In Vivo Macrophage Activation via Locally Delivered Dexamethasone

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Statement of Purpose: Macrophage activation has emerged as a key area in a biomaterials context. Dexamethasone is a well-known glucocorticoid that dampens inflammation and has been widely incorporated into different experimental biomaterials. Macrophages are plastic and can rapidly undergo phenotypic changes. The present prevailing hypothesis in the biomaterials field is that alternatively activated, M2, macrophages support improved outcomes. Macrophage phenotypes have been previously referred to as M1 (classical activation) and M2 (alternative activation) with the M2 phenotype further classified as M2a, M2b and M2c. However, recent recommendations from the macrophage immunology community have requested not using these classifications, but rather indicate the modulator used, e.g. M(Dex) combined with a description of cell surface and protein markers identified. In this study, we have used implanted microdialysis sampling probes to locally deliver dexamethasone to an implant site. We have previously focused on M(Dex) with daily infusions [1]. In this work, we waited until 3 days post implantation to begin the M(Dex) delivery noting that previous studies have suggested the foreign body response needs to be activated for healing to occur. The chemokine, CCL2, was measured from collected dialysates. After the experiment, excised tissue was prepared for qRT-PCR analysis of different markers for macrophage activation.

Methods: Two microdialysis probes (CMA 20) were implanted into the dorsal subcutaneous space of male Sprague Dawley rats (275-325 g). One probe served as the control with a saline solution as the perfusion fluid. The other probe served as the treatment probe with 20 µg/mL of dexamethasone-21-phosphate (Dex) in the saline solution. Three days post implantation, the dialysis probes were infused with Dex for 6 hr and samples were collected once per hr. This continued through Day 6 post implantation. After experimental completion, the probes and surrounding tissue were excised for either qRT-PCR analysis or immunohistochemical staining of macrophage markers CD 163 and CD 206. Dialysate samples were quantified for CCL2 using an ELISA.

Results: On day 3 post implantation, the first infusion of Dex was performed and dialysate concentrations of CCL2 ranged from 500 to 2000 pg/mL (n=8). However, there was no significant difference between the concentrations obtained from control vs. treatment probes. On day 4 post implantation, dialysate concentrations did not become statistically significant ($p < 0.05$) until the 3rd hr of perfusion with Dex (n=8). A similar observation was made on day 5 post implantation (n=8). However, by day 6, while the CCL2 concentrations appear to be lower for the treatment group, the differences between these two groups are not significant (n=6) as shown in Figure 1.

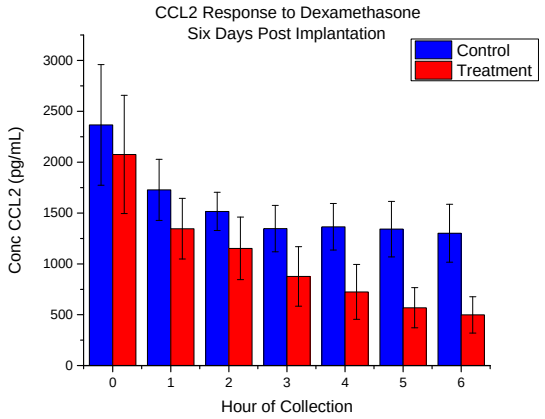

Figure 1. Bar graphs showing the concentration of CCL2 collected from the control and treatment probes perfused at 1 µL/min for each hour. Error bars represent the SEM with *p≤0.05 and n=6 (Day 6) animals. 0 represents the initial flush period (first 15 minutes).

The relative gene expression data from tissue excised around the dialysis probes suggested that only IL-6 expression was significantly upregulated (2 fold), $p<0.001$, in treatment animals compared to controls. CCL2 was also measured, but was not significantly different than controls in this excised tissue at day 6 which is consistent with the protein level observations in Figure 1. Several other markers indicative of the M2c (wound healing) phenotype such as IL-10, iNOS, CD 163 and CD 206 were not significantly upregulated.

Conclusions: Macrophage activation is a relatively new area for biomaterials. This work aims to combine previous work with measuring qRT-PCR and immunohistochemical markers combined with active cytokine protein measurements. The M(Dex) treatment did initially reduce CCL2 concentrations on days 4 and 5, but not on day 3 and 6. This suggests that waiting 3 days post implantation before having Dex be released may be too long. Optimal strategies and timing are still an active area of research for macrophage activation studies in the context of biomaterials research.

References: [1] Keeler GD, Durdik JM, and Stenken JA (in press) Acta Biomaterialia 0.1016/j.actbio.2014.10.022