues against attack by chlorinated oxidants, particularly hypochlorous acid. Taurine is found in high concentrations in human leukocytes, particularly polymorphonuclear leukocytes. Some studies have revealed that taurine possesses potent antimicrobial properties as it is able to increase neutrophil phagocytic ability and respiratory burst activity (Amir Aslani & Ghobadi, 2016). Taurine upregulates constitutive nitric oxide synthase, known to be a cytoprotectant and the only known endogenous inhibitor of inducible nitric oxide synthase. This antioxidant is also thought to be the main mediator that regulates the dramatic systemic hypotension observed in endotoxaemia (Aydın *et al.*, 2016). Egan *et al.* (2001) administered 300 mg/kg of taurine to Suffolk sheep 1 h before endotoxin infusion and revealed that pretreatment with intravenous taurine significantly reduced pulmonary myeloperoxidase activity and peripheral neutropenia and increased neutrophil respiratory burst activity. Their data suggested that taurine may have a therapeutic role in preventing the lung injury seen in ovine endotoxaemia.

N-acetyl-cysteine is yet another antioxidant which is quite famous for its abi-

lity to minimise oxidative stress and the downstream negative effects that are thought to be associated with oxidative stress. N-acetyl-cysteine inhibits granulocyte aggregation and scavenges free radicals (Parker, 2017). Koyama *et al.* (1992) studied the effect of recombinant-human superoxide dismutase, an enzyme that catalyses the dismutation of superoxide anion, on both the physiologic and biochemical lung changes in endotoxic sheep. They reported that this antioxidant agent prevented the endotoxin-induced lung injury in sheep.

Dimethyl sulphoxide, an oxygen free radical scavenger and anti-inflammatory agent, has been employed as an antioxidant substance for the treatment of endotoxaemia in sheep (Samimi *et al.*, 2014). Samimi *et al.* (2014) infused 1 and 2 g/kg of dimethyl sulfoxide in endotoxic sheep, and reported that dimethyl sulfoxide improved clinical signs and reduced acute phase response following endotoxaemia induced by *E. coli* serotype O55:B5 in rams.

Prostaglandins

As important mediators of inflammation, some prostaglandins, especially prostaglandin E (PGE) and prostacyclin (PGI₂), have properties that would theoretically be beneficial in the septic state. Both PGE and PGI₂ are vasodilators and inhibitors of platelet aggregation, used by certain investigators to stabilise membranes in experimental models. Prostaglandins also can inhibit the release of lysosomal enzyme products from leukocytes (Durand & Gutterman, 2013). Researchers have evaluated the effects of prostaglandins on endotoxin induced pulmonary injuries in sheep. Prostaglandins reduced the pulmonary hypertension and the interstitial oedema produced by en-

dotoxin primarily through their vasodilatory properties (Danek & Żurek, 2014). Demling *et al.* (1981) evaluated the effect of PGI₂ on endotoxin-induced lung injury in sheep. They administered PGI₂ at 0.1 to 0.2 µg/kg/min for a 5-hour period, reporting that PGI₂ protected the lung against injury from endotoxin.

Xanthine derivatives

Xanthine derivatives are a group of alkaloids commonly used for their effects as bronchodilators and respiratory centre stimulators. These substances not only affect the airways but also stimulate the heart rate, contraction force, and cardiac arrhythmias at high concentrations. Pentoxifylline, a methylxanthine derivative and a nonspecific phosphodiesterase inhibitor, is a drug widely prescribed for the management of vascular disorders characterised by defective regional microcirculation (Tjon & Riemann, 2001). This drug also possesses other important pharmacological actions such as anti-inflammatory activities in toxin-induced paw oedema, adjuvant-induced arthritis, oedema caused by carrageenan in the rat, and the cutaneous inflammatory response to ultraviolet light (Abdel-Salam *et al.*, 2003). It is stated that pentoxifylline may be beneficial in endotoxaemia (Krysztopik *et al.*, 2000). Ji *et al.* (2004) revealed that pentoxifylline suppressed both endotoxin-induced TNF- α and IL-6 production in the intestine of rats, a suppression achieved by down-regulating the mRNA expression. Pentoxifylline modulates the production of inflammatory cytokines and acute phase proteins induced by endotoxin in sheep (Rahmani Shahraki *et al.*, 2017). Chalmeh *et al.* (2016) evaluated the effects of pentoxifylline (at 30 and 60 mg/kg) on endotoxin-induced acute phase response in sheep. They observed that

pentoxifylline acted as an anti-inflammatory mediator by reducing pro-inflammatory cytokines and hepatic acute phase proteins, and modulated oxidative enzymes activities following endotoxaemia induction in sheep. However, they stated that the efficacy of pentoxifylline at two different doses was fairly similar. Rahmani Shahraki *et al.* (2016) showed that the efficacy of pentoxifylline (30 and 60 mg/kg) on attenuating acute phase response following ovine experimental endotoxaemia was significantly higher and lower compared with dexamethasone (1 mg/kg) and ketoprofen (3 mg/kg), respectively.

