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Hybrid PET/MR imaging of tumors using an oleanolic acid-conjugated nanoparticle

Sung-min Kim [1], Min Kyung Chae [1], Min Su Yim, Il Ha Jeong, Janggeun Cho, Chulhyun Lee**, Eun Kyoung Ryu*

Center of Magnetic Resonance Research, Korea Basic Science Institute (KBSI), Chungbuk 363-883, Republic of Korea

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ABSTRACT

Research into multifunctional nanoparticles is focused on creating an agent for use in an all-in-one multimodal imaging system that includes diagnostic imaging, drug delivery, and therapeutic monitoring. We designed a new dual-modality tumor-targeting agent with a new tumor-targeting molecule, oleanolic acid (OA), which is derived from a natural compound and coupled with a macrocyclic chelating agent such as 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA), iron oxide nanoparticles (IONP), and radiolabeling components such as ^{68}Ga for dual-modality positron emission tomography (PET)/magnetic resonance imaging (MRI). We attempted to obtain fusion PET/MR images with the ^{68}Ga–NOTA–OA–IONP hybrid tumor-targeting imaging agent using colon cancer (HT-29) xenograft mice models. The HT-29 cancer cells showed high uptake of ^{68}Ga–NOTA–OA–IONP, which also had an inhibitory effect on the cells. Moreover, we obtained PET and MRI tumor images as well as fusion PET/MRI images of the tumors using ^{68}Ga–NOTA–OA–IONP. Therefore, the dual-modality cancer-targeting radiolabeled nanoparticle reported here is a potent imaging agent that is suitable for PET, MRI, and PET/MRI-based diagnosis of tumors; it also has the advantage of not only detecting tumor functionality, but also simultaneously aiding in tumor resolution.

1. Introduction

Early detection of disease is a popular and important issue in current medical practice. Several imaging modalities are presently used for the diagnosis of human diseases, but despite the advanced technology used in such diagnostic instruments, it has become increasingly clear that a multimodal imaging modality may be crucial for precise detection of cancer. The combination of different imaging modalities overcomes the inherent disadvantages of each single instrument. Positron emission tomography (PET) has high signal sensitivity, but it does not provide any information regarding anatomical structure, which thus requires the use of single photon emission computerized tomography (CT). CT is useful for obtaining clear *in vivo* images of anatomical structures, and is especially economically favorable compared with other imaging modalities, but it is not useful for soft tissue imaging as it yields poor-quality images of soft tissues [1,2]. Magnetic resonance imaging (MRI) provides excellent spatial resolution without any ionizing radiation, but it has low sensitivity. Consequently, the combination of PET and MRI should allow a more accurate diagnosis by overcoming the limitations particular to each technique without exposing the subject to ionizing radiation; it can also be helpful for improving our understanding of certain malignant and/or benign cancers in soft tissues [3–5].

Recently, multifunctional nanoparticles have been designed for use in multimodal imaging systems that combine diagnostic imaging, drug delivery, and therapeutic monitoring [6,7]. One example is a combination of an anti-cancer drug delivery/PET/MR imaging agent and a PET/MRI/fluorescence imaging agent. Nanoparticles have a large surface area and functional groups can easily be introduced by appropriate surface modification. In addition, nanoparticles have a long circulation time in the body due to their size (≤100 nm) and they accumulate in lesions, which makes them efficient agents for drug delivery [8–11].

To date, many different types of multimodal imaging agents have been reported [12–15]. Usually, these are composed of iron oxide nanoparticles (IONP), tumor-targeting molecules, and radioactive labeling components. Iron oxide nanoparticles are used as MRI contrast agents, and have been coated with substances

* Corresponding author. Tel.: +82 43 240 5091; fax: +82 43 240 5419.
** Corresponding author. Tel.: +82 43 240 5090; fax: +82 43 240 5419.
E-mail addresses: chulhyun@kbsi.re.kr (C. Lee), ekryu@kbsi.re.kr (E.K. Ryu).
[1] These authors contributed equally to this work.

