Nanoparticle Mediated Delivery of Glutamate Enhancers to Restrain Autoimmune Reactions

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Statement of Purpose: Autoimmune diseases often involve immune dysfunction in which self-molecules are recognized as foreign. An evolving area to combat these autoinflamamtory reactions is based on targeting dendritic cells (DCs) with immunomodulatory small molecules that reduce the ability of DCs to activate inflammatory T cells, or polarize the function of T cells (e.g., away from inflammatory T cells and toward regulatory T cells, T_{REGS}). While many candidate drugs have shown some therapeutic efficacy, these drugs are limited by poor solubility and specificity, and dose limiting toxicity. Polymeric nanoparticles (NPs) could allow controlled release of many such drugs, as well as reduce systemic toxicity. Toward the goal of harnessing these benefits, we have developed nanoparticles loaded with [-Phenyl-7-(hydroxyimino)cyclopropa[*b*]chromen-1a-carboxamide] (PHCCC), a novel immunomodulatory small molecule that alters the balance of glutamate receptors on dendritic cells. In Multiple Sclerosis (MS) – an autoimmune disease in which myelin is recognized as foreign – excess glutamate causes toxicity and destruction of host cells. PHCCC selectively enhances receptors that metabolize excess glutamate (e.g., MGluR4). In particular, in mouse models of MS, recent studies demonstrate that daily, systemic treatment with PHCCC modulates glutamate receptors on DCs, ultimately redirecting T cell function away from inflammatory phenotypes and toward T_{REGS}. Thus, we hypothesized that PHCCC encapsulated in controlled release NPs might provide more enduring, less toxic immunotherapy with infrequent dosing.

Methods: PHCCC was encapsulated in poly(lactide-co-glycolide) (PLGA) NPs using nanopreciptiation in acetone. Drug loading and particle size were quantified by UV-Vis spectroscopy and laser diffraction, respectively. To assess the toxicity of PHCCC in soluble or NP form, primary DCs were stimulated with an inflammatory signal (LPS) and treated with PHCCC in soluble or NP form prior to analysis by flow cytometry (FACS). Particle uptake was assessed by incorporating a florescent dye in NPs and viability was measured by staining with DAPI. To test the functional effects of PHCCC NPs, splenic DCs ($CD11c^+$) isolated from mice were stimulated with LPS and treated with each PHCCC formulation. Inflammatory cytokine secretion was measured by ELISAs, and expression of co-stimulatory markers was measured by FACS. The impact of PHCCC on antigen presentation was tested by incubating DCs with LPS and a model antigen (SIINFEKL), along with each PHCCC form. The percent of cells presenting SIINFEKL via major histocompatibility complex I (MHC-I) was then quantified by FACS. The impact of altered DCs activation and antigen presentation was then assessed by co-culturing DCs stimulated with LPS in the presence of myelin and each PHCCC treatment with CSFE-labeled $CD4^+$ T cells isolated from 2D2 mice – a strain where T cells recognize the self-antigen attacked in MS (myelin).

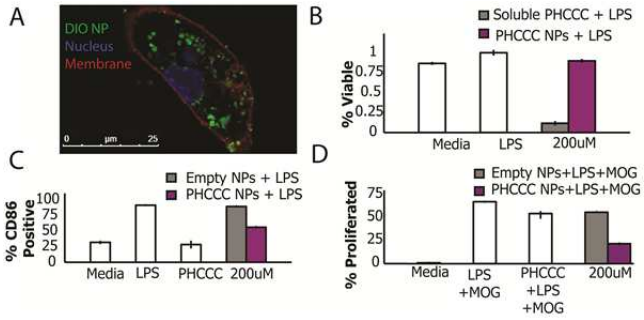

Figure 1: NP mediated delivery of PHCCC (A) reduces toxicity. (B) NPs are endocytosed by DCs. (C) PHCCC NPs reduce DC activation. (D) T cells proliferate less when cultured with PHCCC NPs treated DCs.

Results: PHCCC NPs exhibited a size of 148.5 nm + 47.1 nm and a drug loading level of 73.2 μg PHCCC/mg of NPs. NPs were efficiently endocytosed by primary DCs, as measured by FACS and confocal microscopy (**Fig. 1A**). Critically, cells treated with PHCCC NPs exhibited no significant toxicity, whereas equivalent drug doses in soluble form left only 11.6% of cells viable (**Fig. 1B**). PHCCC NPs reduced inflammatory cytokine secretion (e.g., IL-6) by 45.9 $\pm$ 1.8% compared to empty particles, while also reducing co-stimulatory marker (e.g., CD86) expression (**Fig. 1C**). These effects resulted in a 52.8 $\pm$ 1.1% reduction – relative to empty particles – of SIINFEKL antigen presentation via MHC-I. The functional ability of DCs treated with PHCCC NPs to prime T cells was tested by co-culturing treated cells with $CD4^+$ T cells isolated from 2D2 transgenic mice. In these studies DCs were treated with LPS, myelin antigen, and each PHCCC form, then co-cultured with the CSFE-labeled transgenic $CD4^+$ T cells for 3 days. T cells co-cultured with DCs treated with PHCCC NP proliferated significantly less compared to untreated controls and DCs treated with soluble PHCCC (**Fig. 1D**).

Conclusions: The findings above demonstrate a low energy method for synthesizing PHCCC loaded particles that reduce inflammatory cytokine secretion, DC activation, and T cell proliferation without toxicity. These *ex vivo* studies support a platform that could provide similar effects *in vivo* to help combat autoimmune diseases by restraining inflammation. Importantly, future studies will assess the phenotype of T cells to quantify any shift in function away from inflammatory phenotypes (e.g., T_H17) and toward regulatory populations (e.g., T_{REGS}). This approach could ultimately contribute to therapies that selectively suppress inflammatory reactions without broad immunosuppression hindering current treatments for autoimmune disease.