Insulin therapy

Experimental studies in sheep have indicated that insulin can act as an anti-inflammatory agent through reducing the proinflammatory response and increasing the anti-inflammatory cascade. Insulin treatment was reported to dampen inflammatory and acute phase responses by reducing cytokines and acute phase proteins in ovine endotoxaemia models (Obese *et al.*, 2001; Chalmeh *et al.*, 2013a,c,d,e). Moreover, the administration of insulin subcutaneously has been reported to lower the proinflammatory cytokine expression in the liver and serum levels of TNF- α , IL-1 β and IL-6 in endotoxic rats (Jeschke *et al.*, 2004). Obese *et al.* (2001) reported that insulin can reduce fever at a dose of 12 mU/kg in sheep challenged with LPS. Some studies have revealed the anti-inflammatory effect of insulin by increasing the anti- and decreasing the pro-inflammatory cascade, thereby restoring homeostasis in thermally injured and endotoxaemic rats. In addition, insulin prevented liver damage and preserved liver function in these animals (Jeschke *et al.*, 2004). Antiendotoxic and

anti-inflammatory effects of insulin regular at different doses have been compared with flunixin meglumine at 2.2 mg/kg (Chalmeh *et al.*, 2013c) and dexamethasone at 1 mg/kg (Chalmeh *et al.*, 2013b); the efficacy of insulin regular was significantly higher than both flunixin meglumine and dexamethasone in terms of reducing the acute phase response following experimental endotoxaemia induction in sheep. Kang *et al.* (2003) demonstrated that the antiapoptotic action of insulin is paralleled by reducing the production of reactive oxygen species. The generation of reactive oxygen species in response to insulin is integral to the activation of distal insulin signaling cascade (Mahadev *et al.*, 2001). Insulin is becoming more and more attractive as an agent for improving the outcome of critically ill patients and attenuating the pro-inflammatory cascade (Leffler *et al.*, 2007). Following inflammation, insulin exerts an anti-inflammatory effect on cellular mediators and hepatic acute phase proteins. In an animal model, insulin exerted its anti-inflammatory effects through reducing the pro-inflammatory signal transcription factors and pro-inflammatory cytokines, while increasing anti-inflammatory cytokines (Jeschke *et al.*, 2004). Insulin acts as an anti-inflammatory molecule through direct rather than indirect cellular effects. It alters the intracellular signal cascade in the liver and decreases some pro-inflammatory signal transcription factors (Jeschke *et al.*, 2004). Therefore, it may act as an anti-inflammatory molecule via two different pathways: decreasing pro-inflammatory mediators, and increasing anti-inflammatory mediators. Chalmeh *et al.* (2013d) administered different doses of insulin regular to endotoxic sheep, showing that insulin regular did not induce its effects in a dose-dependent manner;

moreover, the anti- and pro-inflammatory effects of insulin regular at 3 IU/kg were significantly higher than other doses of this drug in terms of decreasing the acute inflammatory conditions in Iranian fat-tailed sheep.

Immunotherapy

LPS can activate biological mediators of shock even if the amount of free, solubilised endotoxin is below detectable levels. Furthermore, the clinical features of Gram-negative bacteraemia such as fever, complement activation, disseminated intravascular coagulation and transient leukopaenia followed by leukocytosis, are identical to the effects of intravenously administered endotoxin, hence the fact that antibiotics cannot reverse these phenomena (Constable *et al.*, 2017). For these reasons, anti-endotoxin or anti-LPS immunotherapy has raised considerable scientific interest as a potential therapeutic mode that might improve survival in Gram-negative bacteremia (Karlsson *et al.*, 2014).

Neutralisation of endotoxin (lipopolysaccharide, LPS) would be of considerable benefit in the treatment of Gram-negative sepsis. Administration of anti-LPS antibodies is an old approach which has been renewed by improvements in monoclonal antibody technology. The antibodies directed at the conserved core region of LPS or at the lipid A which have been studied in humans are discussed in this review. In humans, two main approaches have been used to obtain serum with anti-endotoxin activity, including antibody to endotoxin-core glycolipid (J5 antiserum) and high titer anti-LPS plasma (naturally occurring) (Cross, 2014). Antibody to complete LPS is directed primarily against the side chains, which differs widely from strain to strain. Such antiserum acts against the effects of LPS be-

longing to the immunising bacterial strain, but is much less effective against LPS from other strains. The core regions of most Gram-negative bacteria contain very similar LPS core-structures. Therefore, a strain of bacteria defective in side chains and containing only core elements in its LPS was selected for the production of antiserum (Karlsson *et al.*, 2014). Peake *et al.* (2002) evaluated the effects of anti-ovine interleukin-1 β monoclonal antibody immunotherapy on an ovine model of endotoxaemia. They reported that this therapeutic regimen improved haemodynamic performance, but the beneficial effects were incomplete and survival was not significantly improved.

Chemotherapy

Recent insights into the structure and biosynthesis of the toxic portion of endotoxin, lipid A, may provide new therapeutic insights. Certain *E. coli* mutants deficient in phosphatidylglycerol have been found to accumulate 2,3-diacylglucosamine-1-phosphate, in which P-hydroxymyristoyl moieties are the sole fatty acid substituents. The discovery of this monosaccharide, designated lipid X, helped facilitate the elucidation of the correct structure of lipid A. It was subsequently shown that lipid X and a nucleotide derivative of lipid X are condensed to form the disaccharide backbone of lipid A (Molinaro *et al.*, 2015). Lipid X has some of the activities of lipid A and endotoxin, yet has little toxicity. Like LPS, it causes the *Limulus* amoebocyte lysate gelation, murine B lymphocyte mitogenesis, and macrophage activation, though to a much lesser degree compared with LPS or various lipid A-related disaccharides. Lipid X creates a transient rise in pulmonary artery pressure, and a modest increase in lung lymph flow, in a biphasic manner similar

to that of LPS; however, lipid X does not cause pulmonary vasculature protein permeability as is observed following LPS challenge (Scior *et al.*, 2013). Large doses of lipid X (1,000 $\mu\text{g}/\text{kg}$), minimally pyrogenic in animals, do not kill sheep (Burhop *et al.*, 1985). Golenbock *et al.* (1987) revealed that lipid X did not prevent endotoxin-induced neutropaenia or moderate hypotension in response to LPS in sheep. They also suggested that Lipid X is a potential prototype compound for a new type of chemotherapy directed at blocking the harmful effects of LPS during bacterial septicaemia.

