Targeting blood cell activation and clotting dysfunction in equine endotoxaemia

Simon Bailey and Jennifer Bauquier, Faculty of Veterinary Science, University of Melbourne

Background and aims

Bacterial endotoxaemia results most commonly from gastrointestinal disorders such as colic and colitis (it can also occur with other conditions such as post-foaling metritis in mares) and contributes significantly to the morbidity and mortality of these conditions.1,2 Bacterial toxins enter the bloodstream most commonly from the intestines, causing systemic inflammation.

Non-steroidal anti-inflammatory drugs (NSAIDs) are currently the mainstay of anti-inflammatory treatment in horses with endotoxaemia (along with fluid therapy, plasma transfusions etc). Although NSAID drugs improve some of the clinical signs of inflammation, they are not very effective in preventing activation of leukocytes and the production of pro-inflammatory cytokines.3 Addressing this aspect may be beneficial in reducing tissue damage, organ dysfunction and improving morbidity and mortality rates. (Figure 1).4

![Diagram showing how endotoxin (originating from the intestine or some other septic focus) activates leukocytes and platelets in the blood to cause tissue damage and clotting dysfunction. Pro-inflammatory cytokines and prostanoid mediators play an important role in this process, but non-steroidal anti-inflammatory drugs (NSAIDs; such as flunixin) have very little effect on cytokine release. Novel drugs act through different pathways to target leukocyte activation and cytokine release.](image-url)

Figure 1. Diagram showing how endotoxin (originating from the intestine or some other septic focus) activities leukocytes and platelets in the blood to cause tissue damage and clotting dysfunction. Pro-inflammatory cytokines and prostanoid mediators play an important role in this process, but non-steroidal anti-inflammatory drugs (NSAIDs; such as flunixin) have very little effect on cytokine release. Novel drugs act through different pathways to target leukocyte activation and cytokine release.
Associate Professor Simon Bailey has for many years been investigating novel anti-inflammatory drugs that may be effective alongside NSAIDs by reducing pro-inflammatory cytokine production, leukocyte activation and clotting dysfunction. A promising drug has been investigated in other species but not previously examined in horses. The name of the compound cannot be published at the present time because of commercial and patenting considerations.

The objectives of this project were firstly to investigate the anti-inflammatory effects of the anti-endotoxic drug on the inflammatory effects of endotoxin on equine blood cells in vitro. This involved laboratory assays where the drug was added to equine blood cells which were then stimulated with endotoxin. Then the anti-inflammatory effects of the drug was determined in a low-dose endotoxaemia model in experimental horses. The effects of this agent on blood clotting dysfunction was also examined.

Summary of methods and results

Effect of candidate drug on cytokine production in vitro
Firstly, a whole blood assay was used to assess the effects of the drug on cytokine production from leukocytes (white blood cells) in response to bacterial lipopolysaccharide (endotoxin). In these whole blood stimulation assays, the compound caused a concentration-dependant inhibition of TNFα production, with 100% inhibition at high concentrations.

Preliminary safety/tolerance study in horses
The test compound was given to six Standardbred horses and clinical parameters (heart rate, respiratory rate, rectal temperature, demeanour, mucous membrane colour and capillary refill time, auscultation of gastrointestinal sounds, and indirect blood pressure) and white blood cell counts were monitored over 24 hours. No adverse effects were observed, and all clinical parameters remained unchanged and completely normal in all horses.

Anti-inflammatory effects of the compound in vivo: low-dose endotoxin challenge model
The major phase of the study was to test the candidate drug in an experimental model to stimulate some of the inflammatory changes seen in horses with naturally-occurring clinical disease. This well characterised model of endotoxaemia induces mild signs of malaise similar to transient ‘flu-like’ symptoms which resolve over 4–5 hours.

Standardbred horses were used for the study, which was conducted under the permission of the Animal Ethics Committee of the University of Melbourne. The test compound was administered intravenously immediately prior to the endotoxin infusion. This was a placebo-controlled, blinded study.

The low-dose endotoxin challenge was conducted as previously described. Clinical outcomes including rectal temperature, heart rate, respiratory rate, intestinal sounds and demeanour were recorded immediately prior to pre-treatment and before lipopolysaccharide infusion, then every 15–30 min. The low-dose endotoxin challenge was well tolerated by all horses, and produced mild ‘flu-like’ symptoms which were only transient. However, measureable changes in clinical signs were observable, and also further objective evidence of systemic inflammation and leukocyte activation, which could be used to assess the anti-inflammatory effects of the drug compound.

Clinical signs
The administration of lipopolysaccharide to normal horses caused a transient increase in heart rate which was significantly reduced by the drug treatment. Rectal temperature was also significantly reduced in the treated group compared to control values.

