Cytokine Response to Local Delivery of Dexamethasone-21-Phosphate at an Implant Site

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Statement of Purpose: Dexamethasone is a glucocorticoid steroid which is known to have immuno-suppressive and anti-inflammatory effects. Dexamethasone-21-phosphate disodium salt (Dex-21-P) is used as a replacement for dexamethasone as it is more water soluble and is converted *in vivo* by esterases to dexamethasone. Chemokine (C-C) ligand 2 (CCL2/MCP-1)is produced by macrophages and is responsible for the recruitment of monocytes to a wound site. Once at the wound site, monocytes differentiate into macrophages which are then polarized to an M1 or M2 a,b,c state depending on the cytokines present. While dexamethasone has been shown to shift macrophages to an M2c state *in vitro,* it has not been demonstrated *in vivo*.

In this work, subcutaneously-implanted microdialysis probes are used to locally deliver Dex-21-P in awake and freely-moving rats. Microdialysis sampling allows for the simultaneous delivery of agents to the extracellular space and collection of analytes from the extracellular space allowing quantification of CCL2 in response to Dex-21-P delivery. In addition to CCL2 quantitation in dialysates, qRT-PCR was used to obtain the relative expression of macrophage and cytokine-related genes associated with an M2c response.

Methods: Two CMA 20 microdialysis probes (Harvard Apparatus, Holliston, MA, 100kDa MWCO) were implanted subcutaneously on the dorsal side of male Sprague Dawley rats. One probe served as the control using Ringer's (147 mM NaCl, 4.6 mM KCl, 2.3 mM $CaCl_2$) + 4% w/v Dextran-500 + 0.1% w/v bovine serum albumin. The second probe served as a treatment consisting of the same perfusion fluid as the control plus 2 µg/mL Dex-21-P. An initial flush was performed starting at 3 µL/min and was reduced by 0.5 µL/min every 5 mins until the desired flow rate of 1 µL/min was reached. Collections were then performed in 1 hour intervals for 6 hours. After collections were performed, a final flush using either Ringer's solution (control) or Ringer's solution + 2 µg/mL Dex-21-P (treatment) was performed. This procedure was performed every day for three days starting on the day of implantation (day 0). Following collections on the third day, the animal was euthanized and the tissue surrounding the membrane portion of the microdialysis probe was harvested for qRT-PCR analysis using a 7500 real time PCR instrument (ABI Grand Island, NY). CCL2 concentrations in the dialysate were quantified using a standard ELISA (BD Biosciences San Diego, CA).

Results: The M2c state is characterized by low production of inflammatory cytokines and high production of IL-10 and results in an anti-inflammatory, pro-wound healing and tissue remodeling response. Initial results show that Dex-21-P is able to suppress CCL2 concentrations in the later hours of the collection period (Table 1). Furthermore, qRT-PCR data shows that CCL2 gene expression is significantly down-regulated in response to Dex-21-P (Figure 1). Interestingly, the expression ratios of the other genes tested show less consistency from one animal to the next. While further studies are needed, this data shows that Dex-21-P is capable of decreasing CCL2 concentrations and down-regulating CCL2 gene expression and may be able to induce an M2c state at the implant site.

Table 1: Range of CCL2 concentrations (pg/mL) found in dialysates.

	Animal 1		Animal 2		Animal 3		Animal 4	
	Control	Treatment	Control	Treatment	Control	Treatment	Control	Treatment
Day 0	170-650*	40-135*	50-115**	80-190***	115-225*	90-450*	65-375*	490-815*[++]
Day 1	90-345	45-455*	305-580[N]	50-395	365-895	40-760	415-670	220-740
Day 2	125-535*[N]	45-140****	60-270	40-450**	405-755	35-290**	45-230*	155-475

* Denotes the number of samples with concentrations below the LOD, [+] Denotes the number of samples with concentrations over range, [N] Denotes where there was no sample.

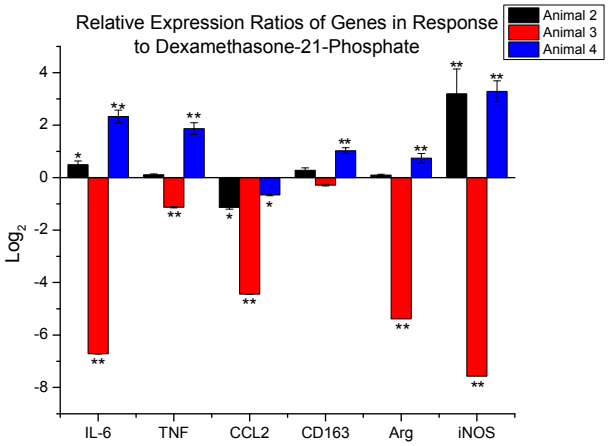

Figure 1: Expression ratios of 6 genes in response to Dex-21-P. *p≤0.05, **p≤0.001

Conclusions: Reduction of CCL2 levels in response to Dex-21-P suggests the drug is capable of reducing inflammatory markers at a wound site. However, the inconsistency in gene expression does not definitively show that an M2c macrophage state is predominating the wound site. Future studies will utilize higher concentrations of Dex-21-P, infusions over a longer period of time (7 days), and immunohistochemistry to identify macrophages at the implant site.

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