Toll-like receptors inhibitors

The recent discovery of the toll-like receptors (TLRs) has furthered the understanding of the signalling pathways for pathogen recognition and immune activation in sepsis, in particular when Gram-negative bacteria are the origin. Lipopolysaccharide, a component of the cell wall of Gram-negative bacteria and a main ligand for TLRs, may play a significant role in the development of sepsis. TLR mediates the actions of lipopolysaccharide through the production and release of cytokines and the stimulation of inflammatory cells (Constable *et al.*, 2017). Anti-TLR4 antibodies in mice reduce mortality following endotoxaemia and experimental polymicrobial sepsis (Daubeuf *et al.*, 2007). Fenhammar *et al.* (2011) pre-treated conscious Texel crossbred ewes with the selective TLR4 inhibitor, TAK-242. They reported that TAK-242 reduced renal injuries following endotoxaemia induction in treatment groups.

Nitric oxide

The endotoxin reaction involves the formation of arachidonic acid metabolites and cytokines. Furthermore, endotoxin

stimulates endothelial and inflammatory cell nitric oxide synthase, producing nitric oxide from L-arginine. Vasodilation secondary to nitric oxide generation, with or without impairment of cardiac output, is believed to be one of the main reasons for the systemic arterial hypotension following endotoxaemia (Bogdan, 2015). The hypotension, but not the impaired cardiac output, is effectively blocked by NG-methyl-L-arginine, a nitric oxide synthase inhibitor. During endotoxaemia, a possible pulmonary vasodilator effect of endogenous nitric oxide may be obscured by opposing vasoconstrictive forces. This is mainly due to thromboxane A₂ in the early phase and release of leukotrienes, and endothelin, the potent endothelium-derived vasoconstrictor protein, during the late phase (Byrne *et al.*, 2018). Previous investigations have shown that inhaled nitric oxide can alleviate the pulmonary hypertension and derangement in gas exchange subsequent to endotoxin lung injury (Parker, 2017). Bjertnaes *et al.* (1998) studied the effects of inhaled nitric oxide on lung lymph flow and protein concentration during the late phase of endotoxaemia in awake sheep. They concluded that inhaled nitric oxide reduces lung fluid filtration by reducing microvascular pressure and, apparently, microvascular permeability during the late phase of endotoxaemia.

Protein C

Protein C is an inactive precursor of the serine protease activated protein C which is catalysed by thrombin/thrombomodulin complex when it is bound to its endothelial receptor. Activated protein C attenuates inflammation by inhibiting the translocation of NF- κ B and activator protein-1 in LPS-stimulated monocytes, thereby suppressing the release of proinflamma-

tory cytokines (Joyce *et al.*, 2004). Moreover, activated protein C reduces leukocyte rolling and adhesion to the endothelium by suppressing adhesion molecules, causing reduced migration and accumulation of pulmonary leukocytes (Nick *et al.*, 2004). Recombinant human activated protein C with its anticoagulant, anti-inflammatory, fibrinolytic and antiapoptotic effects, reportedly reduces the respirator-dependent days and the mortality of patients with severe sepsis. Waerhaug *et al.* (2008) reported that recombinant human activated protein C in awake endotoxic sheep alleviated endotoxin-induced lung injury as characterised by the improvements in oxygenation, coagulation and inflammation, as well as the reversal of pulmonary haemodynamic and volumetric changes.

Fructose-1,6-diphosphate

Fructose-1,6-diphosphate reduces the mortality rate in experimental sepsis, ameliorates haematological and histological alterations and pulmonary function parameters (Hattori *et al.*, 2017), and inhibits respiratory burst and oxyradical generation of activated neutrophils (Markov *et al.*, 2007); it has further been shown that fructose-1,6-diphosphate has immunosuppressive properties (Cohly *et al.*, 2004). Markov *et al.* (2007) evaluated the effect of fructose-1,6-diphosphate on endotoxin-induced lung injuries in sheep. They revealed that fructose-1,6-diphosphate treatment significantly attenuated the characteristic pulmonary hypertension, lung lymph protein clearance, and pulmonary vascular leakage observed in sheep infused with endotoxin.

Methylene blue

A nonselective inhibitor of nitric oxide and soluble guanylate cyclase, methylene blue markedly attenuates the increment in

lung fluid filtration. The effect of methylene blue is associated with reduced pulmonary capillary pressure and permeability surface area product. Furthermore, methylene blue enhances gas exchange and precludes the increase in the lung lymph of endotoxic sheep (Evgenov *et al.*, 2001). Researchers have stated that this substance reduces the early endotoxin-induced declines in stroke volume, left ventricular stroke work and cardiac indices, preventing the mean arterial pressure from falling. Moreover, methylene blue increases pulmonary arterial pressure and pulmonary vascular resistance index, reduces the increments in venous admixture and the falls in oxygen delivery, maintains oxygen consumption, prevents the increase in body temperature and plasma nitrites and nitrates, and delays the increase in plasma lactate (Evgenov *et al.*, 2001). Evgenov *et al.* (2002) investigated the effects of methylene blue on pulmonary haemodynamics in endotoxic sheep. They concluded that methylene blue attenuates the endotoxin-induced pulmonary hypertension and oedema, at least in part, by inhibiting the cyclo-oxygenase products of arachidonic acid.

POTENTIAL THERAPEUTIC PROTOCOLS

Given the pathophysiological aspects of endotoxaemia, which causes acute systemic inflammatory responses and multiple organ dysfunctions, it seems that paying attention to new anti-inflammatory drugs and protocols can be effective in treating endotoxaemia. As mentioned earlier, the sheep is a very suitable model to evaluate endotoxaemia and the effect of drugs on it. Therefore, this animal species can be used in experimental studies in the evaluation of anti-inflammatory or any

other drugs against endotoxaemia and can be generalised to other related species.

