TARGETED MAGNETOLIPOSOMES FOR VISUALIZATION OF HEPATOCYTES

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INTRODUCTION:

Cell labeling strategies can be classified in (a) unspecific uptake of contrast agents, typically to pre-label cell populations before engraftment and (b) those that aim for specific uptake or binding of targeted contrast agents. It is the aim of this study to evaluate the specificity of functionalized magnetoliposomes (ML i.e. lipid coated iron oxide particles) to target hepatocytes in vitro and in vivo. In case of liver cirrhosis or viral hepatitis where hepatocytes are damaged and healing is difficult, differentiation of stem cells into hepatocyte like cells is a potential therapeutic approach (1). In order to make use of these hepatocytes for cell based therapies, it is necessary to isolate hepatocytes from cell mixtures (cultures containing undifferentiated stem cells, cell types from meso- and endoderm and hepatocytes) and potentially follow their location in vivo. In addition, systemic administration of hepatocyte targeting MLs allows non-invasive evaluation of liver function. Thus in this work, we have used galactose-terminal entities which are recognized by asialoglycoprotein receptors (ASGPR), which are hepatic receptors (2).

METHODS:

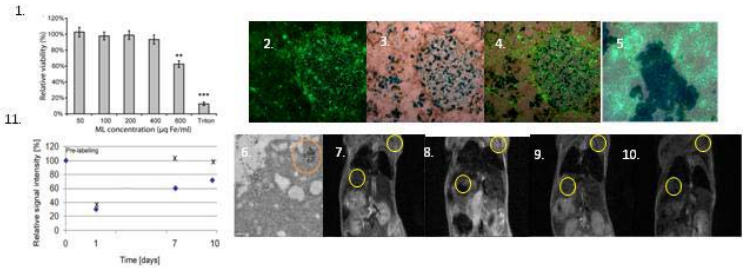
ML synthesis: Cationic (3.33% distearoyltrimethylammonium propane (DSTAP)), anionic (5% dimyristoylphosphatidylglycerol (DMPG)) in a dimyristoylphosphatidylcholine (DMPC) matrix were synthesized as described before [3, 4]. Lactosyl-bearing MLs were produced similarly to the anionic MLs using 95% DMPC and 1% 1,2-dioleoyl-sn-glycero-3-phosphoethanolamine-N-lactosyl (DOPE-lac).
In vitro experiments: For initial *in vitro* labeling studies, HepG2 and C17.2 (negative control) cells were labeled with the three different MLs (cationic MLs as positive (unspecific) control and anionic MLs as negative control) at different concentration (0-600µg Fe/ml). Toxicity (post labeling) was assessed using cell counts, MTT assays, EdU staining (proliferation), formation of reactive oxygen species (ROS, oxidative stress) and calcium levels. Uptake confirmation was done using TEM and Prussian blue staining. Differentiation of mESCs/ rat adult stem cells was performed according to [1], resulting in a mixture of meso- and endodermal cells and hepatocytes. This cell mixture was labeled with the three MLs (100µg Fe/ml). Labeled samples were stained with Prussian blue reagent. For the magnetic cell separation, labeled cells were taken off with dispase and % of positive fraction was determined with respect to the total cell count.
In vivo *experiments:* Proof of principle experiments were done by injecting all three MLs (2.5µg Fe in 15 µl) in black 6 mice intravenously. Animals were scanned at day 1, day 7 and day 10 after injections.
MRI: MR images were acquired using a Bruker Biospec 9.4 Tesla small animal MR scanner (Bruker Biospin, Ettlingen, Germany; horizontal bore, 20 cm) equipped with actively shielded gradients (600 mT m^{-1}). 3D T2*-weighted MRI (FLASH, TE=12ms, TR=150ms) and T2-maps (MSME, 16 TE increments of 8ms) were acquired from agar phantoms and in vivo (respiration triggered).

RESULTS:

In vitro studies of HepG2 and C17.2 revealed no toxic effects up to total Fe concentrations of 400µg/ml. Lactose MLs were marginally taken up by C17.2 cells wherein there was high uptake efficiency by HepG2 cells. TEM revealed that cationic MLs remain membrane associated and lactose MLs showed NPs in lysosomes. Anionic MLs were not taken up by any cell type. Differentiated hepatocytes retrieved from mESCs / rat adult stem cells also did not show any toxic effects at 100µg Fe/ml. Cationic MLs were surface associated to most cell types. Lac MLs were specifically taken up by hepatoblasts, which was confirmed with Prussian blue and TEM. ML uptake was confirmed by overlaying albumin staining with Prussian blue (Figure 4). Magnetic separation with EasySep confirmed that LacMLs target only hepatocytes from the culture which are 25-30% of the cells, which coincides with the positive fraction. In vivo experiments showed that all three particles accumulate in the liver 24hrs post injection. Clearance of anionic and cationic MLs was rapid. However, Lac MLs also remained detectable after the latest time point (day 10, see Figure 7-11).

CONCLUSIONS:

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



(1) Results of an MTT assay. (2) - (4) uptake confirmation (all at 10X): differentiated hepatocytes labeled with lactose MLs. Albumin (green) expressing cells (2) containing iron oxide particles (3), (4) is an overlay of albumin-prussian blue staining. (5) Unspecific uptake by non-albumin expressing cells labeled with cationic MLs. (6) Lac MLs presence in lysosomes of HepG2s. (7)- (8) In vivo scans of lactose MLs injected mice.(9)-(10) In vivo scans of anionic MLs injected mice taken at day1 and 7 after injection respectively. (11) Relative signal intensities were calculated by comparison of liver with respect to muscle contrast, which is indicated for Lac ML (diamonds) and anionic MLs (crosses).

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