Casein Coated Magnetic Iron Oxide Nanoparticles for Targeted Imaging and Drug Delivery

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Purpose

Magnetic iron oxide nanoparticles have shown not only superb MRI contrast enhancement for improving diagnostic sensitivity but also unique capability of imaging guided drug delivery to facilitate the treatment.[1-3] Here we report the development of a novel theranostic nanoplatform that is composed of milk protein casein coated magnetic iron oxide nanoparticles (CNIOs), near infrared dye Cy5.5, chemotherapy agent cisplatin (cis) and folate receptor targeted folic acid (FA) for MRI monitored drug delivery in cancer animal models.

Materials and Methods

The hydrophobic iron oxide nanoparticles (IOs) are prepared from heating iron oxide powder with oleic acid. After exchanging the oleic acid with oligosaccharides on the surface, obtained water-soluble particles (SIOs) were then encapsulated into casein to form protein coated iron oxide nanoparticles (CNIOs). Then folic acid was conjugated on the surface using EDC/NHS via a covalent link of the carboxyl groups of folic acids with the amine groups of the casein on CNIO coating, giving folate receptor targeted FAIO nanoparticles. Near infra-red (NIR) dye molecules, Cy5.5-NHS, were then cross-linked onto FAIOs. Chemotherapeutic agent, cisplatin, was loaded on FAIOs by mixing cisplatin with Cy5.5-FAIO, with carboxyl groups on FAIOs coordinating with cisplatin molecules. Morphology, size distribution and composition of Cy5.5-FAIO-cis were characterized by TEM, DLS, gel electrophoresis, IR, UV-Vis and Bradford assays. Stability, transverse relaxivity and MRI contrast enhancing effects at different pH, and pH-responsive properties were investigated by DLS, zeta potential measurements and multi-echo T_2 weighted spin echo MRI. For in vitro experiment, ovarian cancer SKOV3 cells with over expression of the folate receptor were incubated with FAIO-cis at 37 °C for 72 h. Cell counting kit (cck-8) was used to evaluate the proliferation of the viable cells. For in vivo experiment, mice (n=5) bearing head and neck tumor were treated with FAIO-cis twice a week at the dosage of 0.2 mg Pt/kg mouse weight. Cy5.5-FAIO-cis were intravenous administrated into mice bearing head and neck tumor. NIR and MR images were collected at before and 24 h after the administration of Cy5.5-FAIO-cis.

Results

Developed cisplatin loaded nanoparticle construct (Cy5.5-FAIO-cis) has excellent water solubility and colloidal stability. The casein coated magnetic iron oxide nanoparticles exhibit significantly higher transverse relaxivity, R2, than that of iron oxide nanoparticles with conventional synthetic polymer coating, suggesting a potential to improve the sensitivity of MRI. Target specific binding of Cy5.5-FAIO-cis to cancer cells with overexpression of folate receptor was confirmed by Prussian blue staining and confocal microscopy images (Fig. 1b). Sustained release of cisplatin from Cy5.5-FAIO-cis was observed in vitro in the physiological condition as shown in Fig. 1c. IC_{50} measurements showed that cytotoxicity of Cy5.5-FAIO-cis to cancer cells is much higher than free cisplatin (Fig. 1d), which may allow for applying an effective dosage while reducing the side effect of cisplatin. When treating mice bearing head and neck tumor with Cy5.5-FAIO-cis intravenously, tumor targeted delivery of cisplatin can be monitored by both NIR (Fig. 2a) and MRI (Fig. 2b) to visualize the accumulation of Cy5.5-FAIO-cis. Furthermore, improved therapeutic efficacy by CNIO delivery of cisplatin was observed in comparison with administering free cisplatin. The averaged tumor volume of the groups treated with FAIO-cis or CNIO-cis was about four times smaller than the group treated with free cisplatin. The results indicate that casein coated magnetic iron oxide nanoparticles may provide a promising theranostic platform for imaging guided drug delivery and improving the efficacy of chemotherapy.