CONCLUSION

Sheep are among the species most susceptible to inflammatory conditions following the endotoxaemia, hence the necessity of adopting powerful therapeutic methods. Furthermore, sheep are a common animal model to evaluate the endotoxaemia, with results generalisable to other large animals. Several researchers have experimentally induced ovine endotoxaemia and have evaluated different various treatment modalities, presented in the current review. Introduced drugs have been used in experimental models, however, their use in clinical trials in sheep that are naturally affected with endotoxaemia is also recommended.

REFERENCES

- Abdel-Salam, O. M., A. R. Baiuomy, S. M. El-Shenawy & M. S. Arbid, 2003. The anti-inflammatory effects of the phosphodiesterase inhibitor pentoxifylline in the rat. *Pharmacological Research*, **47**, 331–340.
- Amir Aslani, B. & S. Ghobadi, 2016. Studies on oxidants and antioxidants with a brief glance at their relevance to the immune system. *Life Science*, **146**, 163–173.
- Aydın, A. F., J. Çoban, I. Doğan-Ekici, E. Betül-Kalaz, S. Doğru-Abbasoğlu, & M. Uysal, 2016. Carnosine and taurine treatments diminished brain oxidative stress and apoptosis in D-galactose aging model. *Metabolic Brain Disease*, **31**, 337–345.
- Bogdan, C., 2015. Nitric oxide synthase in innate and adaptive immunity: an update. *Trends in Immunology*, **36**, 161–178.
- Bonanno, L. S., 2018. Early administration of intravenous fluids in sepsis: Pros and cons. *Critical Care Nursing Clinics of North America*, **30**, 323–332.

- Bröking, K., M. Lange, A. Morelli, C. Ertmer, H. V. Aken, M. Luecke, S. Rehberg, N. Böwering, H. G. Bone, D. L. Traber & M. Westphal, 2008. Employing dobutamine as a useful agent to reverse the terlipressin-linked impairments in cardiopulmonary hemodynamics and global oxygen transport in healthy and endotoxemic sheep. *Shock*, **29**, 71–77.
- Burhop, K. E., R. A. Proctor, R. B. Helgerson, C. H. Raetz, J. R. Starling & J. A. Will, 1985. Pulmonary pathophysiological changes in sheep caused by endotoxin precursor, lipid X. *Journal of Applied Physiology*, **59**, 1726–1732.
- Byrne, L., N. G. Obonyo, S. Diab, K. Dunster, M. Passmore, A. C. Boon, L. S. Hoe, K. Hay, F. Van Haren, J. P. Tung, L. Cullen, K. Shekar, K. Maitland & J. F. Fraser, 2018. An ovine model of hyperdynamic endotoxemia and vital organ metabolism. *Shock*, **49**, 99–107.
- Chalmeh, A., 2013. Comparison of effects of insulin regular, dexamethasone and flunixin meglumine on endotoxemia experimentally induced by *Escherichia coli* serotype O55:B5 in Iranian fat-tailed sheep. Post Graduate Thesis in Large Animal Internal Medicine, Shiraz University, Shiraz, Iran.
- Chalmeh, A., K. Badiei, M. Pourjafar & S. Nazifi, 2013a. Acute phase response in experimentally *Escherichia coli* serotype O55:B5 induced endotoxemia and its comparative treatment with dexamethasone and flunixin meglumine in Iranian fat-tailed sheep. *Veterinarski Arhiv*, **83**, 301–312.
- Chalmeh, A., K. Badiei, M. Pourjafar & S. Nazifi, 2013b. Anti-inflammatory effects of insulin and dexamethasone on experimentally *Escherichia coli* serotype O55:B5 induced endotoxemia in Iranian fat-tailed sheep. *Journal of Faculty of Veterinary Medicine, Istanbul University*, **39**, 197–208.
- Chalmeh, A., K. Badiei, M. Pourjafar & S. Nazifi, 2013c. Anti-inflammatory effects of insulin regular and flunixin meglumine on endotoxemia experimentally induced by *Escherichia coli* serotype O55:B5 in an ovine model. *Inflammation Research*, **62**, 61–67.
- Chalmeh, A., K. Badiei, M. Pourjafar & S. Nazifi, 2013d. Modulation of inflammatory responses following insulin therapy in experimentally bolus intravenous *Escherichia coli* lipopolysaccharide serotype O55:B5 induced endotoxemia in Iranian fat-tailed sheep. *Small Ruminant Research*, **113**, 283–289.
- Chalmeh, A., K. Badiei, M. Pourjafar & S. Nazifi, 2014a. Effects of intravenous dexamethasone and flunixin meglumine on *Escherichia coli* serotype O55:B5 induced endotoxemia in ewes. *Online Journal of Veterinary Research*, **18**, 596–606.
- Chalmeh, A., K. Badiei, M. Pourjafar, S. Nazifi, S. M. M. Heidari, M. Heidari & M. Babazadeh, 2014b. Alterations in electrocardiographic parameters and serum cardiac biomarkers in an ovine experimental endotoxemia model. *Journal of Faculty of Veterinary Medicine, Istanbul University*, **40**, 211–219.
- Chalmeh, A., K. Badiei, M. Pourjafar & S. Nazifi, 2014c. The relationship among acute phase response proteins, cytokines and enzymes during ovine experimental endotoxemia. *Iranian Journal of Veterinary Medicine*, **8**, 179–185.
- Chalmeh, A., A. Rahmani Shahraki, S. M. M. Heidari, K. Badiei, M. Pourjafar, S. Nazifi & M. J. Zamiri, 2016. The comparative efficacy of tyloxapol versus pentoxifylline against induced acute phase response in an ovine experimental endotoxemia model. *Inflammopharmacology*, **24**, 59–64.
- Cohly, H., J. Jenkins, T. Skelton, E. Meydrech & A. K. Markov, 2004. Fructose-1, 6-diphosphate suppresses T-lymphocyte proliferation, promotes apoptosis and inhibits interleukins-1, β -actin mRNAs, and transcription factors expression. *Immunological Investigations*, **33**, 407–421.

