**INTRODUCTION**

- Inflammation plays a critical role in painful equine orthopedic disorders.
- Prostaglandin E2 (PGE2) is a potent mediator in inflammatory disease.
- NSAIDs (ibuprofen, aspirin, etc.) inhibit PGE2 by targeting COX enzymes, but cause negative side effects due to non-selective prostanooid blockade.
- Horses are especially sensitive to these side effects, limiting NSAID use.

Thus, more PGE2-specific therapeutic targets are needed in horses.

- Prostaglandin E-synthases (PGES) act downstream of COX in the prostaglandin synthesis pathway.
- Isoforms of PGES differentially mediate basal and induced PGE2 synthesis:
  - Microsomal PGES-1 (mPGES-1): induced PGE2 synthesis in response to inflammatory stimuli.
  - Cytosolic PGES (cPGES): basal, homoeostatic PGE2.
- mPGES-1 inhibition decreases PGE2 and minimizes adverse side effects associated with non-selective targeting in rodent models.
- mPGES-1 is poorly characterized in circulating leukocytes, major effector cells of inflammation, and has not been investigated in horses for anti-inflammatory targeting.

Our goal was to investigate mPGES-1 as an anti-inflammatory target in equine leukocytes using an ex vivo inflammatory model.

**HYPOTHESIS**

mPGES-1 expression is induced by lipopolysaccharide (LPS) and is essential to the induction of PGE2 synthesis in equine leukocytes. We predict that inhibition of mPGES-1 will selectively reduce PGE2 production in these cells when compared to COX-2-selective or non-selective COX inhibitors.

**METHODS**

Leukocyte-rich plasma (LRP) was harvested from equine white blood, and leukocytes were used for the following experiments:

**Ex Vivo Inflammatory Model:**

1. **GM-CSF** (Protein)  
   - Cells were incubated with 1ng/mL GM-CSF for 30 minutes followed by stimulation with 100ng/mL LPS for the indicated periods.
2. **LPS Stimulation**  
   - Real-time PCR: mRNA was isolated from cells and first-strand cDNA synthesis was performed. Real-time PCR was performed with 10ng cDNA and gene-specific Taqman primers and probes (Invitrogen) for equine mPGES-1, COX-1, COX-2, and β-Actin. Data analysis was performed using the 2^-ΔΔCT method with β-Actin used for normalization.
3. **Cell Lysis and Western Blot**  
   - Cells were lysed in RIPA buffer and supernatants containing protein were subjected to SDS-PAGE. Protein was transferred to PVDF membranes, blocked using 5% milk, and incubated with appropriate primary and HRP-conjugated secondary antibodies. Membranes were incubated in ECL substrate and visualized using ChemiDoc-MP image system. Image Lab software was used for normalization to β-Actin and for protein quantification.

**Inhibitor Studies and ELISA**  
- Following priming, LPS was added to cells simultaneously with various concentrations of inhibitors of mPGES-1 (MF-63, COX-2 inhibitors), and a non-selective COX inhibitor (Indomethacin). Cells were collected at 37°C for various time points and PGE2 levels in the supernatants were measured in cell supernatants via a competitive radioligand assay.

**REFERENCES**


**RESULTS**

**Graph A:** GM-CSF priming followed by LPS stimulation induces mPGES-1 and COX-2 mRNA production in equine leukocytes; cPGES and COX-1 mRNA expression is constitutive.

**Graph B:** mPGES-1 protein is constitutively expressed in equine leukocytes.

**Graph C:** mPGES-1 selectively decreases PGE2 production in equine leukocytes.

**Graph D:** mPGES-1 mRNA is induced by LPS stimulation following GM-CSF priming, with maximal induction at 2 hours. cPGES mRNA expression is constitutive.

**Graph E:** mPGES-1 protein is constitutively present and is not induced by LPS stimulation.

**Graph F:** mPGES-1 inhibition with MF-63 leads to significant decrease in PGE2 levels with an IC50 of 0.1125M.

**Graph G:** mPGES-1 inhibition is specific to PGE2, as other eicosanoids (TXA2 and PGI2) were not affected by mPGES-1 inhibition.

**CONCLUSIONS & SIGNIFICANCE**

- mPGES-1 mRNA is induced by LPS stimulation following GM-CSF priming, with maximal induction at 2 hours. cPGES mRNA expression is constitutive.

- mPGES-1 protein is constitutively present and is not induced by LPS stimulation.

- mPGES-1 inhibition with MF-63 leads to significant decrease in PGE2 levels with an IC50 of 0.1125M.

- mPGES-1 inhibition is specific to PGE2, as other eicosanoids (TXA2 and PGI2) were not affected by mPGES-1 inhibition.

**REFERENCES**


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