In vivo Evaluation of Safety and Biocompatibility of Carbohydrate-Functionalized Polyanhydride Nanoparticles

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Statement of Purpose: Surface modification using carbohydrates allows for targeting of pattern recognition receptors (PRRs) on cells that can activate an immune response.[1] In particular, C-type lectin receptors are PRRs that have a specific affinity for carbohydrates and are important in modulating the adaptive immune response. *In vitro*, functionalized particles have shown promising results for their ability to activate dendritic cells (DCs) and macrophages.[2-4] In this work, the *in vivo* safety and biodistribution profiles of carbohydrate-functionalized polyanhydride nanoparticles was analyzed. Toxicity studies are a critical first step to assess the safety of the carbohydrate-functionalized particles prior to efficacy studies. In addition, analyzing the distribution of targeted polyanhydride nanoparticles will enable the rational selection of formulations that target antigen presenting cells (APCs) most effectively.

Methods: Polyanhydrides based on a 50:50 molar ratio of 1,8-bis-(*p*-carboxyphenoxy)-3,6-dioxaoctane (CPTEG) and 1,6-bis-(*p*-carboxyphenoxy)hexane (CPH) were copolymerized using melt polycondensation. Nanoparticles containing 1% AlexaFluor 647 dye were fabricated using anti-solvent nanoprecipitation. Surface functionalization with di-mannose, galactose, or glycolic acid (as a "linker" control) was performed using an amine-carboxylic acid coupling reaction and compared to a non-functionalized (NF) nanoparticle control. In the safety studies, 5 mg of each type of nanoparticle formulation was administered subcutaneously to Swiss Webster mice. Histopathology measurements were conducted to assess kidney or liver damage at two different time points post-immunization. Simultaneously, blood and urine samples were analyzed for biomarkers of kidney and liver function. In the biodistribution experiments, 500 μg of nanoparticles were administered intranasally to Swiss Webster mice. Flow cytometry was performed on cells extracted from lung tissue at four time points to characterize the cellular populations that internalized nanoparticles.

Results: Seven days after the administration of functionalized nanoparticles, no histological differences were observed in the NF or functionalized particles compared to the saline group (data not shown). Kidney and liver function markers (i.e., blood urea nitrogen (BUN), serum creatinine, and albumin) (Fig. 1) in blood samples of mice that received functionalized and NF particles were not significantly different than that in saline-treated mice.

The biodistribution results show that the cellular uptake kinetics of carbohydrate-functionalized particles was chemistry-dependent (Fig. 2A). Compared to NF particles, the functionalized particles were internalized more effectively by cells at earlier time points. NF particles demonstrated enhanced internalization at later time points, particularly 96 hours. Flow cytometry revealed that DCs accounted for at least 50% of the cells that internalized particles, regardless of formulation and generally increased with time (Fig. 2B). The number of epithelial cells, macrophages, and neutrophils that internalized particles was also time dependent (data not shown). Macrophages and epithelial cells exhibited early internalization of NF and functionalized particles; however, after 48 hours, the percentage of these cells that internalized particles was significantly reduced. Very few neutrophils internalized particles at all the time points studied, regardless of nanoparticle formulation.

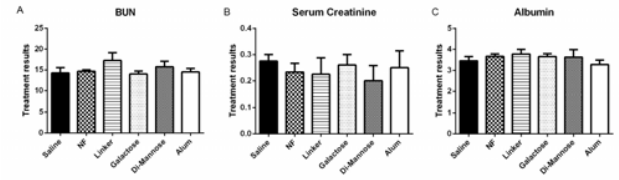

Fig. 1. Markers of liver and kidney function were similar across mice administered nanoparticle formulations, saline, and Alum 7 DPI.

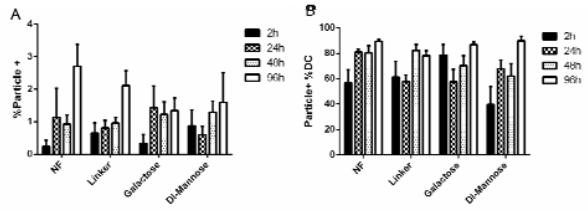

Fig. 2. Cellular uptake of functionalized nanoparticles. A) Particle internalization was analyzed using whole lung homogenate. B) Majority of particle uptake was from DCs.

Conclusions: Biological markers of kidney and liver function as well as with histopathological analysis did not exhibit significant differences in mice treated with functionalized nanoparticle treatments and saline controls, indicating a favorable safety profile for the particles. Furthermore, the studies show that internalization kinetics *in vivo* is both chemistry- and time-dependent for the biodistribution of the functionalized particles. DCs were the dominant cell type that internalized both functionalized and NF particles. Together, these results suggest that functionalization of particles can be used to design safe and efficacious vaccines.

References:

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