2024 CMBEC46
Toronto, ON
May 28-30, 2024
CMBES/SCGB

Examining the Effect of Supraphysiological Insulin in an *In Vitro* Insulin Infusion Cannula Host Response Model

GLM Riddell[1], K Gee[2] and LE Fitzpatrick[1,2]

[1] Department of Chemical Engineering, Queen's University, Kingston, Canada
[2] Department of Biomedical and Molecular Sciences, Queen's University, Kingston, Canada

I. Introduction

Continuous subcutaneous insulin infusion (CSII), also known as "pump therapy", is a widely used insulin replacement strategy for individuals with type 1 diabetes. However, despite advancements made in insulin pump design, the insulin infusion set (IIS) portion of the pump remains a significant limitation [1]. Currently, IIS cannulas have a limited wear time of 2-3 days, after which insulin delivery and glycemic control become impaired, placing individuals at risk for clinical hyperglycemia and diabetic ketoacidosis [2]. This limitation is often attributed to an acute inflammatory host response to the IIS cannula, which is largely carried out by macrophages that interact with the cannula surface and its adsorbed protein layer. Additionally, emerging research highlights a potential role for the insulin molecule itself in causing changes to local tissue surrounding the implant site [3]. However, the exact mechanisms that contribute to IIS related pump failure remain unknown. We hypothesize that the supraphysiological concentrations of insulin present at the infusion site exacerbate the pro-inflammatory macrophage response to the IIS.

II. Methods

Macrophage-like immortalized cell lines (THP-1, THP1-XBlue) were treated with +/- 5U/mL of Humulin N in the presence of TLR agonists (PAM3CSK4 or LPS, 150 ng/ml) for 24 hours to examine inflammatory markers. NF-κB/AP-1 signaling activity was indirectly assessed using a secreted embryonic alkaline phosphatase (SEAP) assay and TNF-α production was detected using ELISA.

III. Results

The Fitzpatrick lab had previously developed an *in vitro* IIS host response model, in which macrophages are cultured on model TCPS/Teflon AF surfaces in the presence of soluble or adsorbed inflammatory agonists to recapitulate the local inflammatory response at the implant site [4]. Here, using this model with soluble Toll-like receptor (TLR) agonists, we demonstrated that both LPS and PAM3CSK4 on their own increased NF-κB signaling and TNF-α concentrations (Fig. 1A, 1B). Interestingly, cells treated with insulin alone did not exhibit a significant increase in inflammatory markers (Fig. 1A, 1B). However, when combined with an inflammatory stimulus, the Humulin-N enhanced both pro-inflammatory signaling and cytokine expression (Fig. 1A, 1B).

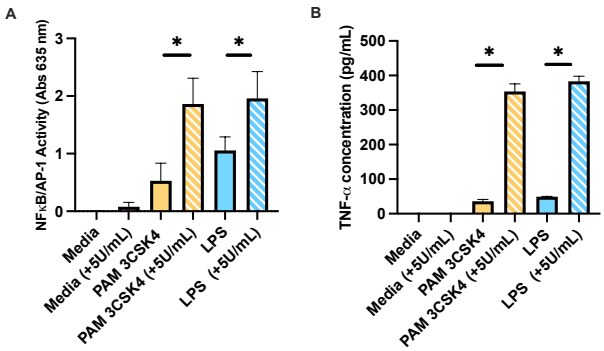

*Figure 1: Humulin N enhances (A) pro-inflammatory NF-kB/AP-1 signaling activity and (B) TNF-α expression in macrophage-like cells cultured in the presence of soluble inflammatory mediators. N=2, *p represents < 0.05.*

IV. Discussion & Conclusions

This work demonstrates that macrophages cultured in the presence of insulin activated pro-inflammatory transcription factors more strongly than macrophages cultured in the presence of TLR agonists alone. Our results also demonstrate an increased production of pro-inflammatory cytokines by macrophages treated with supraphysiological concentrations of insulin. Together, these results suggest that insulin may have a synergistic effect with soluble inflammatory mediators such as PAM3CSK4 and LPS. We propose that this is due to crosstalk between the insulin receptor signaling pathways and the TLR pathways of immune cells present at IIS implant sites, producing an enhanced overall pro-inflammatory response. We believe this is responsible for the exacerbated host response to IIS that results in their limited wear time. Future work will aim to elucidate the signaling pathways that are implicated in IIS failure with the overall goal of improving IIS design.

References

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