$Gd@C_{82}$ Fullerenol

$Gd@C_{82}(OH)_{40}$

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Chemical name:	$Gd@C_{82}$ Fullerenol	
Abbreviated name:	$Gd@C_{82}(OH)_{40}$	
Synonym:	Gd-fullerenol	
Agent category:	Cage molecule	
Target:		
Target category:	Retention	
Method of detection:	Magnetic resonance imaging (MRI)	
Source of signal/contrast:	Gadolinium	
Activation:	No	
Studies:	• *In vitro* • Rodents	No structure is currently available in PubChem.

Background

[PubMed]

Fullerenes, also known as buckyballs, are spheroidal carbon cages with a molecular formula of C_{2n} (n = 30–41) and a diameter of ~1 nm (1). The high chemical stability of fullerenes can resist any potential metabolic cage-opening process and hence prevents them from degradation in various biological conditions (2). Fullerenes contain hollow interiors that can hold atoms or ions as payloads for various biomedical applications (1). For example, Gd atoms or the trimetallic nitride Gd_3N can be trapped inside the cage to form endohedral metallofullerenes denoted as $Gd@C_{2n}$ or $Gd_3N@C_{2n}$, where the "@" symbol refers to the encapsulated nature of the Gd or Gd_3N. For the $Gd@C_{2n}$, the encapsulation of electropositive Gd atom leads to the transfer of electrons from the Gd atom to the electronegative fullerene cage, resulting in a zwitterion [$Gd^{3+}@C_{82}^{3-}$]. For

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$Gd_3N@C_{2n}$, each Gd atom shares one electron with the N atom and donates the other two electrons to the C80 cage that is in I_h-symmetry to produce a zwitterion $[Gd_3N^{6+}@C_{80}{}^{6-}]$ (3, 4). These endohedral metallofullerenes thus lead to the generation of a novel type of contrast agent for magnetic resonance imaging (MRI), which possesses a high biological safety in that the carbon cage completely prevents the release of toxic Gd^{3+} ions into surrounding tissues (5, 6). The surface of fullerenes can be functionalized with a variety of groups or specific ligands; i.e., the addition of hydroxyls or polyethylene glycols (PEG) substantially increases their aqueous solubility (7).

Gd-Fullerenols ($Gd@C_{82}(OH)_{40}$) are water-soluble Gd endohedral metallofullerenes used with *in vivo* MRI (8). The encapsulation of Gd has three electrons transferred to the C82 fullerene cage, resulting in zwitterions $[Gd^{3+}@C_{82}(OH)_{40}{}^{3-}]$ in which seven unpaired electrons are located on the 4f-orbital of gadolinium ion and one unpaired electron is located on the fullerene cage. These unpaired electrons contribute to the paramagnetic moment of $Gd@C_{82}(OH)_{40}$. Nevertheless, the encapsulation of the Gd ion inside the carbon cage prevents direct coordination and exchange of water to Gd^{3+}. The relaxation enhancement of $Gd@C_{82}(OH)_{40}$ is therefore quite different from the classic inner-sphere mechanism in conventional Gd chelates such as Gd-DTPA but depends on its paramagnetic moment and intermolecular aggregation effect (1). The aggregation leads to an increase in the rotational correlation time and alterations in the water exchange kinetics, which both influence the resultant relaxivity.

Synthesis

[PubMed]

Mikawa et al. reported the preparation of $Gd@C_{82}(OH)_n$ in two steps (8). Initially, $Gd@C_{82}$ was obtained by arc-vaporization of 0.8% Gd/graphite rods under helium atmosphere (50 Torr), followed by extraction with carbon disulfide and then purification with high-performance liquid chromatography. The purified $Gd@C_{82}$ was dissolved in toluene and reacted with 50% aqueous NaOH solution in the presence of 15-crown-5 as a catalyst at room temperature to produce brownish $Gd@C_{82}(OH)_n$. The average number of hydroxyl groups (n) was found to be ~40, yielding a molecular formula of $Gd@C_{82}(OH)_{40}$ (one Gd atom per fulluerene). The charge state of encapsulated gadolinium atom was found to be trivalent (Gd^{3+}) using a Gd $M_{4,5}$-edge electron energy loss spectroscopy.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

The proton relaxivity of $Gd@C_{82}(OH)_{40}$ was measured in aqueous solutions with various magnetic fields. The T_1 relaxivity (R_1) was 67 $mM^{-1}s^{-1}$ at 0.47 T, 81 $mM^{-1}s^{-1}$ at 1.0 T, and 31 $mM^{-1}s^{-1}$ at 4.7 T. The T_2 relaxivity (R_2) was 79 $mM^{-1}s^{-1}$ at 0.47 T, 108 $mM^{-1}s^{-1}$ at 1.0 T, and 131 $mM^{-1}s^{-1}$ at 4.7 T. Compared to the 3.9 $mM^{-1}s^{-1}$ (R_1) and 6.2 $mM^{-1}s^{-1}$ (R_2) of Gd-DTPA at 1.0 T, the relaxivity of $Gd@C_{82}(OH)_{40}$ (R_1 or R_2) was increased ~20-fold. In

addition, the relaxivity of $Gd@C_{82}(OH)_{40}$ appeared to be significantly influenced by the pH and ionic strength in the solution; i.e., a bell shape profile was observed for T_1 relaxivity in the pH range of 3–8, and T_1 relaxivity was lower in a 1.0 M aqueous NaCl solution than in water (61 $mM^{-1}s^{-1}$ *versus* 67 $mM^{-1}s^{-1}$).

