Treatment of Endotoxemia

Vernon C. Langston, *DVM, PhD, Diplomate ACVCP*
College of Veterinary Medicine
Mississippi State University
Mississippi State, MS

Abstract

Endotoxemia is a component of a variety of gram-negative bacterial diseases of veterinary importance. Active and passive immunization with anti-core antibodies may neutralize endotoxin and is a useful tool in severe cases. When the GI tract is a primary source of endotoxin, oral activated charcoal will prevent entry of the endotoxin into the circulation. While the effect of NSAID treatments such as flunixin or ketoprofen on animal production or mortality is largely undetermined, they will decrease many of the signs of endotoxemia and are useful in the majority of cases. It appears that glucocorticoids will provide improvement early in the course of the disease but data suggest the eventual mortality rate is unaffected by steroid use. Unless the infection can be deemed self-limiting, appropriate antibiotic therapy is crucial for recovery. For animals in shock, fluid therapy is an essential supportive measure.

Introduction

Despite the availability of potent antimicrobials, gram-negative bacterial infections continue to be significant diseases within human and veterinary medicine. Based on 1987 CDC data, over 100,000 cases of gram-negative septicemia are reported in the U.S. annually in humans, of which approximately 25% die.[1,2] Numerous diseases associated with gram-negative sepsis such as salmonellosis, coliform mastitis, toxic metritis, colisepticemia, and Pasteurella pneumonia are of economic importance in the cattle industry. While it is now recognized that for some gram-negative bacteria exotoxins play an important role, the impact of endotoxin in gram-negative sepsis cannot be overstated. In nonlethal cases endotoxin accounts for significant tissue damage with resultant decreases in production and increased treatment costs and culling. In its most severe form endotoxemia can lead to organ failure, disseminated intravascular coagulation (DIC), and shock with an associated mortality as high as 90%.

Pathophysiology and clinical signs

Endotoxin (lipopolysaccharide, LPS) is an integral part of the outer cell wall of all gram-negative bacteria. The LPS molecule can be subdivided into lipid A and polysaccharide portions. Lipid A is considered responsible for most of the toxic properties of endotoxin whereas the polysaccharide portion determines the antigenicity of the molecule. The polysaccharide can be further subdivided into an inner "core-antigen" that is similar antigenically across gram-negative genus lines, and an outer "O-antigen" that is bacterial strain specific.[3] While the release of endotoxin is primarily associated with lysis of the bacterial cell, up to 18% of a cell's total endotoxin content can be shed while still alive, especially during log growth.[4]

Gram-negative infections anywhere in the body can serve as a source of endotoxin and subsequent absorption into the central circulation. The gastrointestinal tract is unique in that resident bacteria represent a constant potential source of endotoxin that is held in check by bile acids (which bind endotoxin) and the mucosal barrier. The small amount of endotoxin that is absorbed into the portal circulation is removed by the Kupfer cells of the liver. Obstructive cholestasis, liver disease, or any disease affecting GI mucosal integrity may allow absorption of significant concentrations of endotoxin.

Once absorbed, endotoxin triggers a complex cascade of pathophysiologic events. Endothelial damage and activation of the coagulation system may occur as a consequence of direct endotoxin membrane injury. It is however activation of the body's own inflammatory mechanisms by endotoxin that lead to most of the damage. In this regard, activation of neutrophils, platelets, vascular endothelium, mast cells, and most importantly macrophages leads to the release of critical mediators such as tumor necrosis factor (TNF), interleukins (especially IL-1), platelet activating factor, phospholipase, prostaglandins, thromboxane, and leukotrienes. Numerous other mediators and toxins have also been implicated and include histamine, serotonin, beta endorphins, and toxic oxygen metabolites. Cytotoxicity occurs due to direct actions, mediator effects, oxygen radical formation and lysosomal enzyme release.[5]

