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Author/s: Martin Combs

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A novel therapeutic for perennial ryegrass toxicosis

Martin Combs
Charles Sturt University
School of Animal and Veterinary Sciences, Charles Sturt University,
Wagga Wagga, New South Wales, Australia, 2560
A Hamlin, S Edwards, J Quinn

Introduction

Perennial Ryegrass Toxicosis (PRGT) is a clinical syndrome of herbivores in southern regions of Australia and New Zealand grazing pasture with a high proportion of perennial ryegrass. To date no clinically applicable therapeutic has been available to treat clinical cases of PRGT or to prevent disease. PRGT is a complex toxicity with multiple alkaloids involved. Disease presentations range from subclinical productivity losses to a severe neurological syndrome with ataxia, tremor, recumbency and occasionally death. Lolitrem B, the primary toxin responsible for neurological signs, is thought to block calcium activated potassium channels (BK Channels). In the brain this will have a number of effects; generally it will increase neuronal instability. In the cerebellum however the overall effect is to reduce neuronal outputs. As the cerebellum is involved in regulating movement the effect of intoxication is for movement to become less regulated and more exaggerated (cerebellar ataxia) or the classical "ryegrass staggers" presentation. This presentation considers the use of bromide, an accepted therapy for epilepsy in small animals and humans, as a treatment for PRGT and evaluates therapeutic efficacy within murine and ovine models of Perennial Ryegrass Toxicosis (PRGT). Trials demonstrate that bromide is effective at reducing lolitrem B induced tremor and ataxia. Because of its limited side effects, high oral bioavailability, high safety margin and low cost, bromide is a good potential on-farm therapy for PRGT.

What's the problem?

Epichloë festucae var. lolii is a symbiotic fungus of perennial ryegrass. Under certain environmental conditions *Epichloë festucae var. lolii* will produce a wide variety of alkaloids that generate a complex toxicity in grazing animals, known as perennial ryegrass toxicosis (PRGT). Lolitrem B, a diterpenoid alkaloid, is a key toxin producing neurological signs (ryegrass staggers) along with vasoconstriction and gastrointestinal dysfunction [1, 2].

Models and Therapeutic Testing

A problem with studying potential therapeutics for PRGT is the intermittent nature of clinical disease and a high level of variation in clinical expression making clinical testing in the field almost impossible, particularly if you wish to screen a series of drugs in animals with a similar clinical presentation. To address this, two disease models were developed by a team of neurophysiologists and clinicians at Charles Sturt University. In the first model mice were exposed to purified lolitrem B and then measurements were made using a series a behavioural and neurological parameters (specifically tremor and ataxia) [3]. Neuronal activation was also investigated in the forebrain using the immediate early gene cFos as a biomarker. This murine model was used to screen candidate drugs.

Out of the series of therapeutics tested in the murine model, bromide was the most effective at reducing tremor and improving movement after exposure to lolitrem B. Bromide was also able to demonstrate reduced neuronal activation of the central amygdala.

The ovine model was used to test the primary candidate drug coming from murine testing, namely bromide. The ovine model utilised a novel endophyte variety (Ga66 AR98; Grasslanz Technology Ltd, Palmerston North, NZ) that produces a high level of lolitrem B toxin and relatively low level of ergot-alkaloid. By using this toxin we were able to produce a disease model where the neurological effects predominated over effects on peripheral vasculature, thereby better mimicking naturally occurring disease [4]. Sheep were then monitored for a series of neurological and physiological parameters. As onset and progression of clinical signs was highly variable between animals bromide was administered when clinically relevant neurological signs developed rather than at a set timepoint [5].

Results in sheep demonstrated a dramatic improvement in ataxia following bromide therapy and a relative reduction in tremor compared to control animals at the same clinical stage. Neuropathological improvements were not demonstrated with bromide therapy in sheep although the study protocol did not allow similar neuronal activation c-Fos studies as occurred in mice.

Part of the Solution?

Pharmacokinetic studies have demonstrated that bromide is readily absorbed orally, is well tolerated, with a high safety margin. Its low cost and ability to be administered via a wide range of modalities make it an excellent option as an on farm therapeutic [6].

Future Directions

Bromide has demonstrated therapeutic efficacy in a disease model, the next critical stage is to develop clinically applicable therapeutic regimens for the field. These regimens are likely to vary for acutely affected ambulatory and recumbent animals and as a prophylactic to at-risk animals as well as prior to husbandry activities. Studies should include consideration of a broad range of production variables and therapeutic modalities.

References

1. McLeay, L. and B. Smith, *Effects of the mycotoxins lolitrem B and paxilline on gastrointestinal smooth muscle, the cardiovascular and respiratory systems, and temperature in sheep.* New Zealand Grassland Association, Research and Practice series, 1999(7): p. 11-17.
2. Reddy, P., et al., *Tremorgenic effects and functional metabolomics analysis of lolitrem B and its biosynthetic intermediates.* Scientific Reports, 2019. **9**(1): p. 9364.
3. Combs, M., A. Hamlin, and J. Quinn, *A single exposure to the tremorgenic mycotoxin lolitrem B inhibits voluntary motor activity and spatial orientation but not spatial learning or memory in mice.* Toxicon, 2019. **168**: p. 58-66.
4. Combs, M., et al., *Development of a model for investigation of perennial ryegrass toxicosis in sheep.* New Zealand Veterinary Journal, 2018. **66**(6): p. 281-289.
5. Combs, M.D., et al., *Treatment with potassium bromide mitigates ataxia and reduces tremor in lambs with perennial ryegrass toxicosis.* New Zealand Veterinary Journal, 2019: p. 1-22.
6. Quast, T., M. Combs, and S. Edwards, *Pharmacokinetics of bromide in adult sheep following oral and intravenous administration.* Australian Veterinary Journal, 2015. **93**(1-2): p. 20-25.