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such as dopamine, dextran, protein, and a functionalized polyethyleneglycol (PEG)-based polymer due to their stealth effect. These nanoparticles are stable in biological environments and are protected from unwanted non-specific binding and reticuloendothelial system uptake (RES) [16–18].

For multimodal imaging, functionalized IONP are coupled with macrocyclic chelating agents such as 1,4,7-triazacyclononane-1,4,7-triacetic acid (NOTA) and 1,4,7,10-tetraazacyclododecane-*N*-*N*′,*N*″,*N*‴,-tetraacetic acid (DOTA) and are used for PET imaging and with tumor-targeting ligands (i.e., cyclo(Arg–Gly–Asp–D–Phe–Cys) [c(RGDfC)], doxorubicin, and antibodies). The RGD class is the most promising of the tumor-targeting ligands. For example, Chen et al. synthesized an RGD-conjugated radiolabeled IONP and successfully obtained dual PET/MRI images by targeting $\alpha_v\beta_3$ integrin expression by a tumor [12]. An anti-tumor drug delivery and PET/MRI dual-imaging agent comprising radiolabeled IONP, doxorubicin (anti-tumor agent), and cRGD have also been reported [14]. Target-specific binding is an important concept as it improves our understanding of the progression of disease and of drug effects using molecular imaging.

Several criteria have to be considered when developing imaging agents for human applications, including their pharmacokinetics, solubility, lipophilicity, efficacy, and toxicity. We consider toxicity and specific and selective targeting of a tumor to be the most important of these criteria. Oleanolic acid (3b-hydroxy-olea-12-en-28-oic acid, OA) is a non-toxic natural compound that is a member of the triterpenoid family. It is known to have potent hepatoprotective, antibacterial, antifungal, and anti-inflammatory properties [19,20]. Moreover, OA has been shown to inhibit cancer cell proliferation and to induce apoptosis in cancer cells [21,22]. In our previous studies, we found that OA derivatives showed strong inhibition of cancer cells, especially colon cancer cells, and induced apoptosis and cancer cell death. We have since attempted to develop an imaging agent for PET that combines both high tumor uptake and tumor-specific targeting.

In the present study, we report our design of a dual-modality tumor-targeting agent comprising a new tumor-targeting molecule (OA), IONP, and a radioactive chelator (NOTA) for PET/MRI dual imaging. We synthesized IONP by a thermal decomposition reaction and by changing the coating material to a DSPE-mPEG 2000/DSPE-mPEG 2000 amine, which made it possible for the agent to be dispersed in water. In addition, the functional amine groups of the PEG–phospholipid made it possible to introduce a tumor-targeting and radioactive ion chelating ligand for use as an MRI contrast agent. NOTA was used as the radioactive chelating ligand in order to bind radioactive isotopes (e.g., ^{68}Ga, $t_{1/2} = 68$ min) for PET. Finally, we attempted to obtain fusion PET/MR images with this hybrid tumor-targeting imaging agent using xenograft mice models of colon cancer.

2. Materials and methods

2.1. Preparation of IONP–OA–NOTA

2.1.1. Chemicals

All reagents were used without further purification. Oleanolic acid, iron (III) acetylacetonate ([Fe(acac)$_3$]) (99.9%), oleylamine (>70%), and diisopropylethylamine (DIEA) were purchased from Aldrich Chemical Co. (St. Louis, MO, USA). Oleic acid (90%) was purchased from Alfa-Aesar (Ward Hill, MA, USA). 1,2-Distearoyl-*sn*-glycero-3-phosphoethanol-amine-N-methoxy(polyethylene-glycol) 2000 (DSPE-mPEG 2000) and 1,2-distearoyl-*sn*-glycero-3-phosphoethanolamine-N-[amino-(polyethylenglycol) 2000] (DSPE-mPEG 2000 amine) were purchased from Avanti Polar Lipids, Inc. (Huntsville, Ala, USA). *O*-Benzotriazole-*N*,*N*,*N*,*N*′-tetramethyl-uronium-hexafluoro-phosphate (HBTU) and hydroxybenzotriazole (HOBt) were purchased from BeadTech Inc. (Seoul, Korea), and (*p*-SCN-Bn)-NOTA·3HCl (NOTA) was procured from FutureChem Co., Ltd. (Seoul, Korea).

