Efficacy of a Human TNF-alpha Inhibitor in Horses, a Preliminary Study

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Background

• Following an acute injury or in some cases with repetitive loading of a joint, the inflammatory cascade is initiated.[1]

•Pro-inflammatory cytokines, such as TNFα, drive the catabolic processes that can ultimately lead to cartilage destruction if left unchecked. Permanent damage to the cartilage occurs as a result of TNFα induced suppression of proteoglycan and collagen synthesis by chondrocytes as well as increased production of matrix degrading metalloproteinases (MMPs).

•The inhibition of TNF-alpha, through the use of "Biologics" has proved to be an effective means to reduce clinical signs of inflammation due to various types of arthritis in humans.[2]

•Current anti-inflammatory drugs (corticosteroids and Non Steroidal Anti-Inflammatories) used in horses can possess considerable side effects.

Research Goals

1. Conduct a pilot study to characterize the *in vivo* effects of an FDA approved human TNF-alpha inhibitor fusion protein in an experimental model of joint inflammation (synovitis) in horses.

2. Characterize the effects of an FDA approved human TNF-alpha inhibitor fusion protein on concentrations of inflammatory cytokines in an experimental model of joint inflammation (synovitis) in horses.

Methods

•Animals: 4 exercised Thoroughbred research horses (4-7 yrs)
•Synovial inflammation was induced by intra-articular (IA; right antebrachiocarpal joint) injection of 100 ng lipopolysaccharide
•Horses were randomly assigned to receive 25 mg of Etanercept (Enbrel, Amgen, Thousand Oaks, CA) or 0.9% saline IA 2 hours post LPS
•Blood samples were collected prior to drug administration and at various times until 10 days post drug administration
•Synovial fluid samples were collected from the right and left antebrachiocarpal joint after LPS administration and immediately prior to drug administration and on days 4, 7 and 10 for determination of:
- •Drug concentration
- •Concentrations of pro- and anti-inflammatory biomarkers

•Pharmacodynamic assessments on day 0 (prior to LPS), 2 hours and on days 4, 7 and 10 post drug administration included:
- Lameness evaluations (AAEP lameness scoring system)
- Pain free range of motion using a goniometer
- Joint circumference

•Determination of biomarker concentrations conducted using commercially available immunoassays.

Results

- No systemic or local adverse effects were noted following Intra-articular Enbrel administration

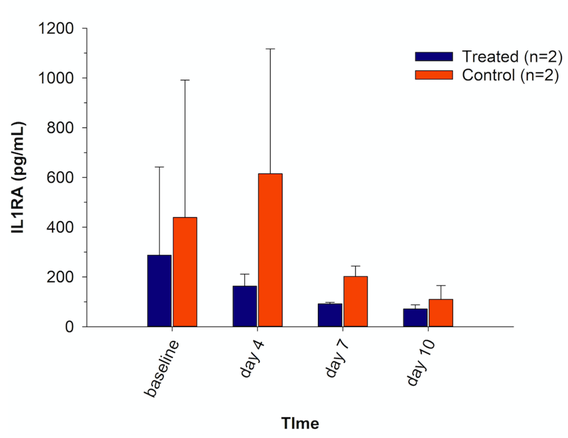
Joint Evaluation (Right Antebrachiocarpal Joint):

	Group	Lameness Score	Joint Circumference (cm)	Range of Motion (degrees)
Day 0	Treated	0.5 ± 0.71	12.1 ± 0.53	143 ± 8.9
	Control	1 ± 1.41	13 ± 0	149.6 ± 0
2 hours	Treated	0.5 ± 0.71	12.1 ± 0.88	148 ± 3.8
	Control	2.25 ± 0.35	13 ± 0	141.5 ± 1.12
Day 4	Treated	1 ± 0	12.5 ± 0.71	147.1 ± 11.1
	Control	0.5 ± 0.71	13.4 ± 0.18	146.3 ± 1.88
Day 7	Treated	0.5 ± 0.71	12.3 ± 0.35	144.5 ± 12.0
	Control	0.5 ± 0.71	13.25 ± 0	145 ± 2.82
Day 10	Treated	0	12.1 ± 0.53	146.3 ± 9.9
	Control	0.5 ± 0.71	13.25 ± 0.35	139 ± 3.77