Endotoxin administration also caused a significant increase in both systolic and diastolic blood pressure. However, following drug treatment, there was no longer a significant increase in blood pressure.
**Leukocyte activation and cytokine production**

Endotoxin caused a transient decrease in the blood white cell count, due to cell activation and adherence to the endothelium. This effect was significantly reduced by drug treatment. The compound also significantly and markedly reduced the sharp spike in pro-inflammatory cytokine levels caused by the endotoxin.

**Effect of the candidate drug on clotting dysfunction**

In addition to measuring prothrombin time (extrinsic clotting pathway) and activated partial thromboplastin time (APTT; intrinsic and common coagulation pathways) a technique called thromboelastography was used. This evaluates the overall clotting function, including fibrin-platelet interaction, the strength of the clot and rate of clot lysis. LPS (endotoxin) induced a hypercoagulable state, similar to that observed in clinical cases of endotoxaemia. This was shown by the reduction in the reaction time, which was the time from start of the analysis to the initiation of clot formation induced by kaolin. The kaolin clotting time is equivalent to the APTT, and represents intrinsic and common coagulation pathways. The candidate drug compound significantly reduced the hypercoagulable effect of LPS as seen by a shortened reaction time.

**Conclusions**

This study has identified a drug which effectively inhibits inflammatory cell activation and cytokine production in experimental horses in response to an endotoxin challenge. This drug has the potential to be a very useful addition to the current therapies available for the treatment of colic and endotoxaemia.

The drug demonstrated excellent anti-inflammatory effects in this model – clinical impressions were that the inflammatory signs of endotoxaemia (increased heart rate, muscle tremors, reduced gut sounds, increased rectal temperature) were obviously blunted or almost completely abolished when the drug had been administered. This was confirmed by the fact that white blood cell activation (TNF-α production and cell margination out of the blood circulation) was significantly reduced. The compound also significantly reduced the hypercoagulable effect of LPS.

**Implications**

This novel compound may potentially be very useful for the treatment of this common and life-threatening condition, improving clinical outcomes for many thousands of horses with colic and colitis. A reduction in the morbidity and mortality associated with colic and other diseases where endotoxaemia results will be extremely important, because in the developed world, colic is a leading cause of euthanasia in horses, making it a highly significant welfare issue and cause of financial loss to the equine industry.

As mentioned, currently used therapies such as non-steroidal anti-inflammatory drugs only inhibit certain parts of the inflammatory pathway. By inhibiting at a higher level in the pathway, drugs like the current compound should have a much more profound anti-inflammatory effect and block the production of more of the inflammatory mediators that amplify the inflammatory process and lead to tissue and organ damage.

Therefore this drug may present significant advantages for the treatment of clinical endotoxaemia, and have a synergistic effect when combined with current therapies.

Because of the transient and mild nature of the inflammatory endotoxaemia model used in this study, the anti-inflammatory drugs tested have to be given at time zero just before the endotoxin infusion begins. This is clearly different to the situation with the naturally occurring disease, where animals present when the signs are already established. Therefore these results will need to be confirmed in naturally occurring cases. A clinical trial is currently being planned; the investigators have now successfully filed a provisional patent on this compound and discussions with a pharmaceutical company partner are ongoing.
References


About the Authors

Simon Bailey is an Associate Professor at the University of Melbourne, Faculty of Veterinary Science. In addition to teaching, his research involves inflammation, novel anti-inflammatory therapies (in horses and other large animal models) and endocrinology. He is a diplomat of the European College of Pharmacology and Toxicology, and has many years of experience researching the mechanisms of equine endotoxaemia and evaluating anti-inflammatory therapies. He moved from the Royal Veterinary College, London, to the University of Melbourne in 2007, and is currently an Associate Editor of The Veterinary Journal, Domestic Animal Endocrinology and the Australian Veterinary Journal.

Dr. Jennifer Bauquier is a recipient of an Australian Postgraduate Award (APA) PhD scholarship from the Australian Government. She is currently undertaking PhD studies supervised by Simon Bailey, investigating treatments for endotoxaemia in horses. Dr Bauquier returned to Australia after working in the USA for several years, including three years of residency training in the specialty of large animal internal medicine at the University of Pennsylvania’s New Bolton Center. She is a diplomat of the American College of Veterinary Internal Medicine, and later this year she will take up a lectureship in equine medicine at the University of Melbourne Equine Hospital.

Researcher Contact Details

Principal Investigator
• A/Prof Simon Bailey, BVMS PhD FHEA DipECVPT MRCVS
  Faculty of Veterinary Science
  University of Melbourne VIC 3030
  Phone: 03 8344 6315
  Fax: 03 8344 7374
  Email: bais@unimelb.edu.au

RIRDC Project PRJ-007906
Pub. No. 14/078