INTRODUCTION

- Glucocorticoids (GC): pleiotropic steroid hormones extensively involved in the regulation of development, metabolism and immune function.
- Corticosteroids (CS): synthetic GCs that possess potent anti-inflammatory properties (e.g. MPL: methylprednisolone).

- Two major mechanisms of CS action:
  1) Transrepression: interaction with transcription factors NFκB and AP-1, inhibit the upregulation of their pro-inflammatory target genes.
  2) Transactivation: direct regulation of transcription of certain genes.
- Glucocorticoid-induced leucine zipper (GILZ): gene that is transactivated by CS; possible mediator of GC-induced anti-inflammation.
- Septic shock: severe systemic inflammatory response to infection; major threat to human health.
- Lipopolysaccharide (LPS): endotoxin administration to rats: model for systemic inflammation; produces a rapid induction of cytokines such as TNF-α and IL-1β.

Previous work demonstrates that treating CS either simultaneously or after administration to rats is ineffective.

HYPOTHESIS:

Enhancement of GILZ by CS in LPS treated animals is suppressed by rapid induction of cytokines. This hypothesis can be tested by assessing the time course of GILZ expression in LPS treated v. non-treated animals.

OBJECTIVES/QUESTIONS ADDRESSED:

1) Develop a highly quantitative TaqMan-based qRTPCR assay according to MIQE standards for GILZ mRNA measurement.
2) In which tissues is GILZ expressed, and are its levels of expression same/different across these tissues?
3) Does endogenous GILZ production show a circadian rhythm of expression in vivo?
4) What are the pharmacodynamics of GILZ expression in response to MPL in lung?
5) Does systemic inflammation alter GILZ expression in lung upon simultaneous treatment with MPL and LPS?

METHODS

I. Animal Experiments

Circadian baseline study MPL-treatment study MPL+LPS study

II. Development of real-time qPCR assay

Aim: To design TaqMan based qRTPCR assay


designed.

TOPO cloning

Dilute cRNAs as standards

One step qPCR

Data analysis

Probes and primers used

Final assay conditions

<table>
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<th>Description</th>
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RESULTS

I. Varying levels of GILZ expression was found across all tissues measured, with maximal expression in lung

II. GILZ mRNA expression in vivo follows a distinct circadian oscillation i.e. entrained to endogenous corticosterone (CST) production

III. GILZ pharmacodynamics in response to MPL: expression in lung is strongly upregulated with peak expression at 6h after injection.

IV. GILZ mRNA expression is NOT suppressed by LPS in the acute inflammation model in lung

CONCLUSION

- The results of this study did not support our hypothesis that GILZ expression is suppressed by LPS.
- Classical dogma contends that the anti-inflammatory effects of CSs are mediated predominantly via transrepression, while its side-effects are caused by transactivation mechanisms. Hence, development of newer "dissociated" CSs which show improved transrepression over transactivation may be beneficial. However, our results show that CS clearly enhance GILZ expression in vivo. Therefore, novel CS agonists which fail to transactivate genes like GILZ may lack important anti-inflammatory properties.

ACKNOWLEDGEMENTS

This work was supported by NIH Grant GM24211.