- Constable, P. D., K. W. Hinchcliff, S. H. Done & W. Gruenberg, 2017. Toxemia and endotoxemia. In: *Veterinary Medicine: A Textbook of the Diseases of Cattle, Horses, Sheep, Pigs and Goats*, 11th edn, Elsevier, USA, pp. 59–67.
- Cross, A. S., 2014. Anti-endotoxin vaccines: back to the future. *Virulence*, **51**, 219–225.
- Danek, J. & U. Žurek, 2014. Changes in domestic animals after endotoxin administration – a review. *Annals of Animal Science*, **14**, 479–489.
- Daubeuf, B., J. Mathison, S. Spiller, S. Hugues, S. Herren, W. Ferlin, M. Kosco-Vilbois, H. Wagner, C. J. Kirschning, R. Ulevitch & G. Elson, 2007. TLR4/MD-2 monoclonal antibody therapy affords protection in experimental models of septic shock. *Journal of Immunology*, **179**, 6107–6114.
- Demling, R. H., M. Smith & R. Gunther, 1981. The effect of prostacyclin infusion on endotoxin-induced lung injury. *Surgery*, **89**, 257–263.
- Dubin, A., B. Maskin, G. Murias, M. O. Pozo, J. P. Sottile, M. Barán, V. S. Edul, H. S. Canales & E. Estenssoro, 2006. Effects of levosimendan in normodynamic endotoxaemia: A controlled experimental study. *Resuscitation*, **69**, 277–286.
- Dubin, A., G. Murias, J. P. Sottile, M. O. Pozo, M. Barán, V. S. Edul, H. S. Canales, G. Etcheverry, B. Maskin & E. Estenssoro, 2007. Effects of levosimendan and dobutamine in experimental acute endotoxemia: a preliminary controlled study. *Intensive Care Medicine*, **33**, 485–494.
- Dubin, A., V. S. Edul, M. O. Pozo, G. Murias, C. M. Canullán, E. F. Martins, G. Ferrara, H. S. Canales, M. Laporte, E. Estenssoro & C. Ince, 2008. Persistent villi hypoperfusion explains intramucosal acidosis in sheep endotoxemia. *Critical Care Medicine*, **36**, 535–542.
- Durand, M. J. & D. D. Gutterman, 2013. Diversity in mechanisms of endothelium-dependent vasodilation in health and disease. *Microcirculation*, **20**, 239–247.
- Egan, B. M., H. Abdih, C. J. Kelly, C. Condrón & D. J. Bouchier-Hayes, 2001. Effect of intravenous taurine on endotoxin-induced acute lung injury in sheep. *European Journal of Surgery*, **167**, 575–580.
- Ertmer, C., H. G. Bone, A. Morelli, H. Van Aken, M. Erren, M. Lange, D. L. Traber & M. Westphal, 2007. Methylprednisolone reverses vasopressin hyporesponsiveness in ovine endotoxemia. *Shock*, **27**, 281–288.
- Evgenov, O. V., G. Sager & L. J. Bjertnaes, 2001. Methylene blue reduces lung fluid filtration during the early phase of endotoxemia in awake sheep. *Critical Care Medicine*, **29**, 374–379.
- Evgenov, O. V., N. V. Evgenov, T. E. Mollnes & L. J. Bjertnaes, 2002. Methylene blue reduces pulmonary oedema and cyclooxygenase products in endotoxaemic sheep. *European Respiratory Journal*, **20**, 957–964.
- Fenhammar, J., M. Rundgren, J. Forestier, S. Kalman, S. Eriksson & R. Frithiof, 2011. Toll-like receptor 4 inhibitor TAK-242 attenuates acute kidney injury in endotoxemic sheep. *Anesthesiology*, **114**, 1130–1137.
- Fink, M. P., 2014. Animal models of sepsis. *Virulence*, **5**, 143–153.
- Garland, A., A. Kumar & J. Waechter, 2010. Early administration of crystalloid fluids reduces mortality in septic shock. *American Journal of Respiratory and Critical Care Medicine*, **181**, A4097.
- Golenbock, D. T., J. A. Will, C. R. Raetz & R. A. Proctor, 1987. Lipid X ameliorates pulmonary hypertension and protects sheep from death due to endotoxin. *Infection and Immunity*, **55**, 2471–2476.
- Hajimohammadi, A., K. Badiei, P. Kheibari, M. Pourjafar & A. Chalmeh, 2018. Effects of polymyxin B on clinical signs, serum TNF- α , haptoglobin and plasma lactate concentrations in experimental endotoxaemia in sheep. *Journal of Veterinary Research (Poland)*, **62**, 79–85.