The cellular damage leads to an escalating cascade of microvascular injury, increased capillary permeability, and decreased tissue perfusion. Though cardiac output is initially maintained by increased rate, as the syndrome progresses toward shock there is a decrease in the systemic vascular resistance and a redistribution of the intravascular volume such that cardiac preload is decreased. The decreased preload coupled with nega-

tive inotropic effects (due to a variety of factors including beta-endorphins) cause a drop in cardiac output with resultant hypotension, tissue hypoxemia, and lactic acidosis. Hyperventilation often occurs due to lactic acidosis and stimulation of Hering-Breuer reflexes in the lung as a result of incipient or overt pulmonary edema (most notable in cattle) from damaged endothelium and pulmonary hypertension. Ventilation-perfusion mismatches in the lung worsen the hypoxemia.[6] Hemoconcentration and leukopenia followed by leukocytosis are common clinical pathologic findings. Electrolyte imbalances are also encountered with hypocalcemia often occurring in cattle. It is worthwhile to note that in some cases, serum calcium may be normal or mildly elevated yet a decrease in ionized calcium (due to acidosis and protein loss) may lead to signs of hypocalcemia.

Clinical signs of endotoxemia include fever, depression, tachycardia, and tachypnea often advancing to combinations of hypotension, hypoxemia, metabolic acidosis, clotting abnormalities (DIC), diarrhea, and death. Treatments for endotoxemia can be divided into those therapies aimed at preventing the binding of endotoxin with its cellular receptor(s), antagonizing the effects of released mediators and toxins, and general supportive care.

Treatments preventing the binding of endotoxin

Physical removal

The most obvious way to decrease endotoxin absorption is physical removal of infected fluids. Frequent stripping of the affected quarter is a worthy recommendation in all cases of toxic mastitis to remove toxins, bacteria, and inflammatory mediators. Likewise, uterine lavage can greatly reduce the signs of endotoxemia in toxic metritis. While other endotoxic diseases often do not lend themselves well to drainage of the infected site, where possible, drainage of abscesses or body cavities may provide substantial improvement. It is prudent to keep in mind that tissue manipulation can cause showering of bacteria that may need to be addressed.

Polymyxin B

Polymyxin B and colistin are related antibiotics used primarily for gram-negative infections. An unusual feature distinct from their antibiotic activity is an ability to bind endotoxin. More specifically, unlike anti-endotoxin antibodies that bind to the core region of the molecule, polymyxin B binds to lipid A. This difference is of theoretical value in that lipid A, the toxic component of endotoxin, can be found disassociated from the core antigen. Thus the prospect of utilizing a drug with excellent antimicrobial activity against gram-negative pathogens and an ability to neutralize lipid A is appealing.

Polymyxin B has been used with some success in humans to decrease the signs of endotoxemia. Unfortunately, the drug is extremely neuro and nephrotoxic, thereby limiting its clinical usefulness to topical administration. Polymyxin B has been studied as a treatment in endotoxin-induced acute mastitis. Although it provided some benefit in minimizing leukopenia and liver enzyme changes, it failed to alter the clinicopathological course of the disease.[7]

To avoid toxicity, plasma-pheresis utilizing polymyxin B bound to a stationary resin has been used successfully in the treatment of experimental endotoxemia.[8] Unfortunately, the cost and logistics of this procedure prohibit its use in veterinary medicine.

Recently, investigators have attempted to conjugate polymyxin B to either dextran or ovalbumin.[9,10] It was hoped that by so doing, these large molecular weight conjugates would be retained within the vascular system, thereby avoiding toxicity while still neutralizing endotoxin. When tested in horses the dextran conjugate unfortunately produced side-effects related to increased thromboxane production.[9] At present, the ovalbumin conjugate has not been tested *in-vivo.*

Activated charcoal

Activated charcoal has an excellent reputation for adsorption of a variety of toxins. Experimentally, hemoperfusion utilizing activated charcoal has been effective in removing endotoxin.[11] Constraints similar to those of polymyxin B plasmapheresis again limit such therapy in animals. However, when the GI tract is the source of endotoxin (e.g., ruminal autointoxication), oral activated charcoal can be extremely beneficial.[12]

Anti-endotoxin antibody

Antibodies (either polyclonal or monoclonal) have been produced from all three regions of the endotoxin molecule. Antibodies to the O-antigen portion are actually protective if that specific strain of bacteria is encountered. The multitude of strains however renders this approach of limited clinical significance.

