SUBCUTANEOUS BOLUS ADMINISTRATION OF META-CRESOL DECREASES INSULIN ABSORPTION IN A SWINE MODEL

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Introduction

- Continuous subcutaneous insulin infusion (CSII) has substantial clinical benefit for Type 1 diabetes management.
- A significant shortcoming of this therapy is *in vivo* device wear time; specifically the abbreviated two to three day wear time of the implanted insulin infusion set [1].
- *In vivo* infusion set dwell time has been shown to correlate to downstream tissue effects such as lipohypertrophy and local site irritation, as well as functional effects like altered insulin pharmacokinetics (PK), and loss of glycemic control [2-6].
- Previous clinical literature has implicated insulin formulation phenolic excipients, phenol and meta-cresol, in inducing inflammation at infusion sites [7].
- Phenol and *m*-cresol have been previously shown to induce rapid and dose-dependent local subcutaneous (SC) inflammation in a large animal model, but it is unclear if these effects alter SC insulin absorption [8].
- LPS-induced inflammation has been shown to alter SC insulin absorption in swine [9].
- **The goals of this study are:**
  - **(1) Assess the effects of bolus SC cresol treatment on insulin absorption.**
  - **(2) Correlate possible changes in insulin absorption to cresol-induced local SC inflammatory response.**

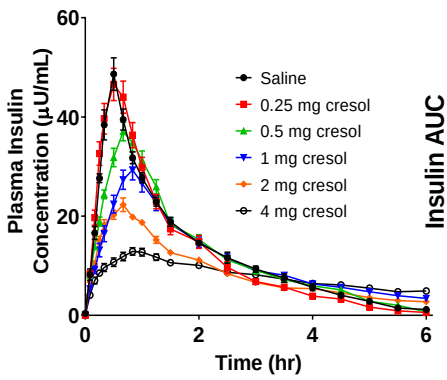
Insulin PK Methodology

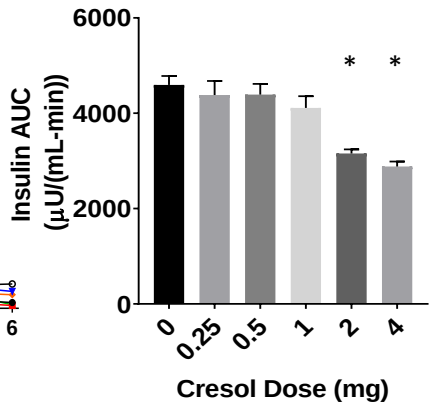
- Non-diabetic female Sinclair swine (n≥5; full crossover design) were given bolus SC injections of two different treatments in varying doses:
  - *m-cresol* – Various doses in 200 µL PBS ; representative excipient dose.
    - 0.25 mg
    - 0.5 mg
    - 1 mg
    - 2 mg
    - 4 mg
  - *0.9% saline* - 200 µL; vehicle dose treatment.
- Twenty-four hours following treatment dosing, an insulin PK study was performed by subcutaneously injecting 4 U of U100 insulin lispro (Humalog, Eli Lilly & Co.; Indianapolis, IN) into the treated site. Blood samples were taken periodically for six hours following insulin injection, serum separated, and frozen at -20°C for analysis.
- Serum samples were analyzed for insulin lispro content via radioimmunoassay using a Perkin Elmer 2470 automatic gamma counter.
- PK metrics (AUC, $AUC_{0\rightarrow60}$, C_{max}) were calculated from the raw plasma insulin lispro data using MATLAB (The MathWorks; Natick, MA) and compared across treatments using appropriate parametric or non-parametric tests.

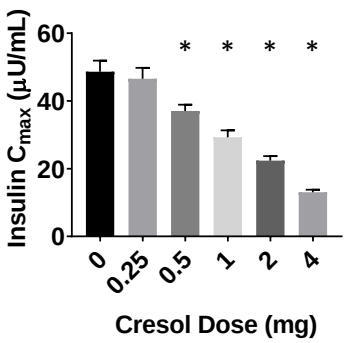
In Vivo Swine IL-6 Assay

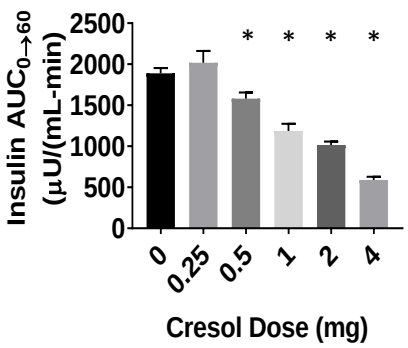
- At the end of the PK study, treated sites were biopsied to assay for the pro-inflammatory cytokine, IL-6.
- Biopsies were homogenized with Tissue Protein Extraction Reagent (T-PER, ThermoFisher Scientific; Rockford, IL) supplemented with HALT protease inhibitor at a ratio of 1:20 w/v of tissue to T-PER reagent.
- Processed biopsy samples were then assayed for the pro-inflammatory cytokine IL-6 (Abcam; Cambridge, MA).
- IL-6 levels were correlated to PK metrics (AUC, $AUC_{0\rightarrow60}$, C_{max}) by calculating the nonparametric Spearman rank correlation coefficient. Correlations were then tested for statistical significance ($p<0.05$).

Effects of Cresol Dose on Subcutaneous Insulin PK



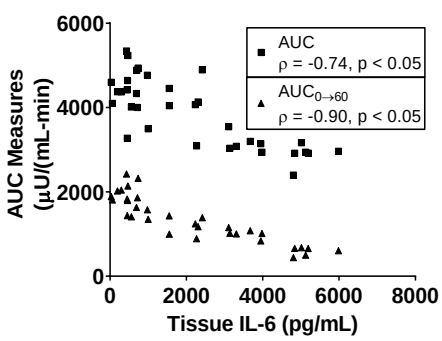


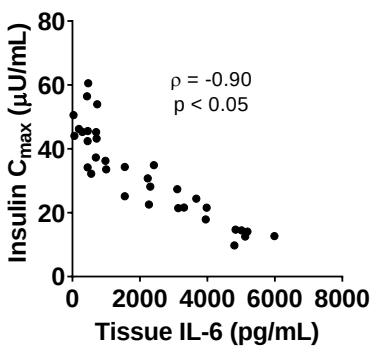




Cresol-induced tissue effects ***significantly altered*** the following PK metrics relative to the saline control ($p<0.05$).

- **AUC (≥ 2 mg cresol)**
- **$AUC_{0\rightarrow60}$ (≥ 0.5 mg cresol)**
- **C_{max} (≥ 0.5 mg cresol)**





Insulin PK metrics (AUC, $AUC_{0\rightarrow60}$, C_{max}) have a ***statistically significant negative correlation*** to subcutaneous inflammatory IL-6 levels.

n≥5; mean±SEM; $p<0.05$ denoted between groups

Conclusions

- Insulin formulation excipients, *m*-cresol and phenol, have been demonstrated to decrease insulin absorption in a ***dose dependent*** manner.
- Decreases in insulin absorption metrics had a statistically significant negative correlation to increases in local pro-inflammatory IL-6 levels.
- Findings suggest local inflammation could affect insulin delivery and downstream glycemic control.
- Future studies should investigate the effect of basal infusion rates on excipient-mediated changes in subcutaneous insulin absorption.

References and Acknowledgements

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