Gd-DOTA-anti-Aβ42-F(ab')$_2$-antibody fragment (putrescine)$_n$

GdAβ42

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Created: November 7, 2008; Updated: December 22, 2008.

Chemical name:	Gd-DOTA-anti-Aβ42-F(ab')$_2$ -antibody fragment (putrescine)$_n$	
Abbreviated name:	GdAβ42	
Synonym:		
Agent category:	F(ab')$_2$ antibody fragment	
Target:	Fibrillar Aβ42	
Target category:	Antibody-antigen binding	
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]

Alzheimer disease (AD) is a neurodegenerative disease characterized by the accumulation of extracellular neuritic or senile plaques and neurofibrillary tangles (NFTs) in the human brain (1, 2). A neuritic plaque contains a dense core formed by amyloid-β peptides (Aβ), which are 39–43 amino acids in length and are derived from proteolytic cleavage of amyloid precursor protein (APP) by β- and γ-secretases. NFTs are formed by intraneuronal bundles of paired helical filaments mainly from the aggregates of hyperphosphorylated tau protein (2). Both types of deposits, each with a diameter of ~10

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NLM Citation: Zhang H. Gd-DOTA-anti-Aβ42-F(ab')$_2$-antibody fragment (putrescine)$_n$. 2008 Nov 7 [Updated 2008 Dec 22]. In: Molecular Imaging and Contrast Agent Database (MICAD) [Internet]. Bethesda (MD): National Center for Biotechnology Information (US); 2004-2013.

nm, are caused by aggregation of highly hydrophobic peptides and can generate significant neurotoxicity (3). The mechanism of Aβ plaque has been extensively studied in various animal models. For instance, four genes have been identified as related to the early onset of AD, including APP, human presenilin 1 (PS1), human presenilin 2 (PS2), and apolipoprotein E (ApoE) (2). Several transgenic models such as the APP single transgenic mouse (Tg2576), the PS1 single transgenic mouse (M146L6.2), or the APP/PS1 double transgenic mouse, produced by mating a Tg2576 mouse with a M146L6.2 mouse, have been developed to study AD (4). These animal models readily produce Aβ plaques in their brains and generate behavioral deficits (5, 6).

One of the promising therapeutic strategies for amyloid clearance is immunization against Aβ, either with active vaccination *via* Aβ injection or with intravenous administration of anti-Aβ antibodies (7). IgG4.1 is an anti-Aβ antibody that binds to the first 15 residues in soluble or fibrillar Aβ40/42, an Aβ that is 40 or 42 amino acids in length (4). A fragment with smaller molecular weight, $F(ab')_2$4.1, can be obtained by removal of the Fc portion in IgG4.1. Use of this fragment can minimize inflammatory response and reduce molecular weight for better diffusion through the blood brain barrier (BBB). In addition, polyamines such as putrescine (1,4-diaminobutane) can be conjugated to the fragment, which can increase the permeability at the BBB many fold (1). Gd-1,4,7,10-tetra-azacyclododecane-1,4,5,10-tetraacetic acid (DOTA)-anti-Aβ42-$F(ab')_2$-antibody fragment $(putrescine)_n$ (GdAβ42) is a magnetic resonance imaging (MRI) agent used for imaging Aβ plaques *in vivo* (4). GdAβ42 consists of three components: Gd chelates (Gd-DOTA) for T_1-relaxivity enhancement, an anti-Aβ42 antibody fragment $F(ab')_2$4.1 to bind Aβ plaques, and putrescine molecules that are covalently attached to the antibody fragment to enhance the BBB permeability.

Synthesis

[PubMed]

Ramakrishnan et al. reported the preparation of GdAβ42 (4). Initially, antibody fragment $F(ab')_2$4.1 was obtained by digesting monoclonal antibody IgG4.1 using ficin at pH 6.5. Then the chelating agent DOTA was coupled to $F(ab')_2$4.1 *via* carbodiimide reaction in two steps: DOTA was activated with carbodiimide, followed by conjugation with the ε-amino group of lysine residues in the $F(ab')_2$4.1. The number of DOTA molecules was controlled to be 1–2 per $F(ab')_2$4.1. The obtained DOTA-$F(ab')_2$4.1 was reacted with $GdCl_3$ to form Gd-DOTA-$F(ab')_2$4.1. The protein carboxylic acid groups of Gd-DOTA-$F(ab')_2$4.1 were further activated with 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride followed by conjugation with the primary amine group of putrescine to produce GdAβ42.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Ramakrishnan et al. examined the binding of GdAβ42 to amyloid plaques *in vitro* (4). GdAβ42 was radiolabeled with ^{125}I using the chloramine-T procedure. After incubation of ^{125}I-labeled GdAβ42 with unfixed brain sections of an APP/PS1 double transgenic mouse for 3 h, the brain sections were dipped in an autoradiographic emulsion for ^{125}I. The amyloid plaques in the mouse brain sections were found to be labeled with GdAβ42. Then the contrast enhancement of GdAβ42 in MRI was examined *in vitro*. GdAβ42 was incubated with unfixed brain slices (500 μm thick) from a 29-month-old APP transgenic mouse for 2 h, at which time T_1-weighted images were collected with a 9.4-T imager with a voxel dimension of 60×60×120 μm^3. In the slices containing hippocampus, the T_1-weighted images exhibited many hyperintense foci (bright spots) throughout the cortex and hippocampus, which further confirmed the binding of GdAβ42 to the amyloid plaques. As a control, brain slices from a wild-type mouse were imaged, but they did not show any hyperintense foci in T_1-weighted images.