Lipid A antibodies have also been produced. While able to neutralize free lipid A, these antibodies thus far have failed to cross-react with endotoxin. Thus, in whole endotoxin, the lipid A epitopes are not expressed, or alternately, steric hindrance prevents binding to lipid A.[13]

Antibodies to the core antigen are produced in response to bacterins of gram-negative bacteria lacking the O-specific side chain. Experimentally they are quite effective *in vivo* both in passive (hyperimmune plasma) and active (toxoid) protection.[14,15] Results from clinical trials have generally been encouraging but individual cases may fail to respond. The reasons for this are probably multifactorial but one explanation is that wild strains of gram-negative bacteria are not deficient in

their synthesis of the O-specific side chain resulting in steric hindrance with immunoglobulin binding.[16] Despite this, immunization has shown a benefit in reducing the incidence and severity of endotoxemia.[17,18] While fewer reports exist to document the benefit of hyperimmune anti-endotoxin plasma transfusions in cattle, reported studies indicate that where cost and logistics are feasible, such therapy may be worthwhile.[14,19]

Antagonizing endogenous mediators and toxins

Cyclo-oxygenase inhibitors

For many years cyclo-oxygenase inhibitors (nonsteroidal anti-inflammatory drugs, NSAIDs) have been an important part of the treatment of endotoxemia. Their benefit appears due to an ability to decrease prostaglandin and thromboxane production, major mediators of endotoxemia. While a variety of NSAIDs including ibuprofen, indomethacin, salicylates, and phenylbutazone have shown a benefit in experimental endotoxemia, flunixin has received the greatest evaluation and is widely used for this purpose.

Studies in horses and cattle have shown notable improvement in clinical signs and some improvement in clinical pathological indicators of inflammation following flunixin administration in endotoxin challenge models.[20,21] Because of the risks of GI ulceration and renal ischemia (particularly in the face of dehydration), flunixin was evaluated to see if lower doses of the drug could attenuate the mediators from LPS. Studies in horses indicated that flunixin in doses as low as 0.25 mg/kg could block the increases in thromboxane, 6-keto-PGF_{1a} and blood lactate seen in control horses given intravenous endotoxin.[22] This has given rise to the use of a so called "anti-endotoxin" dose of flunixin of 0.25 mg/kg every 8 hours. Such utilization is primarily aimed at prevention of laminitis in high risk equine patients. However, low dose flunixin often fails to block many of the signs of endotoxemia (fever, tachypnea, altered capillary refill) normally attenuated by conventional dose flunixin (1 to 2 mg/kg iv or im every 8 to 12 hours) and as such, in overt endotoxemia conventional dose flunixin therapy is usually recommended.

Ketoprofen is another NSAID shown to have similar benefit to flunixin in experimental *E. coli* mastitis. In a clinical trial of naturally occurring coliform mastitis, cows receiving ketoprofen intramuscularly at 2 grams per cow per day of treatment had a significantly higher recovery rate than did the placebo treated animals.[23]

Glucocorticoids

Although glucocorticoids have been recommended to treat shock due to a variety of causes, only septic shock is felt to respond on a reasonably consistent basis. The major actions of glucocorticoids occur at protein transcription and translation where TNF synthesis is blocked and lipocortin is produced to prevent the activation of phospholipase.[24] This early interruption of mediator synthesis may explain why the steroids are one of the few agents shown to consistently have benefit in acute endotoxemia.

