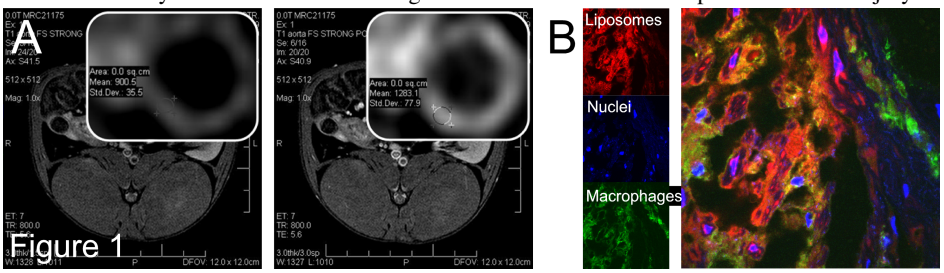
Novel nanomedicine for anti-inflammatory therapy of atherosclerosis monitored by multimodality imaging

M. E. Lobatto[1], S. Silvera[1], E. Vucic[1], V. Mani[1], M. Banciu[2], R. M. Schiffelers[2], K. Nicolay[3], G. Storm[2], J. T. Fallon[4], J. H. Rudd[1], V. Fuster[5], E. A. Fisher[6], Z. A. Fayad[1], and W. J. Mulder[1]

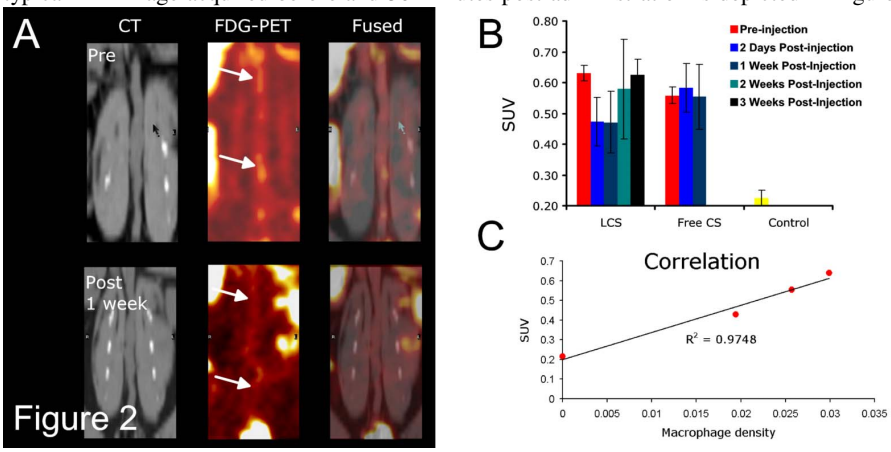
[1]Department of Radiology, Mount Sinai School of Medicine, New York, New York, United States, [2]Utrecht Institute of Pharmaceutics, Utrecht University, Utrecht, Netherlands, [3]Biomedical NMR, Eindhoven University of Technology, Eindhoven, Netherlands, [4]Department of Pathology, Mount Sinai School of Medicine, New York, New York, United States, [5]The Zena and Michael A. Wiener Cardiovascular Institute, Mount Sinai School of Medicine, New York, New York, United States, [6]Department of Medicine, New York University School of Medicine, New York, New York, United States

Introduction: Atherosclerosis is an inflammatory disease. One of the main factors contributing to the buildup of this disease is macrophage accumulation into the arterial vessel wall (1). Macrophage infiltration has been identified as a key event in the progression of this disease that may ultimately result in clinical events such as stroke and myocardial infarction. One of the drug classes that has been studied in the treatment of inflammation in developing atherosclerotic lesions are glucocorticoids. As of yet, they have not been applied for treatment of atherosclerosis in the clinic due to a variety of reasons. Free circulating glucocorticoids have a very low circulatory half-life and poor pharmacokinetics, causing low drug concentrations at sites of desired action, rendering them ineffective in treatment therefore dictating high dosages and frequent administration. In addition to having a short circulation half-life, glucocorticoids cause an array of adverse systemic effects. To overcome these shortcomings, we have encapsulated glucocorticoids in long circulating PEG-liposomes (LGC), which enhances the circulation half-life of glucocorticoids, providing LGC the opportunity to extravasate from the circulation and accumulate in atherosclerotic lesions. In the current study we applied LGC to atherosclerotic rabbits and used MRI to study their delivery. FDG-PET/CT, an emerging nuclear imaging modality for visualizing plaque inflammation (2), was used to monitor the effects of LGC. MRI, PET, and CT were performed on clinical scanners.

