Engineering of a MRI Theranostic Agent for Detection and Treatment of Cerebrovascular Amyloid

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Target Audience: Clinicians and researchers interested in novel MRI contrast agents serving as theranostic nanovehicles for cerebral amyloid angiopathy.

Introduction: In Alzheimer's Disease, the buildup of amyloid beta (Aβ) 40 and 42 proteins in the cerebral arteries, known as cerebral amyloid angiopathy (CAA), is common. This buildup leads to cerebrovascular inflammation and microhemorrhage, which can lead to lobar hemorrhage and stroke [1]. Conventional iron-based MRI contrast agents have been developed to target cerebrovascular amyloid (CVA) for detection [2,3] but lack therapeutic ability. Ultrahigh field MRI has excellent potential to detect CVA due to enhanced sensitivity and contrast; however, to facilitate the detection of CVA, a MRI contrast agent is needed. This study describes the development of a theranostic nanovehicle (TNV) capable of generating MRI contrast that specifically target the CVA while at the same time functioning as a drug delivery vehicle for supplying localized effective doses of immunosuppressant or anti-inflammatory substances [4,5].

Purpose: Development of a multimodal TNV for effective detection of CVA that also has anti-inflammatory drug delivery capabilities.

Methods: A polymeric chitosan core was formed with hydroxypropyl-beta-cyclodextran (HPβCD) to encapsulate an anti-inflammatory substance (curcumin or cyclophosamide). Using an ionic gelation technique, the HPβCD complex was added with a Gd-DTPA-chitosan dispersion and crosslinked to form the nanoparticles. For CVA targeting, an IgG4.1 antibody was covalently bound to the freshly prepared particle [3] and centrifuged to remove any free IgG4.1. Western blot was used to confirm successful conjugation. Nanoparticle characterization was made to determine morphology, size and zeta potential. The Gd-DTPA loading was determined by measuring the difference in absorption with and without Gd-DTPA using 0.2M Arsenazo III. All imaging was performed using the 21.1-T, 900-MHz ultra-widebore magnet at the National High Magnetic Field Laboratory. Relaxometry measurements were carried out with dilutions of the Gd-DTPA conjugated contrast agent using T_1 and T_2 weighted 2D (128x128) spin echo sequences with 9 incrementing repetition times (TR = 25– 15,000 ms) and 16 incrementing echo times (TE = 8 – 124 ms) using a 10-mm birdcage coil. The images were analyzed with regions of interest for each respective dilution and fitted in SigmaPlot 7.1 (SPSS Inc, Chicago, IL) using single exponential growth or decay functions, respectively. For *ex vivo* MRI characterization, 200 µL containing 17 mg of the nanoparticle was injected through the femoral vein in 12-24 month old APP transgenic mice (Tg2576) (Taconic, Germantown, NY) [4] or in DutchAβ40 [5] treated mice. As a control, aged matched WT mice were used. The mice were perfused transcardinally three hours later and stored in 4% paraformaldehyde. Prior to MRI, the brains were washed, placed in individual conical tubes and imaged in unison using a 33-mm birdcage coil using a 3D gradient recalled echo (GRE) sequence acquired with 50-µm isotropic resolution and with TE/TR = 10/150 ms. The data was processed with a Gaussian filter using Amira 5.33 (Visage Imaging, CA). Amira was also used to compare brain structures and contrast generated from the nanoparticle.

Table 1: Relaxation and relaxivity of the GD-DTPA labeled TNV

Concentration (mM)	R_1 (s^{-1})	R_2 (s^{-1})
2	10.1	42.7
0.2	1.29	24.3
0.07	0.556	23.6
0.02	0.441	23.1
0.007	0.382	22.4
Relaxivity ($mM^{-1}s^{-1}$)	**4.9**	**10.0**

Results and Discussion:

Figure 1a illustrates the circular shape of the TNV with an atomic force microscope and reveals a diameter of the nanoparticles below 250 nm for all variations. An Arsenazo III assay determined that the nanoparticles carries 61 ± 9% Gd-DTPA. Relaxation measurements show the expected shortening of T_1 and T_2 relaxation times with a relaxivity sufficient for detection at 21.1 T (Table 1). The capability of the TNV to bind to Aβ fibrils was shown with ELISA and QCM-D methods [5] as well as with *in vitro* human blood brain barrier model [4,5] using DutchAβ40 treated human microvascular endothelial cells (Fig 2). Here, the red Alexa Flour 647 is clearly correlated with the blue DutchAβ40 treated cells. In Fig 3A, e*x vivo* coronal MRI images show hypointense signal from the Gd-DTPA labeled TNV as indicated by yellow arrows. The signal loss from the Gd-based contrast agent is due to the accumulation in the endothelium of arterioles, creating a more dominant T_2 contrast. Enhancement is seen in cortical regions where CVA is expected to be present. As expected, the WT mouse shows no contrast (Fig 3B). Radiolabeling of the TNV with ^{125}I revealed a nearly two fold increase of TNV in the cerebrovascular arteries compared to WT mice [4]. In Fig 3C, histological analysis confirms the location of the TNV in brain arterioles by Alexa Flour 647 labeling and correlated with the amyloid plaques using Thioflavin S (green). The amount of curcumin encapsulated was 0.03 ± 0.01 µg/(mg TNV), and ~75% of the total amount of curcumin encapsulated in the nanovehicles was released within 90 h. For cyclophosphamide, the drug concentration per TNV was estimated to be 22± 1% (w/w), and release over 24 h was linear with $\sqrt{time}$.

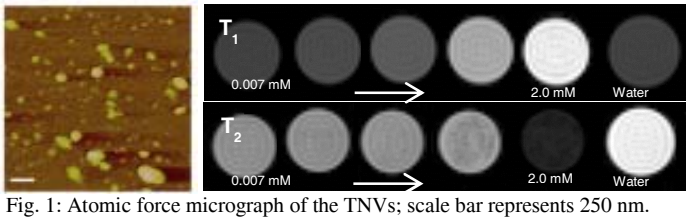

Fig. 1: Atomic force micrograph of the TNVs; scale bar represents 250 nm. (B) T_1 and T_2 images of the TNV showing increasing contrast with increasing Gd-DTPA concentration. [5]

Conclusions:

This work describes the design and engineering of a TNV with both diagnostic and therapeutic capabilities. The incorporation of Gd-DTPA clearly demonstrates the TNV as a MRI contrast agent with T_1 and T_2 contrast enhancement in phantoms. The increased sensitivity gained at 21.1 T facilitates a rapid scan time (< 12 h) with high resolution to detect the TNV targeted Aβ plaques. The TNV is able to entrap hydrophobic drugs while at the same time targeting CVA deposits selectively. In addition, they are large enough to be retained in the cerebral vasculature and small enough to penetrate the blood brain barrier. This novel approach not only serves to detect CVA pre-mortem but also reduce inflammation associate with CAA and limit microhemorrhage [4,5].

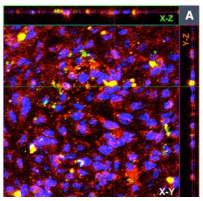

Fig. 2: Alexa Flour 647 labeled TNVs (red) in DutchAβ40 treated human cells (blue) [4]

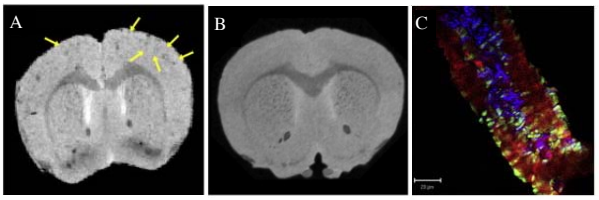

Fig. 3: Ex vivo MRI 3D GRE images with 50-µm resolution showing the hypointense signal in a transgenic mouse (A) compared to wild type where no contrast is seen (B). Fig 3C show a histological correlating the nanovehicle (red) with amyloid plaque by Thioflavin (green).

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