Engineering Theranostic Nanovehicles to Target Cerebrovascular Amyloid Deposits

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Introduction

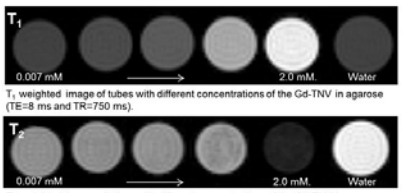
Cerebral amyloid angiopathy (CAA) is characterized by the deposition of amyloid beta (Aβ) proteins within the walls of the cerebral vasculature with subsequent aggressive vascular inflammation leading to recurrent hemorrhagic strokes. The objective of this study was to develop theranostic nanovehicles (TNVs) capable of a) targeting cerebovascular amyloid; b) providing magnetic resonance imaging (MRI) contrast for the early detection of CAA; and c) treating cerebrovascular inflammation resulting from CAA. This work is under review at the Journal of Controlled Release.

Experimental

The TNVs comprised of a polymeric nanocore made from Magnevist® (MRI contrast agent) conjugated chitosan. The nanocore also was loaded with cyclophosphamide (CYC), an immunosuppressant shown to reduce the cerebrovascular inflammation in CAA. The putrescine modified F(ab')2 fragment of the anti-amyloid antibody, IgG4.1 (pF(ab')24.1), was conjugated to the surface of the nanocore to target cerebrovascular amyloid. The TNV was characterized in regards to zeta potential, bindings to amyloid beta proteins, uptake by cells using an *in vitro* CAA model and with an *in vivo* animal model. The MRI contrast was characterized both with solution phantoms and *in vitro* using the 21.1-T ultra-wide bore magnet at the NHMFL.

Results and Discussion

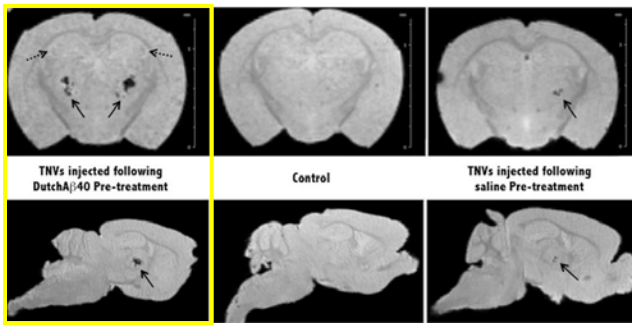
The average size of the control chitosan nanoparticles (conjugated with albumin and devoid of Magnevist®, CYC, and pF(ab')24.1) was 164 ± 1.2 nm and that of the TNVs was 239 ± 4.1 nm. The zeta potential values of the control chitosan nanoparticles and TNVs were 21.6 ± 1.7 mV and 11.9 ± 0.5 mV, respectively. The leakage of Magnevist® from the TNVs was a modest 0.2% over 4 days, and the CYC release from the TNVs followed Higuchi's model that describe sustained drug release from polymeric matrices. The *in vitro* and *vivo* model demonstrated the ability of TNVs to target cerebrovascular amyloid and provide MRI as well as single photon emission computed tomography (SPECT) contrast.



T_1 weighted image of tubes with different concentrations of the Gd-TNV in agarose (TE=8 ms and TR=750 ms).

T_2 weighted image of tubes with different concentrations of the Gd-TNV in agarose (TE=72 ms and TR=5000 ms).

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**
Goodness of fit (R^2)	0.999	0.998



Top: T_1 and T_2 weighted MRI contrast and relaxivity measurements (table) of the TNV. **Bottom:** High resolution *ex vivo* imaging of the CAA animal model (yellow frame) showing its ability to target amyloid plaques for detection with MRI.

Conclusions

Studies conducted with the *in vitro* model using human microvascular endothelial cell as well as in mice *in vivo* have demonstrated the ability of TNVs to target cerebrovascular amyloid. In addition, the TNVs provided contrast for imaging cerebrovascular amyloid using MRI and SPECT. Moreover, the TNVs were shown to reduce pro-inflammatory cytokine production by the Aβ challenged blood brain barrier (BBB) endothelium more effectively than the cyclophosphamide alone.

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