- Halmagyi, D. F. J., B. Starzeki & G. J. Horner, 1963. Mechanism and pharmacology of endotoxin shock in sheep. *Journal of Applied Physiology*, **18**, 544–4.
- Hattori, Y., K. Hattori, T. Suzuki & N. Matsuda, 2017. Recent advances in the pathophysiology and molecular basis of sepsis-associated organ dysfunction: Novel therapeutic implications and challenges. *Pharmacology and Therapeutics*, **177**, 56–66.
- Heidari, S., K. Badiei, A. Chalmeh, M. Pourjafar, A. R. Rahmani Shahraki, S. Nazifi & M. J. Zamiri, 2016. Impact of tyloxapol to combat acute phase response in comparison with betamethasone and flunixin meglumine following an ovine experimentally induced endotoxemia model. *Veterinary Science Development*, **6**, 58–64.
- Henry, C. L. Jr., M. L. Ogletree, K. L. Brigham & J. W. Jr. Hammon, 1991. Attenuation of the pulmonary vascular response to endotoxin by a thromboxane synthesis inhibitor (UK-38485) in unanesthetized sheep. *Journal of Surgical Research*, **50**, 77–81.
- Hessler, M., B. B. Pinto, P. H. Arneemann, T. G. Kampmeier, L. Seidel, A. Morelli, H. V. Aken, M. Westphal, S. Rehberg & C. Ertmer, 2019. Differential effects of selective and nonselective potassium channel inhibitors in ovine endotoxemic shock (macrocirculation) and in a rat model of septic shock (microcirculation). *Shock*, **51**, 247–255.
- Jacobson, K. A., 2015. New paradigms in GPCR drug discovery. *Biochemical Pharmacology*, **98**, 541–555.
- Jeschke, M. G., D. Klein, U. Bolder & R. Einspanier, 2004. Insulin attenuates the systemic inflammatory response in endotoxemic rats. *Endocrinology*, **145**, 4084–4093.
- Ji, Q., L. Zhang, H. Jia & J. Xu, 2004. Pentoxifylline inhibits endotoxin induced NF-kappa B activation and associated production of proinflammatory cytokines. *Annals of Clinical Laboratory Science*, **34**, 427–436.
- Joyce, D. E., D. R. Nelson & B. W. Grinnell, 2004. Leukocyte and endothelial cell interactions in sepsis: Relevance of the protein C pathway. *Critical Care Medicine*, **32**, S280–S286.
- Kang, S., J. Song, H. Kang, S. Kim, Y. Lee & D. Park, 2003. Insulin can block apoptosis by decreasing oxidative stress via phosphatidylinositol 3-kinase- and extracellular signal-regulated protein kinase-dependent signaling pathways in HepG2 cells. *European Journal of Endocrinology*, **148**, 147–155.
- Karlsson, S. L., E. Ax, E. Nygren, S. Källgård, M. Blomquist, A. Ekman, J. Benktander, J. Holmgren & M. Lebens, 2014. Development of stable *Vibrio cholerae* O1 Hikojima type vaccine strains co-expressing the Inaba and Ogawa lipopolysaccharide antigens. *PLoS One*, **9**, e108521.
- Karpnich, N. O., S. L. Hoopes, D. O. Kechele, P. M. Lenhart & K. M. Caron, 2011. Adrenomedullin function in vascular endothelial cells: Insights from genetic mouse models. *Current Hypertension Reviews*, **7**, 228–239.
- Kingsley, S. M. & B. V. Bhat, 2016. Differential paradigms in animal models of sepsis. *Current Infectious Disease Reports*, **18**, 26.
- Koyama, S., T. Kobayashi, K. Kubo, M. Sekiguchi & G. Ueda, 1992. Recombinant-human superoxide dismutase attenuates endotoxin-induced lung injury in awake sheep. *American Review of Respiratory Disease*, **145**, 1404–1409.
- Kowalczyk, A., P. Kleniewska, M. Kolodziejczyk, B. Skibska & A. Goraca, 2015. The role of endothelin-1 and endothelin receptor antagonists in inflammatory response and sepsis. *Archivum Immunologiae et Therapiae Experimentalis*, **63**, 41–52.
- Krejci, V., L. B. Hildebrand & G. H. Sigurdson, 2006. Effects of epinephrine, norepinephrine, and phenylephrine on micro-

