Nanoparticles for Hepatocyte-targeted Delivery

Jamie M. Bergen, Horst A. von Recum, Thomas T. Goodman, Archna P. Massey, Suzie H. Pun,
Department of Bioengineering, University of Washington, Seattle WA 98195, USA

Statement of Purpose:

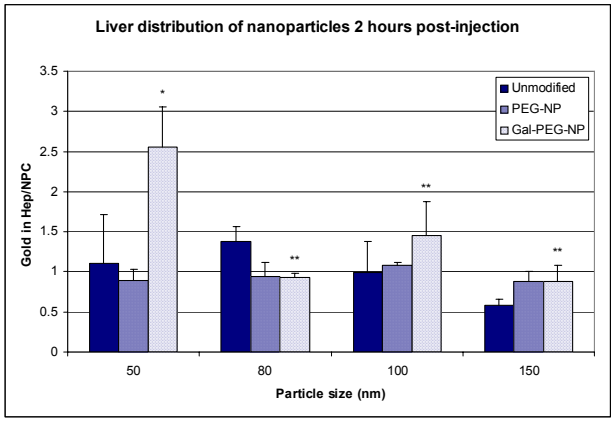
The development of synthetic gene carriers targeted to hepatocytes would potentially impact the treatment of liver-related diseases such as hemophilia. In addition to the effect of targeting ligands, the properties of the nanoparticulate vehicle itself may influence successful targeting *in vivo*. Here, we demonstrate the utility of a gold nanoparticle-based platform for systematic examination of physicochemical properties such as size, charge, PEGylation, and ligand density on targeted delivery. Nanoparticles of varying size, extent of PEGylation, and galactose ligand density were prepared and characterized. Galactose-mediated uptake by hepatoma cells was investigated in cultured cells. Hepatocyte targeting of select formulations was demonstrated by systemic administration to C57Bl/6 mice.

Methods:

Gal-PEG-PDP was synthesized by reaction of 4-aminophenyl-β-D-galactopyranoside with OPSS-PEG_{5000}-SPA. Product purity was confirmed by HPLC and H^1 NMR analyses. Gold nanoparticles 50, 80, 100, and 150 nm in diameter were left unmodified, or were surface-modified by reaction with PEG_{5000}-thiol or gal-PEG-PDP. Particle stability in the presence of physiological salt concentrations was monitored using dynamic light scattering with a ZetaPlus analyzer (Brookhaven Instruments; Holtsville, NY). Galactose ligand density was determined by lectin-induced aggregation assay [1]. For *in vitro* uptake studies, nanoparticles were exposed to HepG2 for 20 minutes in the presence and absence of asialofetuin (competitive ligand). Cell-associated gold was measured by instrumental neutron activation analysis (INAA) and the gold content in each sample was normalized by total protein content, as determined by BCA assay. The *in vivo* liver distribution of nanoparticles was investigated by administration of nanoparticles to C57BL/6 mice via low pressure tail vein injection. Livers were perfused 2 hours post-injection and hepatocytes and liver nonparenchymal cells (NPCs) were isolated by differential centrifugation. Gold contents in blood, hepatocyte and NPC samples were measured by INAA and the gold content in each sample was normalized by total protein content, as determined by BCA assay. All INAA analyses were conducted by the Washington State University Nuclear Radiation Center (Pullman, WA).

Results / Discussion:

PEGylated nanoparticles (PEG-NPs) and galactose-PEG-modified nanoparticles (gal-PEG-NPs) were prepared by reaction of gold colloids with PEG-thiol and gal-PEG-PDP, respectively. Nanoparticles of 4 sizes (50, 80, 100, and 150 nm diameter) were prepared. In the presence of physiological salt concentrations (150 mM NaCl), unmodified nanoparticles aggregated, as expected. However, assembly of a layer of the hydrophilic polymer PEG on the particle surface either by addition of PEG-thiol or gal-PEG-PDP afforded steric stabilization of gold nanoparticles in salt. To demonstrate that the ligand density on the surface of gold nanoparticles can be easily controlled, nanoparticles were surface-modified with mixtures of PEG-thiol and gal-PEG-PDP containing from 0% to 100% gal-PEG-PDP. The degree of lectin-induced aggregation of nanoparticles increased with the percentage of gal-PEG-PDP, thus demonstrating that the ligand density on the surface of gold nanoparticles can be precisely controlled. The effects of NP size and galactose ligand incorporation on hepatocyte targeting were evaluated *in vivo*. The concentration of PEG-NPs in the blood was two to three orders of magnitude higher than that of unmodified NPs. Gal-PEG-NPs were depleted from the circulation to a greater degree than unmodified NPs, presumably due to uptake into hepatocytes via the asialoglycoprotein receptor (ASGP-R). The extent of hepatocyte targeting in the liver was determined by taking the ratio of normalized gold concentration in hepatocytes to NPC (see figure below). PEG-NPs showed no preferential uptake by hepatocytes (gold in hepatocyte/gold in NPC ~ 1); gal-PEG-NP that were 50 nm in diameter conferred preferential hepatocyte targeting, resulting in a 2.5-fold increase in the ratio of gold in hepatocytes to gold in liver.



Conclusions: This work demonstrates the use of gold nanoparticles as a platform for systematically testing physicochemical parameters relevant for optimal cell-specific targeting *in vitro* and *in vivo*. Our results confirm the ability of galactose-modified nanoparticles to target hepatocytes *in vitro*, but reveal a critical size cutoff (~50 nm) for galactose-mediated hepatocyte targeting *in vivo*. These principles can be applied to other synthetic gene delivery biomaterials to increase liver-targeted delivery.

References:

1. Takae, et al. (2005) *Biomacromolecules* v6:818-824.