Magnetite Liposomes as Potential Theranostic Agents for MRI and Magnetic Hyperthermia of Vascular Inflammation

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Introduction: Inflammation plays a critical role in the progression of atherosclerosis. Magnetic nanoparticles have been used for magnetic hyperthermia of cancer cells and for cellular MR imaging. Thus, they may have the potential to both image and treat vascular inflammatory cells.

Purpose: To evaluate magnetite liposomes for macrophage uptake, hyperthermia, and MRI of mouse carotid atherosclerosis at 7T.

Methods: *1) Magnetite Liposomes (ML)* – Liposomes (1,2) were constructed around a colloidal magnetite core (average size 10 nm) with either a phosphatidylcholine/ phosphatidylcholamine (2:1 ratio) lipid membrane (ML-PP) or a *N*-(α-trimethylammonioacetyl) didodecyl-*D*-glutamate chloride, dilauroylphosphatidylcholine, and dioleoylphosphatidylethanolamine (1:2:2 ratio) lipid membrane (ML-TDD). The average overall size was 94 nm for ML-PP and 150 nm for ML-TDD.

2) In vitro macrophage uptake – Mouse macrophage cells (RAW, $1x10^6$) were incubated with ML-PP or ML-TDD at a concentration of 20 μg/mL for 4 hours. Cells were washed and uptake was quantified and examined by Prussian blue staining.

3) In vitro magnetic hyperthermia – Macrophages (RAW, $1x10^6$) were again incubated with ML-PP or ML-TDD at a concentration of 20 μg/mL for 4 hours. After washing cells, tubes of cells at room temperature were placed at the center of an alternating-magnetic-field (AMF) generator and exposed to AMF for 30 minutes.

4) In vivo MRI - A total of 4 FVB mice were studied. After a high fat diet for one month, diabetes was induced by 5 daily intraperitoneal injections of streptozotocin. Two weeks after diabetes induction, left carotid artery ligation was performed. Two weeks after carotid ligation, mice were imaged on a 7T MRI scanner (Varian, Inc., Walnut Creek, CA) using a gradient echo sequence (TR/TE=50/4.2, slice thickness=0.5mm, FOV=3cm, matrix=256x256, FA=50, Spacing=0.5mm, NEX 10). ML-PP was injected via tail vein (2mg/mouse; n=3, 4mg/mouse; n=1) with further MRI performed at 24 and 48 after injection. Detection of ML accumulation was assessed by measuring the extent of T2*-induced reduction in carotid lumen size (% reduction of carotid lumen area). Perl's iron was also performed to confirm accumulation of ML in vascular macrophages.

Results: MLs were taken up by macrophages in vitro: 14.8% of cells for ML-PP and 44.7% for ML-TDD. Magnetic hyperthermia of macrophages was more effective with ML-TDD (Figure 1), resulting in 80% macrophage cell death (Figure 2). After ML-PP injection, serial MR images showed a reduction in lumen size of ligated left carotid arteries at 48 hrs, consistent with signal loss from ML accumulation (Figures 3, 4). Non-ligated right carotid arteries and sham-operated left carotid arteries did not show lumen reduction. Perl's iron staining demonstrated ML in the neointima.

Conclusions: MLs show macrophage uptake and effective magnetic hyperthermia and cell death in vitro. ML also imaged mouse carotid vascular inflammation by 7T MRI. Further optimization of MLs for macrophage uptake, hyperthermia, and MR is warranted. MLs are promising "theranostic" agents for diagnosing and treating vascular inflammation.

Reference:

1. Hamaguchi S, et al. Cancer Sci. 2003; 94: 834-839.

2. Shinkai M, et al. Jpn J Cancer Res. 1996; 87: 1179-1183

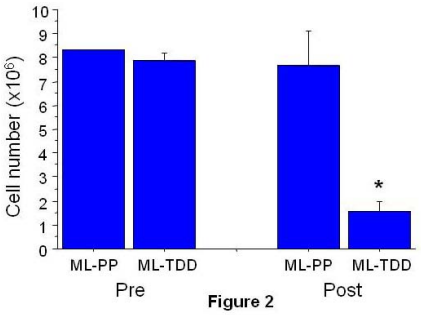
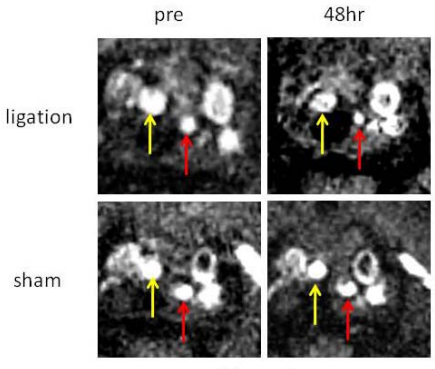


Figure 2



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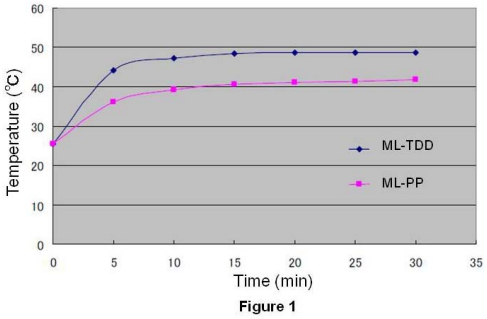

Figure 1

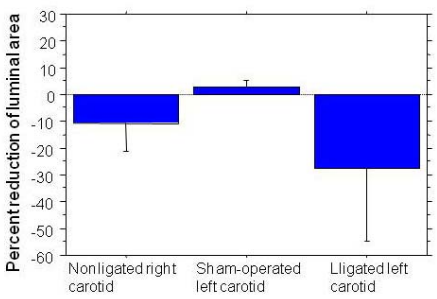

Figure 4