A Metallofullerene Nanoparticle Platform for Imaging and Treatment of Glioblastoma Multiforme Tumors

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Abstract

Glioblastoma multiforme (GBM) brain tumors occur as masses with infiltrative margins that are extremely invasive. GBMs result in a high recurrence rate with a poor prognosis for patients who are newly diagnosed with only a ~14 month median survival rate. During the last few years, we have focused on the development of a trimetallic nitride templated endohedral metallofullerene (TNT EMFs) nanoparticle platform for magnetic resonance (MR) imaging,[1-3] brain tumor brachytherapy[3] and targeting.[4-5] One of the major goals of this work has been the preparation of a number of different radionuclides (^{177}Lu) encapsulated within the fullerene cage and/or attached via external ligands.[3-5] Our recent results and strategies for imaging, targeting, and treatment of GBM tumors is the focus of this paper.

Background

There are several unique advantages of nanoparticles as next-generation cancer diagnostic and therapeutic nanoparticle agents. As pointed out in a recent review by Reineke, "Due to the altered anatomy of tumor vessels, nanosized particles can easily extravasation from the blood pool into tumor tissues and be retained due to poor lymphatic drainage. This phenomenon of selective accumulation of nano-sized particles near tumor tissues is termed the enhanced permeability and retention (or EPR) effect." [6] This article also points out the high surface area-to-volume ratios and high loading capacity of nanoparticles. This enables nanoparticles to be loaded with therapeutic drugs and imaging agents. The introduction of targeting ligands may also help to increase the target-to-background contrast in imaging and improve the local concentration of the therapeutic nanoparticle at the target of interest. The fullerene and metallofullerene carbonaceous nanoparticles offer a new opportunity for encapsulating metal clusters for imaging and therapeutic agents (**Figure 1**). In 1999, our laboratory reported a new family of trimetallic nitride template endohedral metallofullerenes (TNT EMFs) $A_3N@C_{80}$ (A = Group III and rare-earth metals) as illustrated in **Figure 1**.[7]

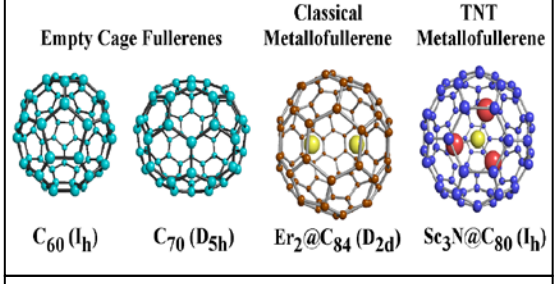

Figure 1. Representative Fullerenes and Endohedral Metallofullerenes (EMFs).

endohedral metallofullerenes (TNT EMFs) $A_3N@C_{80}$ (A = Group III and rare-earth metals) as illustrated in **Figure 1**.[7] The TNT EMFs are four atom molecular cluster endohedral metallofullerenes which are formed via a trimetallic nitride template within the carbon cage. In previous studies, it has been established that the $Gd_3N@C_{80}$ EMF is a highly stable species in which the Gd_3N cluster is bound inside the C_{80} cage by a strong binding energy, making it extremely unlikely for the cluster to break and release Gd atoms.[1-3] Thus, the fullerene carbon cage has inherent advantages because of its high stability and characteristic resistance to any potential metabolic cage-opening process. This prevents the release of toxic metal ions from the TNT EMF into surrounding tissue, serum, and other biological components. The ^{1}H MRI relaxivity, diffusion rates and quantitative concentration distribution maps for the functionalized Gd_3N-EMFs have been reported, and water ^{1}H NMR relaxivities (r_1) for $Gd_3N@C_{80}$ [DiPEG5000$(OH)_x$] were found to be significantly higher than those for commercial agents (*e.g.*, Omniscan^{TM}).[1-3] This increased relaxivity allows the use of significantly lower concentrations (10-100-fold) of $Gd_3N@C_{80}$ as a new contrast agent. These