Selection of a steroid for use in endotoxic shock is based on rapid onset, desired duration of effects, and whether abortifacient activity is an issue. Although anecdotal reports of beneficial effects from anti-inflammatory doses of steroids exist, very large doses of water-soluble formulations are usually recommended. If cost is not an issue, then 10 - 30 mg/kg of prednisolone or methylprednisolone as either the sodium succinate or sodium phosphate formulations are often preferred. This dose may be repeated in 4 to 6 hours and generally lasts 18 to 24 hours. Alternately, a single dose of dexamethasone at 1 - 3 mg/kg can be given which is beneficial for approximately 48 hours. Another factor entering into the selection of a glucocorticoid is reproductive status. If maintenance of pregnancy is a prime concern then prednisolone will pose far less risk of abortion than dexamethasone (though abortion still may occur due to endotoxemia). Lastly, dexamethasone is more prone to worsen laminitis in horses than is prednisolone. Whether this is true in cattle has not been determined, but in cases prone to laminitis (ruminal autointoxication), prednisolone may be preferred.

Due to their immunosuppressive effects multiple doses of glucocorticoids are not recommended. Animals suffering from infectious diseases controlled primarily by cell-mediated immunity (i.e., systemic mycoses, mycobacterial infections), should not receive steroids unless absolutely necessary and appropriate antifungal/antimycobacterial therapy is instituted. To address the gram-negative infection, bactericidal antimicrobials may be preferred to bacteriostatic agents because of glucocorticoid-induced dysfunction of phagocytes. However, in E. coli-induced mastitis, dexamethasone surprisingly did not adversely influence the clearance of bacteria from challenged glands.[25]

While numerous studies in animal models have indicated improved survival in endotoxemia, the benefit of steroids in altering mortality has been debated for many years. In humans, Sprung *et. al.* reported that treatment with either methylprednisolone or dexamethasone resulted in a substantial benefit early in the course of endotoxic shock, but that eventual mortality rates were unaffected.[26] Subsequent studies have affirmed this result and as such while steroids may provide an early benefit, unless the underlying sepsis is controlled the clinical outcome may be unaffected.

Oxygen radical scavengers

The generation of toxic oxygen metabolites (e.g.,

superoxide radicals) produced during tissue injury incurred during endotoxemia has prompted studies of compounds which might either scavenge or decrease the production of oxygen radicals. Unfortunately, agents such as superoxide dismutase, vitamin E, allopurinol, and others have failed to show a consistent benefit.

Perhaps the most widely used free radical scavenger, intravenous dimethyl sulfoxide (DMSO), is the least tested for this condition. While the compound has shown a benefit in a variety of ischemic tissue injuries, its utility in endotoxemia remains speculative. Because intravenous DMSO causes vasodilation by nonspecific histamine release, worsening of hypotension is a concern. To minimize these effects, fluid loading and dilution of the DMSO to a 10% solution are prudent precautions.

Other therapies

Naloxone, an opioid antagonist, showed great promise in early endotoxic models where it improved cardiovascular status, decreased hemoconcentration, decreased acidosis, improved oxygenation, and increased survival times. However, subsequent studies and human trials gave conflicting results and the benefit of naloxone remains speculative.

Pentoxyfylline, antiproteases, antihistamines, and calcium channel blockers have been investigated as treatments in endotoxemia. While all showed benefit in experimental models of endotoxic shock, the results are either too preliminary or were not so clear cut as to warrant recommendations for use in clinical cases.

Anti-TNF antibody therapy can block many of the effects of endotoxemia and is continuing to be evaluated.

Supportive care

Antibiotics: cidal vs. static

Although many of the therapies thus far discussed will mitigate the signs of endotoxemia, the eventual recovery of the patient is dependent on ridding the body of the underlying infection. While antibiotic therapy can often be avoided in coliform mastitis due to its self-limiting nature (most gram-negative bacteria except *Pseudomonas* are tissue non-invasive), appropriate antibiotic therapy is crucial in most gram-negative diseases for cure and minimization of tissue damage.