- circulatory blood flow in the gastrointestinal tract in sepsis. *Critical Care Medicine*, **34**, 1456–1463.
- Krysztopik, R. J., P. J. Matheson, D. A. Spain, R. N. Garrison & M. A. Wilson, 2000. Lazaroid and pentoxifylline suppress sepsis-induced increases in renal vascular resistance via altered arachidonic acid metabolism. *Journal of Surgical Research*, **93**, 75–81.
- Kuklin, V., M. Kirov, M. Sovershaev, T. Andreassen, O. C. Ingebretsen, K. Ytrehus & L. Bjertnaes, 2005. Tezosentan-induced attenuation of lung injury in endotoxemic sheep is associated with reduced activation of protein kinase C. *Critical Care*, **9**, R211–R217.
- Lagoa, C. E., L. F. P. de Figueiredo, R. J. Cruz, E. Silva & M. R. Silva, 2004. Effects of volume resuscitation on splanchnic perfusion in canine model of severe sepsis induced by live *Escherichia coli* infusion. *Critical Care*, **8**, R221–R228.
- Lange, M., C. Szabo, H. Van Aken, W. Williams, D. L. Traber, F. Daudel, K. Bröking, A. L. Salzman, H. G. Bone & M. Westphal, 2006. Short-term effects of glipizide (an adenosine triphosphate-sensitive potassium channel inhibitor) on cardiopulmonary hemodynamics and global oxygen transport in healthy and endotoxemic sheep. *Shock*, **26**, 516–521.
- Lange, M., K. Bröking & C. Hucklenbruch, 2007a. Hemodynamic effects of titrated norepinephrine in healthy versus endotoxemic sheep. *Journal of Endotoxin Research*, **13**, 53–57.
- Lange, M., W. Williams, H. G. Bone, H. Van Aken, K. Bröking, A. Morelli, C. Hucklenbruch, F. Daudel, C. Ertmer, H. Stubbe, D. L. Traber & M. Westphal, 2007b. Continuously infused glipizide reverses the hyperdynamic circulation in ovine endotoxemia. *Shock*, **27**, 701–706.
- Lange, M., C. Ertmer, S. Rehberg, A. Morelli, G. Köhler, T. G. Kampmeier, H. Van Aken & M. Westphal, 2011. Effects of two different dosing regimens of terlipressin on organ functions in ovine endotoxemia. *Inflammation Research*, **60**, 429–437.
- Leffler, M., T. Hrach, M. Stuerzl, R. E. Horch, D. N. Herndon & M. G. Jeschke, 2007. Insulin attenuates apoptosis and exerts anti-inflammatory effects in endotoxemic human macrophages. *Journal of Surgical Research*, **143**, 398–406.
- Mahadev, K., X. Wu, A. Zilbering, L. Zhu, J. T. Lawrence & B. J. Goldstein, 2001. Hydrogen peroxide generated during cellular insulin stimulation is integral to activation of the distal insulin signaling cascade in 3T3-L1 adipocytes. *Journal of Biological Chemistry*, **276**, 48662–48669.
- Maitland, K., S. Kiguli & R. O. Opoka, 2011. Mortality after fluid bolus in African children with severe infection. *New England Journal of Medicine*, **364**, 2483–2495.
- Markov, A. K., E. T. Warren, H. H. Cohly, D. J. Sauls & T. N. Skelton, 2007. Influence of fructose-1,6-diphosphate on endotoxin-induced lung injuries in sheep. *Journal of Surgical Research*, **138**, 45–50.
- Molinaro, A., O. Holst, F. Di Lorenzo, M. Callaghan, A. Nurisso, G. D'Errico, A. Zamyatina, F. Peri, R. Berisio, R. Jerala, J. Jiménez-Barbero, A. Silipo & S. Martín-Santamaría, 2015. Chemistry of lipid A: At the heart of innate immunity. *Chemistry*, **21**, 500–519.
- Morales, J., L. Li, F. J. Fattah, Y. Dong, E. A. Bey, M. Patel, J. Gao & D. A. Boothman, 2014. Review of poly (ADP-ribose) polymerase (PARP) mechanisms of action and rationale for targeting in cancer and other diseases. *Critical Reviews in Eukaryotic Gene Expression*, **24**, 15–28.
- Morresey, P. R. & R. J. Mackay, 2006. Endotoxin-neutralizing activity of polymyxin B in blood after IV administration in horses. *American Journal of Veterinary Research*, **67**, 642–647.
- Naylor, D., A. Sharma, Z. Li, G. Monteith, T. Sullivan, A. Canovas, B. A. Mallard, C. Baes & N. A. Karrow, 2020. Characterizing ovine serum stress biomarkers

- during endotoxemia. *Journal of Dairy Science*, **103**, 5501–5508.
- Newnham, J. P., S. G. Kallapur, B. W. Kramer, T. J. Moss, I. Nitsos, M. Ikegami & A. H. Jobe, 2003. Betamethasone effects on chorioamnionitis induced by intra-amniotic endotoxin in sheep. *American Journal of Obstetrics and Gynecology*, **189**, 1458–1466.
- Nick, J. A., S. D. Coldren, M. W. Geraci, K. R. Poch, B. W. Fouty, J. O'Brien, M. Gruber, S. Zarini, R. C. Murphy, K. Kuhn, D. Richter, K. R. Kast & E. Abraham, 2004. Recombinant human activated protein C reduces human endotoxin-induced pulmonary inflammation via inhibition of neutrophil chemotaxis. *Blood*, **104**, 3878–3885.
- Obese, F. Y., B. K. Whitlock, B. Steele, T. H. Elsasser & J. L. Sartin, 2001. Effect of insulin on fever in endotoxemic sheep. *Bulletin of Animal Health and Production in Africa*, **59**, 435–439.
- Parker, R. E., 2017. Sheep as an animal model of endotoxemia-induced diffuse lung injury. In: *Handbook of Animal Models of Pulmonary Disease*, ed J. O. Cantor.
- Passmore, M. R., N. G. Obonyo, L. Byrne, A. C. Boon, S. D. Diab, K. R. Dunster, Y. L. Fung, M. M. Spanevello, M. H. Fauzi, S. E. Pedersen, G. Simonova, C. M. Anstey, K. Shekar, J. P. Tung, K. Maitland & J. F. Fraser, 2019. Fluid resuscitation with 0.9% saline alters haemostasis in an ovine model of endotoxemic shock. *Thrombosis Research*, **176**, 39–45.
- Patel, U. D., J. H. Patel, R. D. Varia, H. B. Patel, S. K. Bhavsar & A. M. Thaker, 2012. Disposition kinetic of levofloxacin in experimentally induced febrile model of sheep. *Journal of Toxicological Pharmacology*, **7**, 11–19.
- Peake, S. L., J. Pierides, P. I. Leppard & G. R. Russ, 2002. Anti-ovine interleukin-1 β monoclonal antibody immunotherapy in an ovine model of gram-negative septic shock. *Critical Care Medicine*, **30**, 171–181.
- Pittet, J. F., C. M. Pastor & D. R. Morel, 2000. Spontaneous high systemic oxygen delivery increases survival rate in awake sheep during sustained endotoxemia. *Critical Care Medicine*, **28**, 496–503.
- Poli-de-Figueiredo, L. F., A. G. Garrido, N. Nakagawa & P. Sannomiya, 2008. Experimental models of sepsis and their clinical relevance. *Shock*, Suppl 1, 53–59.
- Rahal, L., A. G. Garrido, R. J. Cruz Jr, E. Silva & L. F. Poli-de-Figueiredo, 2009. Fluid replacement with hypertonic or isotonic solutions guided by mixed venous oxygen saturation in experimental hypodynamic sepsis. *Journal of Trauma and Acute Care Surgery*, **67**, 1205–1212.
- Rahmani Shahraki, A. R., M. Pourjafar, A. Chalmeh, K. Badiei, S. M. M. Heidari, M. J. Zamiri & S. Nazifi, 2016. Attenuating the endotoxin induced acute phase response by pentoxifylline in comparison with dexamethasone and ketoprofen in sheep. *Small Ruminant Research*, **136**, 1–5.
- Rahmani Shahraki, A. R., M. Pourjafar, A. Chalmeh, K. Badiei, S. M. M. Heidari, M. J. Zamiri & S. Nazifi, 2017. The efficacy of pentoxifylline against induced acute phase response in an ovine experimental endotoxemia model. *Veterinary Science Development*, **7**, 1–5.
- Ranjan, R., B. K. Roy, A. Ranjan & S. K. Singh, 2011. Effect of *E. coli* endotoxin induced fever on the pharmacokinetic profile and dosage regimen of ceftriaxone in sheep (*Ovis aries*). *Veterinarski Arhiv*, **81**, 423–432.
- Redl, G., S. Abdi, L. D. Traber, R. J. Nichols, J. T. Flynn, D. N. Herndon & D. L. Traber, 1991. Inhibition of thromboxane synthesis reduces endotoxin-induced right ventricular failure in sheep. *Critical Care Medicine*, **19**, 1294–1302.
- Rivers, E., B. Nguyen & S. Havstad, 2001. Early goal-directed therapy in the treatment of severe sepsis and septic shock. *New England Journal of Medicine*, **345**, 1368–1377.

