Fuctionalized Magnetoliposomes for visualization of Hepatocytes in vitro and in vivo

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INTRODUCTION:

End stage liver disease is the final stage of acute or chronic liver damage and is associated with irreversible liver failure. Orthotopic liver or hepaotcyte transplantations are hindered due to paucity of healthy liver donors, only 20-30% cell survival post transplantation and limitation in culturing hepatocytes for longer durations etc. (1). Thus, differentiation of stem cells into hepatocyte like cells could be a potential therapeutic approach. Stem cell differentiation results in mixed cell populations containing undifferentiated stem cells, cells from meso- and endoderm and hepatocytes (2). Labeling of hepatocytes with targeted MR contrast agents will allow following their location in vivo. It is the aim of this study to evaluate the specificity of functionalized magnetoliposomes (MLs i.e. iron oxide particles cpated with a phospolipid bilayer) to target hepatocytes in vitro and in vivo from mixed culture. We have used galactose-terminal entities which are specifically recognized by asialoglycoprotein receptors (ASGPR) abundantly present on hepatcoytes. In addition, systemic administration of Lactose- MLs will allow non-invasive evaluation of liver function.

METHODS:

ML synthesis: Cationic (DSTAP), anionic (DMPG) in a dimyristoylphosphatidylcholine (DMPC) matrix were synthesized as described before. Lactosyl-bearing MLs were produced similarly to the anionic MLs using 95% DMPC and 1% 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-lactosyl (DOPE-lac) (3, 4).

In vitro experiments: Differentiation of m ESCs was performed according to (1). Differentiated cells were labeled with all 3 types of MLs (100µg Fe/ml). Labeled cell samples were collected for RT-PCR, electron microscopy, immunostaining, MRI and magnetic cell separation to confirm the specificity and uptake quantification in hepatocyte like cells.

In vivo experiments: Initial experiments were performed by injecting MLs (200µg Fe/ml) in C57bl6 mice intravenously. Animals were scanned and livers were isolated for immunostaining and TEM at 4 hrs, day 2, 4, 6, 8 and 10 post injections. Tissue sections were stained for Prussian blue stain to check the uptake specificity and wash out at different time points.

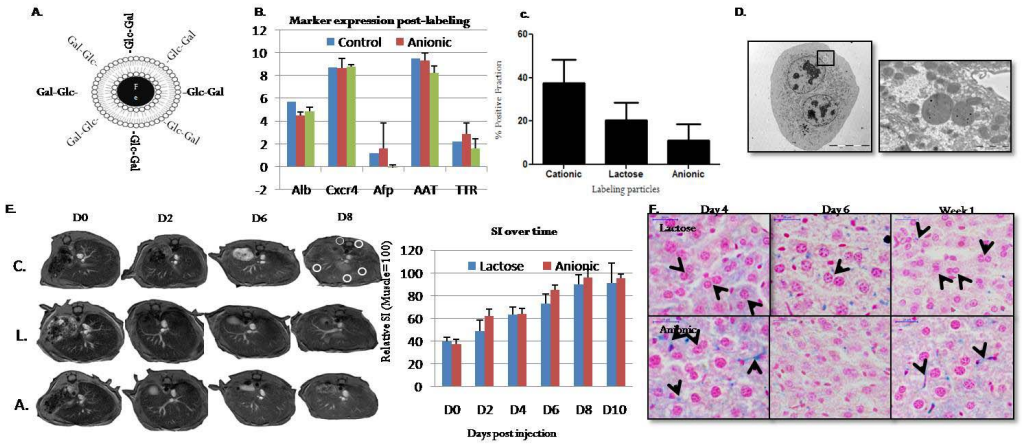
MRI: MR images were acquired using a Bruker Biospec 9.4 T small animal scanner. 3D-T2* weighted MRI FLASH (TE=12ms, TR=150ms) and T2-maps (MSME, with 8 echoes, TE increments of 10ms) were acquired with respiration gating.

RESULTS:

Differentiated mESCs did not show any toxic effects on total cell count and marker expression post labeling. Uptake specificity was confirmed by TEM (presence of iron oxide particles in endosomes of binucleated hepatocytes). Positive fractions post magnetic separation showed 25-30% of the total cell count which coincides with the percentage of ASGPR+ cells detected by FACS. These positive fraction cells were also detectable in MRI. In vivo dynamic contrast enhancement studies showed particles clearance through blood was very fast and all particle types ended up in the liver almost immediately after injection. There was no significant difference observed in the signal intensities of FLASH scans from anionic and lactose injected livers, indicating retention of particles in the liver cells for longer duration. However, histology confirmed that anionic MLs were mainly taken by Kupffer and stellate cells (only very small fraction in hepatocytes) and also confirmed by TEM. Lac MLs were mainly taken up by hepatocytes and kupffer, stellate immediately after injection but by later time points (week 1) particles were present only in hepatocytes (this observation correlated well with TEM images). Further, marker specific immnunostaining experiments will be done to determine uptake specificity.

CONCLUSIONS:

Initial *in vitro* experiments revealed high specificity in uptake of lactose-functionalized MLs by hepatocyte like cells. This can be used for magnetic cell separation and subsequent MR imaging post engraftment of those cells.. The potential of Lac MLs as a contrast agent for the evaluation of liver function was also confirmed by initial in vivo experiments.



(A). Typical layout of Lac-MLs; (B) No effect on marker expression post labeling; (c) % Positive fraction post magnetic separation; (D) Presence of iron oxide particles in bi-nucleated hepatocytes and week 1 liver samples; (E) FLASH scans of Cationic, Anionic and Lactose injected mice livers (axial) at D0, D2, D6, D8 and D10 post injections and their respective intensities wrt muscle. (F) Prussian Blue staining of liver samples at different time points.

References: (1) Kuo T. et.al. Gastroenterology 2008; 134(7): 2111-21; (2) Roelandt P et al. Plos One 2010;5(8): e12101; (3) Stockert RJ. Physiol.Rev. 1995;75:591-609; (4) De Cuyper M et al. Methods Mol. Biol. 2010;605:97-111; (5) Soenen SJ et al. Chembiochem 2007;8: 2067-2077.