A common discussion relative to antibiotic therapy in the face of endotoxemia is whether a bactericidal versus a bacteriostatic drug should be chosen. It has long been realized that many patients with gram-negative sepsis will initially worsen following antibiotic administration, presumably due to rapid antibiotic-induced lysis of gram-negative bacteria. Indeed, in septicemic humans free endotoxin continues to increase despite antibiotic-induced clearance of gram-negative bacteremia.[27]

This observation led to the assertion that bacteriostatic antimicrobials might be preferred in endotoxic patients. Though theoretically sound, this suggestion ignores certain confounding considerations. First, many patients suffering from gram-negative sepsis are neutropenic, a complication that will severely decrease the effectiveness of a bacteriostatic antibiotic. Second, the supposed benefit of a static over a cidal agent remains largely unproven as there are no studies comparing their effects using antibiotics likely to be administered in large animal medicine. The few reports that have been published concentrated on the bactericidal agents used in human intensive care scenarios such as ciprofloxacin, aminoglycosides, imipenem, and third-generation cephalosporins. The only bacteriostatic agent studied was chloramphenicol. While one study indicated that during chloramphenicol exposure, free endotoxin correlated well with the number of viable bacteria (supporting the cidal lysis theory), other studies showed that chloramphenicol dramatically increased free endotoxin in excess of the viable bacteria.[28,29,30] This latter result suggests that even bacteriostatic antibiotics can induce shedding of endotoxin. One important finding was that use of an antibiotic that failed to inhibit replication of the bacteria resulted in greater free endotoxin concentrations than did effective bactericidal therapy.[21] Thus, while selection of a static drug might be ideal, from a practical view point selecting an agent with a high probability of sensitivity and clinical efficacy remains paramount, even if the agent is bactericidal.

A significant toxicity that should be kept in mind is the synergistic nephrotoxicity seen with tetracycline administration in the face of endotoxemia. The tetracyclines are normally only mildly nephrotoxic; however, when administered in high doses (i.e., 20 mg/kg) during endotoxemia, severe nephrosis has been reported both experimentally and clinically.[31]

Fluid therapy

That intravascular volume support is imperative in endotoxic shock is accepted. This is usually accomplished with isotonic electrolyte solutions, hypertonic saline, or plasma transfusions.

Isotonic electrolyte intravenous (iv) infusions have been the mainstay of fluid therapy in all species for years. A variety of formulations can be used including isotonic saline or multielectrolyte solutions, though the latter is often preferred as electrolyte imbalances and metabolic acidosis are common complications in endotoxic shock. When glucose supplementation is required, dextrose should be added to these solutions rather than used alone (D5W) since the majority of the dextrose (and hence the accompanying water) is redistributed to the intracellular space thereby making D5W an ineffective

volume expander. Large volumes (40-60 liters per day) of iv fluids are commonly required in adult cattle often creating economic and logistic problems for this form of therapy. Although ineffective in overt shock, oral fluid therapy can be used in certain cases to supplement intravenous fluid administration.

Hypertonic saline (7.2% NaCl) administered intravenously at 4 to 5 ml/kg over 3 to 10 minutes has a number of advantages over isotonic fluid therapy. By virtue of its high osmolarity, it draws fluid from the interstitium into the vascular space; thereby rapidly restoring cardiac output and vascular volume. This fact, along with its low volume and cost make it both appealing and appropriate in the management of peracute and acute hemorrhagic or septic shock. It must be remembered however that the positive hemodynamic effects generally last less than 2 hours.[32] As such, further support in the form of either intravenous (preferred) or oral fluid therapy (if voluntary water consumption is inadequate) must follow hypertonic saline treatment. Relative contraindications to the use of hypertonic saline treatment would include moderate to severe dehydration, hypernatremia, hyperosmolarity, hypokalemia, or cardiogenic shock.

Anti-endotoxin hyperimmune plasma is a viable treatment to reduce concentrations of circulation endotoxin and has been previously discussed. However, the routine use of plasma as a resuscitative fluid is troublesome in the cow due to difficulties in blood collection, plasma separation, and cross-matching. Despite this fact, plasma transfusions should be considered in cases associated with hypoproteinemia and capillary leakage with pulmonary or peripheral edema. Because plasma stays within the vasculature, the amount required is generally about 1/3 of the equivalent crystalloid solution that would have been administered. When hypocoagulable DIC is encountered, fresh plasma incubated with heparin (5 to 10 units/kg) for 30 minutes prior to administration will provide depleted antithrombin III.[33]

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