Trastuzumab-dextran iron oxide nanoparticles

Trastuzumab-dextran NP

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Chemical name:	Trastuzumab-dextran iron oxide nanoparticles	
Abbreviated name:	Trastuzumab-dextran NP	
Synonym:	Herceptin-dextran NP	
Agent Category:	Humanized monoclonal antibody	
Target:	HER2 or ErbB2/neu receptor	
Target Category:	Antibody-receptor binding	
Method of detection:	Magnetic resonance imaging (MRI)	
Source of Signal/Contrast:	Iron oxide nanoparticles	
Activation:	No	
Studies:	• *In vitro* • Rodents	Click here for the human nucleotide and protein sequence of HER2.

Background

[PubMed]

Breast cancer tumors that express high levels of human epidermal growth factor receptor 2 (commonly known as HER2/neu or the ErbB-2 receptor; also designated as the cluster of differentiation 340 (CD340)) indicate a poor prognosis for the patient because this receptor promotes rapid proliferation and spread of cancer cells into the surrounding tissues (1-3). Trastuzumab, a humanized monoclonal antibody (MAb) against HER2/neu, is commercially available in the United States. This MAb is approved by the United States Food and Drug Administration for the treatment of the primary and metastatic forms of breast cancer. This MAb is also being evaluated in clinical trials for the treatment of a variety of cancers. Because of its specificity for tumors expressing the HER2/neu receptor, trastuzumab has been conjugated with a variety of radionuclides and used for the

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diagnosis of various cancer types, including breast cancer, using different imaging techniques (4, 5).

Investigators have recently developed a series of new contrast agents that contain superparamagnetic iron oxide (SPIO) particles that can be used to visualize different tissue pathologies, including cancers, with magnetic resonance imaging (MRI) (6, 7). The particles have also been used in several *in vivo* applications such as immunoassays, disease diagnosis, drug delivery, and biological fluid detoxification (8-11). To make the SPIO particles biocompatible with the mammalian system, the particles are chemically cross-linked with polymers such as dextran, polyethylene glycols, and polyvinylpyrrolidines that have themselves been used as drug carriers. Chen et al. used dextran cross-linked iron oxide nanoparticles (CLIO NPs) to conjugate trastuzumab and used the trastuzumab-NPs for the *in vitro* detection of HER2/neu expression in breast cancer cell lines (12). The NPs were also used for the *in vivo* MRI imaging of mice bearing breast cancer tumors.

Synthesis

[PubMed]

The synthesis of trastuzumab-dextran iron oxide NP was described by Chen et al. (12). Briefly, the SPIO particles were prepared by mixing dextran-T40 with an aqueous solution of ferric and ferrous chloride at room temperature followed by the addition of ammonium hydroxide. The mixture was stirred for 1 hr and centrifuged to remove any aggregated particles. The SPIO NPs were then purified from excess dextran with gel filtration chromatography on a Sephacryl S-300 column. The dextran-coated SPIO NPs were reported to elute in the void volume under these chromatographic conditions. Iron concentration of the SPIO NPs was determined as described by Chen et al. (12). The cross-linking of dextran on the SPIO NPs to obtain the CLIO NP was achieved by stirring the dextran-coated SPIO NPs with a solution of epichlorohydrin and sodium hydroxide for 24 h at room temperature. The CLIO NPs were subsequently purified with dialysis against several changes of distilled water. The CLIO NPs were then incubated with 2,2'-(ethylenedioxy)bisethylamine (EDBE) for 24 h at room temperature to generate the primary amine groups at the end of dextran and to obtain the CLIO-EDBE NPs. Purification of the CLIO-EDBE NPs was achieved with dialysis against distilled water as described above. Generation of the various NPs (SPIO, CLIO, and CLIO-EDBE) was confirmed with Fourier transform infrared spectroscopy.

To generate the trastuzumab-NPs, the CLIO-EDBE NPs were incubated with commercially available trastuzumab in a solution containing 1-hydroxybenzotriazole and (benzotriazole-1-yloxy)tripyrrolidinophosphonium hexafluorophosphate at room temperature for 24 h (12). Unbound trastuzumab was removed with gel filtration chromatography on a Sephadex-G25 column, and the trastuzumab-NPs were eluted in phosphate-buffered saline (PBS) (the pH of PBS used for the elution was not mentioned in the publication).