Animal Studies

Rodents

[PubMed]

Ramakrishnan et al. measured the plasma pharmacokinetics of GdAβ42 in mice (4). GdAβ42 was radiolabeled with ^{125}I and injected intravenously into a 6-month-old wild-type mouse at a dose of 100 μCi (3.7 MBq). Series blood samples were collected over a period of time. ^{125}I-GdAβ42 had a distribution rate constant of 0.037 ± 0.012 min^{-1} in the α-phase, an elimination rate constant of 0.005 ± 0.002 min^{-1} for the β-phase, a clearance of 0.0469 ± 0.0084 ml/min, and a maximum plasma concentration of 21.52 ± 0.76 μCi/ml (0.796 ± 0.028 MBq/ml). After the final blood sample, the mouse was euthanized and tissues were harvested for quantification of ^{125}I radioactivity. The biodistribution of ^{125}I-GdAβ42 was ~2% in brain and liver, <1% in heart and lung, ~3% in spleen, and ~ 4% in kidney. The permeability coefficient × surface area product (PS; $ml{\cdot}g^{-1}s^{-1} \times 10^{-6}$) was evaluated for six brain tissues: 39.8 ± 1.7 in cortex, 26.8 ± 1.2 in caudate-putamen, 48.0 ± 2.2 in hippocampus, 42.7 ± 1.9 in thalamus, 57.8 ± 2.6 in brainstem, and 56.0 ± 2.5 in cerebellum. Their corresponding vascular space (V_p; μl/g) values were 24.8 ± 6.5 in cortex, 13.8 ± 4.3 in caudate-putamen, 32.7 ± 9.6 in hippocampus, 27.0 ± 7.9 in thalamus, 39.0 ± 9.4 in brainstem, and 38.9 ± 10.7 in cerebellum. Compared to the PS of 1–2 × 10^{-6} $ml{\cdot}g^{-1}s^{-1}$ for native $F(ab')_2$4.1, the BBB permeability of ^{125}I-GdAβ42 increased 18–33-fold, whereas their V_p appeared to be similar.

Ramakrishnan et al. also examined the *in vivo* labeling of amyloid plaques with ^{125}I-GdAβ42 (4). APP/PS1 double transgenic mice (n = 5; 9 months old) were injected intravenously with ^{125}I-GdAβ42 at a dose of 2 mCi (74 MBq). The animals were euthanized 4 h later and brain tissues were sectioned. The amyloid plaques were stained with anti-Aβ antibody, and the radiolabeled immunoglobins were detected with emulsion microautoradiography. The amyloid plaques were found to be labeled with ^{125}I-GdAβ42

and distributed throughout the cortex and hippocampus, arising from an increased permeability of GdAβ42 at the BBB.

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.

NIH Support

NS 57091

References

1. Wengenack T.M., Curran G.L., Poduslo J.F. Targeting alzheimer amyloid plaques in vivo. Nat Biotechnol. 2000;18(8):868–72. PubMed PMID: 10932157.
2. Villemagne V.L., Fodero-Tavoletti M.T., Pike K.E., Cappai R., Masters C.L., Rowe C.C. The ART of loss: Abeta imaging in the evaluation of Alzheimer's disease and other dementias. Mol Neurobiol. 2008;38(1):1–15. PubMed PMID: 18690556.
3. Goedert M., Spillantini M.G. A century of Alzheimer's disease. Science. 2006;314(5800):777–81. PubMed PMID: 17082447.
4. Ramakrishnan M., Wengenack T.M., Kandimalla K.K., Curran G.L., Gilles E.J., Ramirez-Alvarado M., Lin J., Garwood M., Jack C.R. Jr, Poduslo J.F. Selective contrast enhancement of individual Alzheimer's disease amyloid plaques using a polyamine and Gd-DOTA conjugated antibody fragment against fibrillar Abeta42 for magnetic resonance molecular imaging. Pharm Res. 2008;25(8):1861–72. PubMed PMID: 18443900.
5. Poduslo J.F., Ramakrishnan M., Holasek S.S., Ramirez-Alvarado M., Kandimalla K.K., Gilles E.J., Curran G.L., Wengenack T.M. In vivo targeting of antibody fragments to the nervous system for Alzheimer's disease immunotherapy and molecular imaging of amyloid plaques. J Neurochem. 2007;102(2):420–33. PubMed PMID: 17596213.
6. Radde R., Duma C., Goedert M., Jucker M. The value of incomplete mouse models of Alzheimer's disease. Eur J Nucl Med Mol Imaging. 2008;35Suppl 1S70–4. PubMed PMID: 18270700.

7. Raymond S.B., Treat L.H., Dewey J.D., McDannold N.J., Hynynen K., Bacskai B.J. Ultrasound enhanced delivery of molecular imaging and therapeutic agents in Alzheimer's disease mouse models. PLoS ONE. 2008;3(5):e2175. PubMed PMID: 18478109.