- Rivers, E. P., V. Coba, A. Visbal, M. Whitmill & D. Amponsah, 2008. Management of sepsis: Early resuscitation. *Clinics in Chest Medicine*, **29**, 689–704.
- Sadaka, F., M. Juarez, S. Naydenov & J. O'Brien, 2014. Fluid resuscitation in septic shock: The effect of increasing fluid balance on mortality. *Journal of Intensive Care Medicine*, **29**, 213–217.
- Samimi, A., A. Hajimohammadi, M. Fazeli & S. Nazifi, 2014. Effect of dimethyl sulfoxide on clinical signs and acute phase proteins during endotoxemia induced by *Escherichia coli* serotype O55:B5 in sheep. *Online Journal of Veterinary Research*, **18**, 188–200.
- Scharte, M., J. Meyer, H. Van Aken & H. G. Bone, 2011. Hemodynamic effects of terlipressin (a synthetic analog of vasopressin) in healthy and endotoxemic sheep. *Critical Care Medicine*, **29**, 1756–1760.
- Scharte, M., J. R. Nofer, H. V. Aken, R. Waurick, J. Meyer & H. G. Bone, 2003. Nicotinamide increases systemic vascular resistance in ovine endotoxemia. *Intensive Care Medicine*, **29**, 989–994.
- Scior, T., J. Lozano-Aponte, V. Figueroa-Vazquez, J. A. Yunes-Rojas, U. Zähringer & C. Alexander, 2013. Three-dimensional mapping of differential amino acids of human, murine, canine and equine TLR4/MD-2 receptor complexes conferring endotoxic activation by lipid A, antagonism by Eritoran and species-dependent activities of Lipid IVA in the mammalian LPS sensor system. *Computational and Structural Biotechnology Journal*, **7**, e201305003.
- Setayesh, A., M. Pourjafar, A. Hajimohammadi, K. Badieli & A. Chalmeh, 2018. Effect of intravenous colistin administration on *Escherichia coli* liposaccharide-induced endotoxemia in sheep. *Philippine Journal of Veterinary Medicine*, **55**, 85–94.
- Silverstein, D. C. & K. A. Beer, 2015. Controversies regarding choice of vasopressor therapy for management of septic shock in animals. *Journal of Veterinary Emergency and Critical Care*, **25**, 48–54.
- Snapper, J. R., J. S. Thabes, P. L. Lefferts & W. Lu, 1998. Role of endothelin in endotoxin-induced sustained pulmonary hypertension in sheep. *American Journal of Respiratory Critical Care Medicine*, **157**, 81–88.
- Staub, N. C. Sr., K. E. Longworth, V. Serikov, E. H. Jerome & T. Elsasser, 2001. Detergent inhibits 70–90% of responses to intravenous endotoxin in awake sheep. *Journal of Applied Physiology*, **90**, 1788–1797.
- Tjon, J. A. & L. E. Riemann, 2001. Treatment of intermittent claudication with pentoxifylline and cilostazol. *American Journal of Health System Pharmacology*, **58**, 485–493.
- Waerhaug, K., V. N. Kuklin, M. Y. Kirov, M. A. Sovershaev, B. Langbakk, O. C. Ingebretsen, K. Ytrehus & L. J. Bjertnaes, 2008. Recombinant human activated protein C attenuates endotoxin-induced lung injury in awake sheep. *Critical Care*, **12**, R104.
- Westphal, M., H. Stubbe, H. G. Bone, F. Daudel, S. Vocke, H. Van Aken & M. Booke, 2002. Hemodynamic effects of exogenous adrenomedullin in healthy and endotoxemic sheep. *Biochemical and Biophysical Research Communications*, **296**, 134–138.
- Westphal, M., H. Stubbe, A. W. Sielenkämper, C. Ball, H. Van Aken, R. Borgulya & H. G. Bone, 2003. Effects of titrated arginine vasopressin on hemodynamic variables and oxygen transport in healthy and endotoxemic sheep. *Critical Care Medicine*, **31**, 1502–1508.
- Westphal, M., A. W. Sielenkämper, H. Van Aken, H. D. Stubbe, F. Daudel, R. Schepers, S. Schulte & H. G. Bone, 2004. Dopexamine reverses the vasopressin-associated impairment in tissue oxygen supply but decreases systemic blood pressure in ovine endotoxemia. *Anesthesia and Analgesia*, **99**, 878–885.

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