The core size of the trastuzumab-NPs was 3.5 ± 0.3 nm as determined with transmission electron microscopy (12). The number of trastuzumab molecules/NP was not reported in the publication (12). The pH-dependent stability of trastuzumab-NP was investigated by dispersing the NPs in PBS (pH 7.4) and in buffers with pH values ranging from 4 to 10 (12). The NPs were reported to show no aggregation at any pH for at least 4 months as confirmed with the dynamic light-scattering technique. The investigators also reported that the r_1 and r_2 relaxivity values, as measured at 20 MHz with a relaxometer, showed little change over 4 months at 37.0 ± 0.1°C.

In Vitro Studies: Testing in Cells and Tissues

[PubMed]

Human breast cancer cell lines SKBR3, BT-474, MDA-MB-231, and MCF-7 that express HER2/neu receptors were used to study the *in vitro* cytotoxicity and receptor targeting of trastuzumab-NP (12). For this study the KB human nasopharyngeal epidermal carcinoma cell line, which does not express HER2/neu receptors, was used as a control. For the cytotoxicity study an equal number of cells of the respective cell lines were exposed to trastuzumab-NP at concentrations ranging from 1 to 10 mM Fe. It was reported although trastuzumab-NP was not cytotoxic to any cell line at the lowest concentration (1 mM Fe), it was observed to be toxic to all cell lines at higher concentrations (5 and 10 mM Fe) (12). The receptor targeting of trastuzumab-NP was studied with the same cell lines mentioned above using a concentration of 1 mM Fe (12). After exposure to trastuzumab-NP, the cells were treated with an anti-human IgG (γ-chain) conjugated to fluorescein isothiocyanate (anti-human IgG-FITC). The cells were washed with PBS and fixed with paraformaldehyde for observation under a confocal microscope. Only cells expressing the HER2/neu receptors (BT-474, SKBR-3, MDA-MB-231, and MCF-7) were reported to show fluorescence (MDA-MB-231> BT-474> SKBR-3≥ MCF-7) with the anti-human IgG-FITC conjugate, indicating that the trastuzumab-NPs specifically bound to the HER2/neu receptors on these cells.

In another study the different cell lines were respectively exposed to trastuzumab-NP (0.3 mM Fe), washed with PBS, and scanned with a magnetic resonance scanner (12). As expected, only cells expressing the HER2/neu receptor were reported to show a negative enhancement of the MRI signal. No such negative enhancement was evident with the control KB cells.

Animal Studies

Rodents

[PubMed]

Nude mice (n = 5) bearing SKBR-3 (HER2/neu expressing) and KB (HER2/neu non-expressing) cell tumors in the left and right lateral thighs, respectively, were used to investigate the targeting of HER2/neu expressing and non-expressing tumors by

trastuzumab-NP (12). The T_2-weighted fast spin-echo imaging was performed on the mice 1 h after an intravenous injection of trastuzumab-NP. Compared with the signal with the KB cell tumors, a significant drop in the T_2-weighted signal was reported only with the SKBR-3 cell tumors (*P* values were not reported). This indicated that the trastuzumab-NPs specifically targeted the tumors expressing HER2/neu. No competition study using trastuzumab-NP after a pre-treatment of the animals with trastuzumab alone was reported.

The biodistribution of trastuzumab-NP was investigated in 18 male ICR mice after an intravenous injection (20 μmol/kg) of the NPs (12). The animals were euthanized at various time intervals up to 24 h after injection, and the blood, brain, heart, lungs, liver, spleen, and the kidneys from the mice were harvested. The respective organs were washed with PBS, and the Fe concentration of each whole organ was measured with inductively coupled plasma mass spectrometry. The results were reported as a percentage of injected dose per gram of tissue (% ID/g). The serum Fe concentration was reported to increase for 1 h after injection, and it remained stable up to 1.5 h. Subsequently, at 24 h the serum Fe concentration was reported to drop to background levels. The brain showed almost no accumulation of the NPs. The heart showed an increase in Fe concentration up to 30 min after injection, and the increase in concentration remained fairly stable up to 24 h. The lungs showed an increase in accumulation of the NPs from 15 to 30 min, and subsequently the concentration was reduced by 24 h after the injection. Compared to the other organs, the liver and kidneys showed a lower and fairly stable accumulation of the NPs up to 24 h after treatment. The spleen showed an increased NP accumulation, reaching a maximum of ~600% ID/g; however, this organ had a high background Fe level (~300% ID/g).

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.

Supplemental Information

[Disclaimers]

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