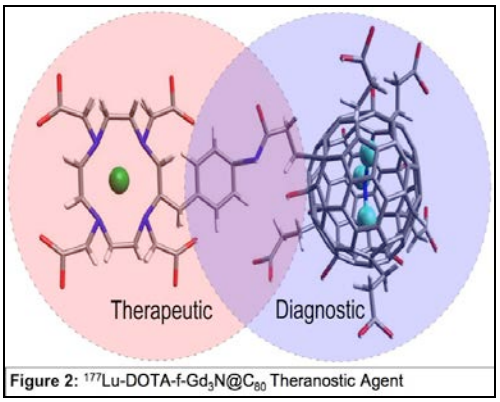

Figure 2: ^{177}Lu-DOTA-f-$Gd_3N@C_{80}$ Theranostic Agent

results demonstrate that water-soluble $Gd_3N@C_{80}$ species functionalized with poly (ethylene glycol) and multihydroxyl groups offer great potential for serving as MR imaging contrast agents because of their T_1 and T_2 relaxivity characteristics – *approximately 40 times greater than conventional gadolinium-containing MR imaging contrast agents.* The $Gd_3N@C_{80}$ nanoparticle was also compared *in vivo* to a typical Gd-based contrast agent injected into a rat brain glioblastoma multiforme (GBM) tumor model.[3]

Results and Discussion

We have recently reported a new multi-modal "theranostic" radiolabeled ^{177}Lu-DOTA-f-$Gd_3N@C_{80}$ EMF platform, (**Figure 2**).[3] This nanoparticle platform involves external functionalization chemistry and subsequent treatment with a radiolabeled $^{177}LuCl_3$ sample (Perkin-Elmer). In a preliminary study, tumors (U87 glioma orthotopic xenograph) were implanted (using CED) in the caudate putamen of 20 female athymic nude mice. On the 8th day post-inoculation, the ^{177}Lu-DOTA-f-$Gd_3N@C_{80}$ (^{177}Lu 30 µCi, 0.25mM $Gd_3N@C_{80}$) was CED-infused in 10 mice and the other 10 mice were infused with 0.25 mM f-$Gd_3N@C_{80}$ (only the MRI diagnostic). **Figure 3** shows the T_1- and T_2-weighted images, in a typical case, out to 16 days post-infusion of the f-$Gd_3N@C_{80}$ agent. The MR contrast characteristics of f-$Gd_3N@C_{80}$ are unique, in that when an optimal concentration is delivered, it is possible to track the material through both T_1 (bright contrast) and T_2 (dark contrast) weighted imaging. This is due to the relaxivity ratio (r_1:r_2) being in a range that allows for T_1 enhancement while not over-shortening T_2 and quenching the signal. Conventional contrast agents are typically only suitable for T_1- (Gd chelates) or T_2- (iron oxide) weighted imaging. Between day 12 and day 16 post-infusion, the tumor volume increases greatly and signs of peritumoral edema become evident. This added water content causes brightening on the T_2 image, which cancels out some of the dark contrast provided by the f-$Gd_3N@C_{80}$'s; however, a bright ring of contrast encompassing the tumor remains in the T_1-weighted image on day 16 (note arrows). Immediately after acquiring the images on day 16, the conventional contrast agent OMNISCAN™ was given intravenously for comparative purposes. The tumor volume highlighted by this agent matched the tumor volume still outlined by the f-$Gd_3N@C_{80}$'s. The survival curves after 60 days for this study are quite remarkable, increasing the median survival from 21 to 52 days. We have also completed a larger study (~100 mice) and established the Lu-177 minimum dosage for effective treatment. Recently, we also published papers that describe the same nanoparticle platform consisting of a gadolinium EMF MRI contrast agent conjugated with the cytokine Interleukin-13 (IL-13) that successfully targets U87-MG glioma cells.[4,5]

