Avidin-gadolinium

Avidin-Gd

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Chemical name:	Avidin-gadolinium	
Abbreviated name:	Avidin-Gd	
Synonym:		
Agent Category:	Protein	
Target:	Biotinylated anti-HER-2/*neu* antibody	
Target Category:	Protein–protein binding	
Method of detection:	Magnetic resonance imaging (MRI)	
Source of Signal/Contrast:	Gadolinium	
Activation:	No	
Studies:	• *In vitro* • Rodents	No information is available.

Background

[PubMed]

Biotin is a protein found in mammalian cells and is considered to be a vitamin because it has an important function in the cellular metabolism of lipids and carbohydrates. Avidin is a protein that is found primarily in the egg white or in bacteria (known as streptavidin if isolated from bacteria; *Streptomyces avidinii* is the source of this protein). Although the two proteins have different origins, they are known to have a very strong non-covalent interaction (discovered almost seven decades ago) with a dissociation constant (K_d) of 4×10^{-14} M (1, 2). The avidin–biotin interaction has been described in detail by Wilchek et al. (3). Only harsh conditions (e.g., treatment with formaldehyde at high temperature) are known to break the avidin/streptavidin–biotin bond, and the released proteins cannot be reused for any other work due to deterioration of the samples (4). Because of their strong

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interaction, the avidin/streptavidin–biotin system has been extensively studied and is widely used after conjugation of either protein to other proteins, nucleic acids, or other matrices for research and diagnostic purposes, including imaging techniques (5-8).

Because of its overexpression, the human epidermal growth factor receptor 2 (HER2/*neu*) is an important target for the diagnosis, treatment, and prognosis of cancers such as those of the breast, lungs, etc (9, 10). As a result, humanized monoclonal antibodies (MAbs) labeled with different nuclides or imaging and contrast agents targeted toward HER2/*neu* have been used for the *in vitro* and *in vivo* imaging of cancers (11-13). Some humanized MAbs against HER2/*neu* alone or their conjugated derivatives are used, or are in development, as immunotherapeutic agents for the treatment of this ailment (14, 15). The avidin–biotin system has been used to visualize HER2/*neu*-positive tumors in transgenic mice by some investigators with magnetic resonance imaging (MRI) (16, 17). To achieve this, the investigators prelabeled the cells expressing HER2/*neu*, under *in vitro* and *in vivo* conditions, with a commercially available biotinylated mouse MAb. The biotinylated MAb was allowed to wash or clear out from the respective test systems, and this was followed by the administration of avidin conjugated to gadolinium (avidin-Gd) to the cells or animals. MRI was subsequently performed, and it was demonstrated that a high positive T1 contrast could be achieved under the *in vitro* and *in vivo* conditions used.

Synthesis

[PubMed]

The synthesis of avidin-Gd was performed as described by Artemov et al. using a modified procedure of Hnatowich et al. (13, 18). Briefly, 20-fold excess cyclic anhydride of diethylenetriaminepentaacetic acid (DTPA) was allowed to react with avidin for 24 h at 4°C. Excess low molecular weight reagents from the conjugated avidin (avidin-DTPA) were removed by centrifuge filtration through a device with a 10K cut-off limit. The avidin-DTPA was then reacted with Gd-(1-(*2*-naphthoyl)-3,3,3-trifluoroacetonate)$_2$ to obtain avidin-GdDTPA (avidin-GdDTPA) as described above. Avidin-GdDTPA was separated from excess low molecular weight compounds as detailed above. On average, 12.5 GdDTPA groups were reported to be attached to each avidin molecule.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

For *in vitro* studies, NT-5 cells expressing HER-2/*neu* were derived from spontaneous mammary tumors of female HER-2/*neu* transgenic mice (13). In the same study, mouse carcinoma EMT-6 cells that do not express HER-2/*neu* were used as controls. Expression of HER-2/*neu* by the two cell lines was confirmed with fluorescence-activated cell-sorting analysis using appropriate reagents (13). The cells were exposed to biotinylated anti-HER-2/*neu* MAb in phosphate-buffered saline (PBS) containing bovine serum albumin (BSA) for 30 min at room temperature. The cells were then washed with PBS several times and incubated with avidin-GdDTPA for 5 min at room temperature. Control cells were

exposed to a non-specific MAb or to avidin-GdDTPA. The cells were washed again as detailed above and fixed in 2% paraformaldehyde in PBS in a nuclear magnetic resonance tube. Subsequently, T1-weighted images (repetition time, TR = 1 s; spin echo time, TE = 8 ms) were obtained with an MRI probe. Only the NT-5 cells that were exposed to the biotinylated anti-HER-2/*neu* MAb showed a positive T1 contrast with avidin-GdDTPA, and the EMT-6 cells had a low background signal.

Animal Studies

Rodents

[PubMed]

In vivo MRI studies were performed with severe combined immunodeficient mice bearing NT-5 and EMT-6 cell tumors (13). All experimental animals (n = 5/time point) were treated with the biotinylated anti-HER-2/*neu* antibodies, and the control animals bearing NT-5 tumors (n = 3) were treated with BSA in saline. The test animals were injected with the avidin-GdDTPA conjugate 12 h after the biotinylated MAb treatment. Control animals were given avidin-GdDTPA *via* intravenous injection. Parameters for the T1-weighted images were as follow: TR = 300 ms, TE = 15 ms, flip angle = 90° with a field view of 32 mm; 16 slice images were acquired over 15 min with a slice thickness of 2 mm. MRI images were obtained at 1, 8, 24, and 48 h after contrast treatment. The NT-5 tumors were reported to retain the contrast for 8–24 h after treatment, but the contrast decreased in the EMT-6 cell tumors 1 h after treatment. Also, the NT-5 tumors that were not pre-treated with the biotinylated MAb were reported to show no retention of the contrast agent. A statistically significant ($P < 0.05$) increase in signal was observed in the tumors treated with NT-5 (~1.7 relative units for the cells treated with NT-5 *versus* ~1 relative unit for the NT-5 control and EMT-6 cells) under these experimental conditions.

Other Non-Primate Mammals

[PubMed]

No references are currently available.

Non-Human Primates

[PubMed]

No references are currently available.

Human Studies

[PubMed]

No references are currently available.

Supplemental Information

[Disclaimers]

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