Material and Methods: Eighteen male New Zealand White (NZW) rabbits were used in this study. Aortic atherosclerotic plaques were induced in 16 NZW rabbits by a combination of a high cholesterol diet and a repeated balloon injury of the aorta. FDG-PET/CT was used to quantify the inflammation in the aorta of the animals. To that end, two healthy rabbits and six hyperlipidemic rabbits were used to optimize the method and verify the mean standard uptake values (SUV) of FDG. Liposomal GC were synthesized as described previously (3), but were modified to allow MRI and fluorescence microscopy of the material. To that aim, a paramagnetic amphiphile (Gd-DTPA-BSA, 10%) and a fluorescent amphiphile (PEG-DSPE-Rhodamine, 0.3%) were included in the liposomal formulation. Next, 10 balloon injured and hyperlipidemic rabbits were randomized and used for treatment of a single injection of free GC (n=3) or liposomal GC (n=7) at a dose of 15 mg/kg. Before and 2, 7, 14, 21 days post administration of the liposomal GC T1-weighted MRI and 18F-FDG-PET/CT scans of the abdominal aorta of these animals were performed. For the animals that were treated with free GC FDG-PET/CT scans were acquired before administration and 2 and 7 days post administration. A week after treatment, 2 liposomal GC treated animals were sacrificed for immunohistochemistry and fluorescence microscopy of sections of the aorta. All the free GC treated animals were sacrificed one week after application of the agent. The macrophage density for the different groups was determined histologically and compared to the FDG SUV determined with PET/CT. In addition, we localized the liposomes in the vessel wall sections using fluorescence microscopy.



Results and Discussion: The delivery and accumulation of the liposomal GC in the vessel wall was determined in vivo using T1-weighted MRI. A typical MR image acquired before and 30 minutes post administration is depicted in Figure 1A. Immunofluorescence was performed on 8 µm thick aortic sections (Figure 1B) and revealed liposomes to be found mainly associated with macrophages. To establish the therapeutic efficacy of liposomal GC (as compared to free GC) we used FDG-PET/CT in vivo. In Figure 2A coronal CT and FDG-PET images of the aorta of an atherosclerotic rabbit are shown. Hotspots of FDG uptake are clearly visible throughout the aorta before administration of liposomal GC (top), while a reduced FDG uptake was observed one week after administration (bottom), demonstrating the effectiveness of the liposomal GC. The mean SUV of the different groups, i.e. liposomal GC treated, GC treated, and healthy animals at base level are shown in Figure 2B. For the liposomal GC treated animals a significant decrease in SUV was observed as soon as 2 days post administration that was maintained up till 7 days. At 14 days post administration a moderate, but not significant decrease, was monitored, while at 21 days the SUV was back to base level. For animals that were treated with free GC no differences were found in SUV before and at 2 and 7 days post administration. In addition, we performed immunohistochemistry based determinations of the macrophage densities. The mean values were compared with the mean SUV of the same animals and showed a high degree of correlation (Figure 2 C).



Conclusions: In this study we have shown (I) the applicability of long circulating liposomes for efficient drug delivery to atherosclerotic plaques and were able to (II) monitor their delivery by MRI. (III) FDG-PET/CT could be used to monitor therapeutic responses of the GC liposomes and it was found that (IV) the liposome-encapsulated glucocorticoids have shown therapeutic efficacy within two days, lasting up to two weeks.

References

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