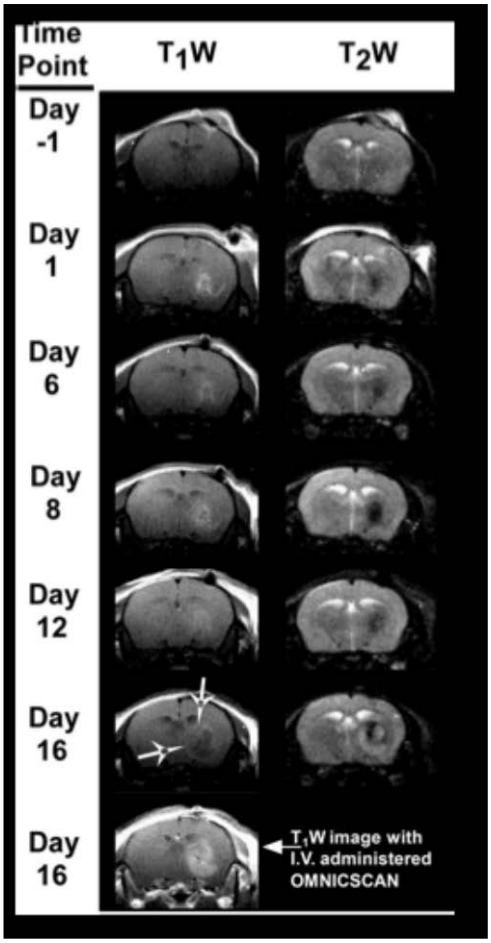

Figure 3: Longitudinal MR images of an untreated tumor bearing mouse brain following a single f-$Gd_3N@C_{80}$

Conclusions

In summary, in this paper we have described a dose-dependent increase in survival in a human GBM

orthotopic tumor-bearing mouse models treated with the radiolabeled metallofullerene, ^{177}Lu-DOTA-f-$Gd_3N@C_{80}$. In addition, this nanoplatform has the potential to be further functionalized to concomitantly deliver other radiosensitizing and other therapeutic and targeting agents. These co-treating agents can be selected to increase the tumor specificity of the therapy, thereby reducing the possibility of normal tissue damage. Along with its MR imaging attributes, the nanoplatform presented may offer advantages to help overcome GBM resistance to current therapies.

References:

1. Shu, C.; Corwin, F.D.; Zhang, J.; Chen, Z.; Reid, J.E.; Sun, M.; Xu, W.; Sim, J.H.; Wang, C.; Fatouros, P.P.; Esker, A.R.; Gibson, H.W.; Dorn, H.C. *Bioconjugate Chemistry*, 20(6), 1186-1193. (2009)

2. Zhang, J.; Shu, C.; Reid, J.; Owens, L.S.; Cai, T.; Gibson, H.W.; Long, G.L.; Corwin, F.D.; Chen, Z.-J.; Fatouros, P.; Dorn, H.C. *Bioconjugate Chemistry*. 24(4), 610-615. (2010)

3. Shultz, M.D.; Wilson, J.D.; Fuller, C.E.; Zhang, J.; Dorn, H.C.; Fatouros, P.P. *Radiology*, 261(1), 136-144. (2011).

4. Shultz, M.D.; Duchamp, J.C.; Wilson, J.D.; Shu, C.; Ge, J.; Zhang, J.; Gibson, H.W.; Fillmore, H.L.; Hirsch, J.I.; Dorn, H.C.; Fatouros, P.P. *Journal of the American Chemical Society*. 132(14), 4980-4981. (2010)

5. Fillmore, H.L.; Shultz, M.D.; Henderson, S.C.; Cooper, P.; Broaddus, W.C.; Chen, Z.-J.; Shu, C.Y.; Zhang, J.; Ge, J.; Dorn, H.C.; Corwin, F.D.; Hirsch, J.I.;Wilson, J.D.; Fatouros, P.P. *Nanomedicine*, 6(3), 449-458 (2011)

6. Kelkar, S. S.; Reineke, T. M. *Bioconjugate Chemistry*, 22 (10), 1879-1903. (2011)

7. Stevenson, S.; Rice, G.; Glass, T.; Harich, K.; Cromer, F.; Jordan, M.R.; Craft, J.; Hadju, E.; Bible, R.; Olmstead, M.M.; Maitra, K.; Fisher, A.J.; Balch, A.L.; Dorn, H.C. *Nature*, 401, 55-57. (1999)