Delivery of acetylsalicylic acid to dendritic cells using degradable micropaticles

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Introduction

Degradable microparticles have the potential to protect and release drugs over extended periods of time and if sized appropriately can be targeted to phagocytic cells[1]. Dendritic cells (DCs) are phagocytes known to play a key role in the immune system. Their expression of costimulatory and co-inhibitory molecules drives the antigen-dependent activation of T cells[2]. Therefore, manipulation of the ratio of stimulatory and inhibitory signals would allow for shaping of the subsequent T cell mediated defensive immune responses in applications such as tissue engineering and autoimmune diseases. In this study we focus on the intracellular delivery of acetylsalicylic acid (ASA) to DCs using poly(lactic-co-glycolicacid) (PLGA) degradable microparticles. As part of its anti-inflammatory effects, ASA hinders antigen presentation by DCs. We hypothesize therefore that a depot of ASA will maintain immaturity of DCs,upregulate inhibitory signals, and prevent antigen presentation to T cells.

Methods

PLGA microparticles were synthesized using a single emulsion/evaporation technique with encapsulation of ASA followed by characterization including size distribution, loading efficiency and release kinetics. The microparticle system was then incubated with murine myeloid derived dendritic cells and necrosis and apoptosis assessed using flow cytometry. The levels of co-stimulatory molecules (CD80/CD86), MHC-II and co-inhibitory molecules (PD-L1/PD-L2) were measured using flow cytometry and luminex. Finally, T-cell activation and proliferation were measured in a mixed lymphocyte reaction using a BrdU assay.

Results

Herein, we present our ability to obtain an ASA loading efficiency of approximately 20% (**Fig.1)**, the release profile of our formulation showing a burst of ASA in the first six days followed by sustained release over a period of forty days (**Fig. 2**)) and decreased levels of CD80 and CD86 (**Fig. 3**) markers on dendritic cells.

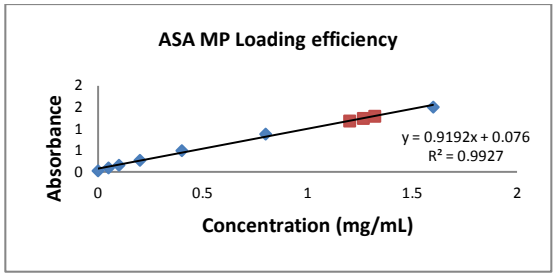

Figure. 1 Loading efficiency of ASA microparticles

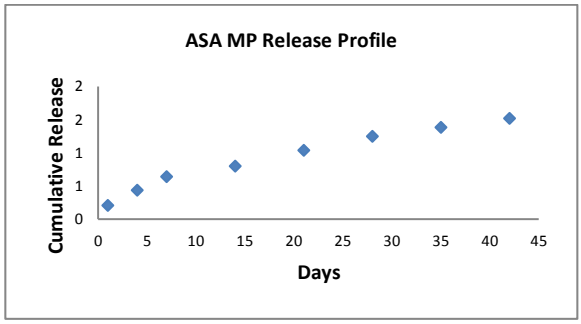

Figure 2. Release profile of ASA microparticles

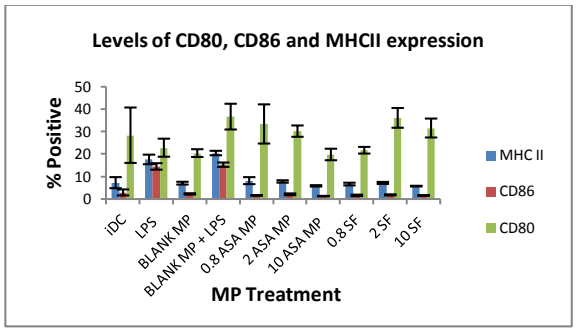

Figure 3. Levels of MHC II, CD86 , CD80

Conclusion

From this study we can conclude that PLGA microparticles are able to load significant levels of ASA and release it at a constant rate over a period of forty days. We can also conclude that the delivery of ASA using a particulate system decreases the levels of costimulatory molecules as well as MHC-II. Future studies will verify the upregulation of co-inhibitory molecules (PD-L1/PD-L2) and the effects of our system on T-cells.

References [1]Jhunjhunwala, S. J. Control Release 2009;133(3):191-7 [2] Muzammal H. Int. Immunopharmacology 2012;12:10-20.