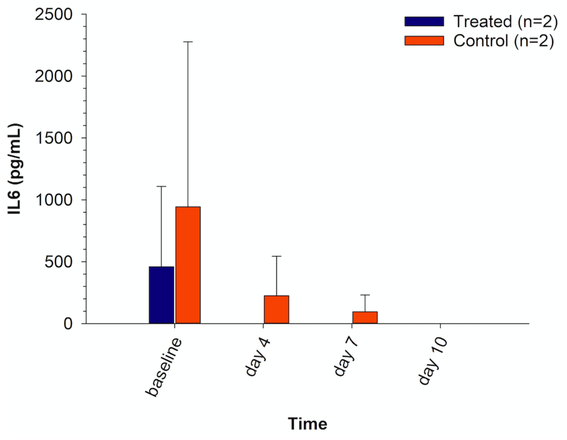
Day 0, prior to LPS administration; day 2, prior to drug administration

Concentrations of Cytokines

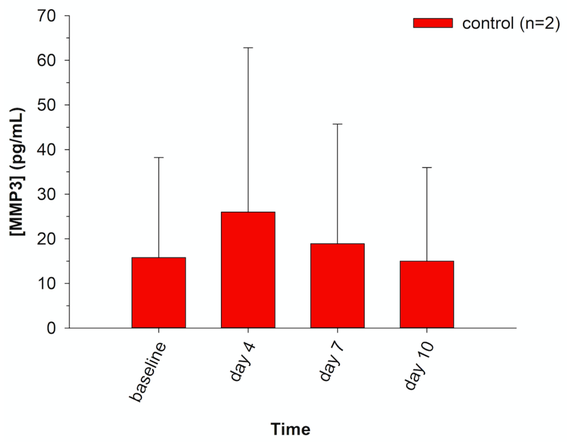
IL1-RA



IL-6



MMP-3



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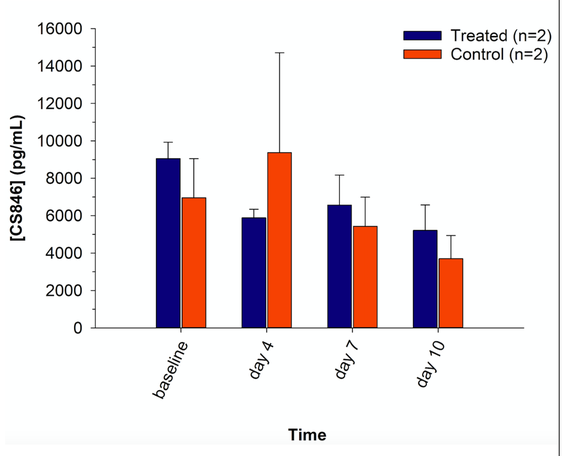

TNF-Alpha

***In Progress*

Drug Concentration Determination and Pharmacokinetics:

- Plasma and synovial fluid concentration determination by liquid chromatography-tandem mass spectrometry is ongoing

Limitations, Conclusions and Future Directions

Limitations:
- Limited number of horses
- Single dose
- Human TNF alpha specific drug

Conclusions:
- Etanercept (Enbrel) was well tolerated following intra-articular administration
- Anti-inflammatory effects are inconclusive and further study is necessary
- Drug concentration determination is ongoing

Future Directions:
- Assessment of anti-inflammatory effects of an equine specific anti-TNF alpha biologic

References

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2. Mitoma, H., Horiuchi, T., Tsukamoto, H., Ueda, N. (2016) Molecular mechanism of action of anti-TNF-alpha agents-comparison among therapeutic TNF-alpha antagonists. *Cytokine*, pii: S1043-4666.

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