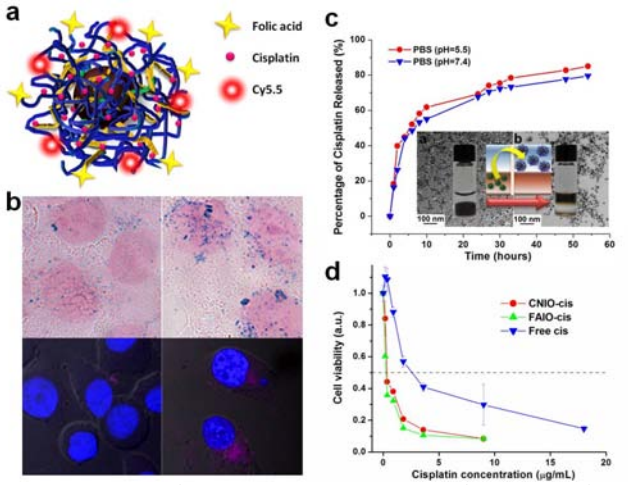

Fig 1. a) Illustration of Cy5.5-FAIO-cis; b) right: specific targeting of Cy5.5-FAIO-cis to FR-expressing SQCC-Y1 cells, comparing with non-targeted Cy5.5-CNIO-cis (left), assessed by Prussian Blue staining (up) and confocal microsope (bottom); c) release profile of FAIO-cis in PBS (pH=7.4) and in pH=5.5 PBS at 37 °C , half life time is evaluated to be ~5 h, inset is the typical TEM images of IOs before modification (left) and after modification (right); d) IC50 assay of FAIO-cis by co-incubating with SKOV3 cell lines at 37 °C for 72 h, drug loaded on nanovehicles (CNIO-cis, FAIO-cis) shows higher therapeutic efficacy compared with free cisplatin.

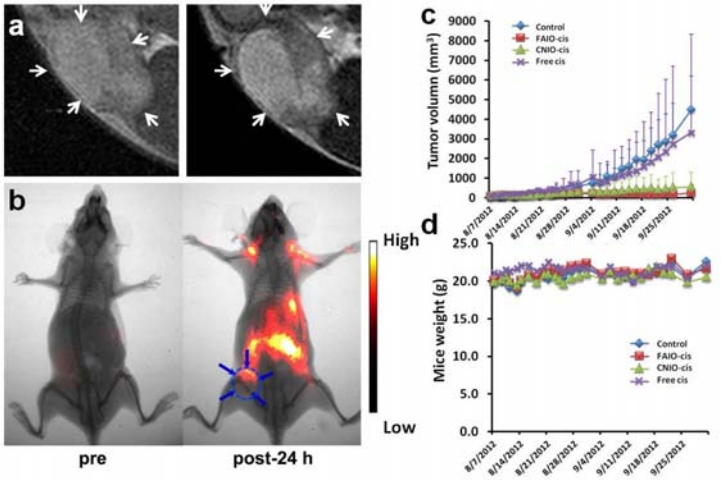

Fig 2. a) In vivo MRI and b) NIR imaging of mice bearing head and neck tumor model. Cy5.5-FAIO-cis was administrated through tail veil injection at the dosage of 10 mg Fe/kg mouse weight. Images were performed at pre-injection and 24 h after the injection. c) Therapeutic effect of drug loaded nanostructures to the mice bearing head and neck tumors. The dosage is 0.2 mg Pt/kg mouse weight. Targeted nanovehicles, together with non-targeted nanovehicles showed stronger tumor suppression, compared with free drug. However, there is no significant difference between targeted and non-targeted nanostructures.

Conclusions

A new class of multifunctional magnetic nanoparticle construct, combining NIR/MRI imaging, nanoparticle drug delivery, chemotherapeutics and tumor targeting, has been developed based on milk protein casein coated iron oxide nanoparticles. These Cy5.5-FAIO-cis nanoparticles exhibit higher r_2 relaxivity for MRI detection and can be used for MRI monitored drug delivery. We demonstrated that developed Cy5.5-FAIO-cis are highly efficient in drug delivery and inhibiting tumor growth. Furthermore, the drug delivery process could be monitored by in vivo NIR/MR imaging.

References:

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