Anderson et al. used $Gd@C_{82}(OH)_{40}$ to label human cervical carcinoma cells (HeLa) and human mesenchymal stem cells (MSCs) (9). Cells were incubated for 10 h with 0.5 µmol Gd/ml $Gd@C_{82}(OH)_{40}$ in the presence of the transfection agent protamine sulfate. The labeled cells exhibited ~100% labeling efficiency and viability with cellular Gd content of 1.19×10^{-10} mg per cell (7.5×10^{-13} mmol Gd per cell). The cellular proliferation was evaluated with 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay; the MTT ratio was 74–78% for day 2 through day 6 and ~100% by day 9. The internalized $Gd@C_{82}(OH)_{40}$ nanoparticles were found with electron microscopy to be aggregated within the endosomes in cytoplasm. In addition, the labeled MSCs demonstrated normal adipogenic, chondrogenic, and osteogenic differentiation compared with the unlabeled control cells. The relaxation enhancement efficiency was examined *ex vivo* in the labeled HeLa cells with a 7-T MR imager. An increase of ~200% in the signal intensity was observed in the T_1-weighted images compared to unlabeled cells.

Animal Studies

Rodents

[PubMed]

Mikawa et al. examined the biodistribution of $Gd@C_{82}(OH)_{40}$ in mice with *in vivo* MRI, followed by Gd assay with inductively coupled plasma-atomic emission spectrometry (ICP-AES) (8). CDF1 mice (*n* = 3, 8 wks old, ~20 g) were injected intravenously with $Gd@C_{82}(OH)_{40}$ at a dose of 5 µmol Gd/kg (compared to the typical dose of 100 µmol Gd/kg for Gd-DTPA). T_1-Weighted images were collected with a 4.7-T imager before injection and at 30 min, 1 h, and 24 h after injection. Strong signal enhancement was observed in the lung, liver, spleen, and kidney. The Gd content in these organs was quantified with ICP-AES: ~60 µg Gd/g tissue in the lung, ~8 µg Gd/g tissue in the liver, ~5 µg Gd/g tissue in the spleen, and ~3 µg Gd/g tissue in the blood at 30 min; ~25 µg Gd/g tissue in the lung, ~10 µg Gd/g tissue in the liver, ~8 µg Gd/g tissue in the spleen, and <2 µg Gd/g tissue in the blood at 1 h; <1 µg Gd/g tissue in the lung, ~9 µg Gd/g tissue in the liver, ~12 µg Gd/g tissue in the spleen, and fell into background in the blood at 24 h. These results demonstrated that $Gd@C_{82}(OH)_{40}$ was readily trapped in the reticuloendothelial system.

Anderson et al. examined the relaxation enhancement efficiency of $Gd@C_{82}(OH)_{40}$-labeled cells *in vivo* with MRI (9). Rats were injected intramuscularly with 3×10^6 $Gd@C_{82}(OH)_{40}$-labeled MSCs in the thigh and imaged with a 7-T imager. A 33% increase in imaging contrast *versus* muscle was observed in the T_1-weighted images. As a comparison, a rat was injected intramuscularly with 4×10^6 $Gd@C_{82}(OH)_{40}$-labeled

MSCs (~1.19×10^{-10} mg Gd per cell or 7.5×10^{-13} mmol Gd per cell) in the thigh and imaged at 1.5 T. These labeled cells demonstrated an 83% increase in signal intensity compared to the muscle in the T_1-weighted images.

Other Non-Primate Mammals

[PubMed]

No publication is currently available.

Non-Human Primates

[PubMed]

No publication is currently available.

Human Studies

[PubMed]

No publication is currently available.

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References

1. Bolskar R.D. Gadofullerene MRI contrast agents. Nanomed. 2008;3(2):201–13. PubMed PMID: 18373426.
2. Fatouros P.P., Corwin F.D., Chen Z.J., Broaddus W.C., Tatum J.L., Kettenmann B., Ge Z., Gibson H.W., Russ J.L., Leonard A.P., Duchamp J.C., Dorn H.C. In vitro and in vivo imaging studies of a new endohedral metallofullerene nanoparticle. Radiology. 2006;240(3):756–64. PubMed PMID: 16837672.
3. Aihara J. Kinetic stability of metabllofullerenes as predicted by bond resonance enery model. Phys. Chem. Chem. Phys. 2001;3:1427.
4. Ge Z., Duchamp J.C., Cai T., Gibson H.W., Dorn H.C. Purification of endohedral trimetallic nitride fullerenes in a single, facile step. J Am Chem Soc. 2005;127(46): 16292–8. PubMed PMID: 16287323.
5. Lu J., Sabirianov R.F., Mei W.N., Gao Y., Duan C.G., Zeng X. Structural and magnetic properties of Gd3N@C80. J Phys Chem B. 2006;110(47):23637–40. PubMed PMID: 17125320.
6. Krause M., Dunsch L. Gadolinium nitride Gd3N in carbon cages: the influence of cluster size and bond strength. Angew Chem Int Ed Engl. 2005;44(10):1557–60. PubMed PMID: 15674866.
7. MacFarland D.K., Walker K.L., Lenk R.P., Wilson S.R., Kumar K., Kepley C.L., Garbow J.R. Hydrochalarones: a novel endohedral metallofullerene platform for

enhancing magnetic resonance imaging contrast. J Med Chem. 2008;51(13):3681–3. PubMed PMID: 18558670.

8. Mikawa M., Kato H., Okumura M., Narazaki M., Kanazawa Y., Miwa N., Shinohara H. Paramagnetic water-soluble metallofullerenes having the highest relaxivity for MRI contrast agents. Bioconjug Chem. 2001;12(4):510–4. PubMed PMID: 11459454.
9. Anderson S.A., Lee K.K., Frank J.A. Gadolinium-fullerenol as a paramagnetic contrast agent for cellular imaging. Invest Radiol. 2006;41(3):332–8. PubMed PMID: 16481917.