2.1.2. Synthesis of Fe_3O_4 nanoparticles

Fe_3O_4 nanoparticles were synthesized by a reductive thermal decomposition reaction. Fe(acac)$_3$ (1.42 g, 4.02 mmol), oleic acid (4 mL, 12.6 mmol), oleylamine (4 mL, 12.4 mmol), and 1,4-hexadecanediol (5.15 g, 20 mmol) were mixed and stirred at 120 °C with vigorous stirring for 2 h and then partially vacuumed to simultaneously remove moisture and oxygen. The solution was then heated to 200 °C under argon and kept at this temperature for 2 h. Thereafter, the solution temperature was rapidly increased to 300 °C and kept at this temperature for 30 min. The solution turned a dark-brown color and was cooled down to room temperature and washed with ethanol. The nanoparticles were redispersed into hexane and precipitated by the addition of excess ethanol and then purified by centrifugation. The final products were dispersed into hexane and stored under an argon atmosphere.

2.1.3. Coating of Fe_3O_4 nanoparticles (IONP) with PEG–phospholipid

A total of 1 mL of Fe_3O_4 nanoparticles (3.3 mg) in $CHCl_3$ was mixed with 1 mL of $CHCl_3$ containing 76.5 mg (4:1 ratio of DSPE-mPEG 2000:DSPE-mPEG 2000 amine) of DSPE-mPEG 2000 and DSPE-mPEG 2000 amine. The reaction mixture was then mixed for 1 h at room temperature. Thereafter, the mixture was dried under argon gas and left in a vacuum oven at 40 °C for 1 h to remove all traces of the organic solvent.

2.1.4. Coupling of the nanoparticles and oleanolic acid (OA–IONP)

The IONP film was dispersed in DMF (1 mL) and mixed with OA (2 mg), *O*-benzotriazole-*N*,*N*,*N*,*N*′-tetramethyl-uronium-hexafluoro-phosphate (HBTU, 4 equiv.) and hydroxybenzotriazole (HOBt, 4 equiv.). The solution was then incubated in the basic condition (diisopropylethylamine, DIEA) at room temperature overnight. Next, the reaction mixture was washed with distilled water using a 100 kDa ultrafiltration unit, the Amicon Ultra-4 (Millipore Corp.). The final OA–IONP complex was stored in 10 mM borate buffer at 4 °C.

2.1.5. Coupling of OA–nanoparticles and NOTA (NOTA–OA–IONP)

The OA–IONP solution was buffer-changed with 0.1 M Na_2CO_3 using a 100 kDa ultrafiltration unit. NOTA (3.6 mg) was added into the OA–IONP solution and mixed for 2 h. The reaction mixture was then washed in distilled water by using a 100 kDa ultrafiltration unit, and finally purified through a PD-10 column with 10 mM borate buffer.

2.2. Radiolabeling of NOTA–OA–nanoparticles (^{68}Ga–NOTA–OA–IONP)

The radioisotope, ^{68}Ga, was produced with a ^{68}Ge/^{68}Ga generator (Eckert & Ziegler, Berlin, Germany) using 0.1 N HCl solution. For ^{68}Ga labeling of the nanoparticles (^{68}Ga–NOTAOA–IONP), NOTA–OA–IONP (5.8 μmol) was dissolved in 300 μL of 0.5 M phosphate buffer (pH 7.4) added to the ^{68}Ga/0.1 N HCl solution. The mixture was then adjusted to a pH of 5.0–5.5, stirred for 20 min to induce a reaction at room temperature, and then purified using size exclusion chromatography (PD-10 column).

2.3. Characterization of the synthesized nanoparticles

2.3.1. Measurement of the nanoparticles

A sample for transmission electron microscopy (TEM) analysis was prepared by drying a dispersion of the synthesized nanoparticles in solvent on amorphous carbon-coated copper grids. The particles were imaged using an FEI Tecnai G2F30ST microscope. The size distribution of the nanoparticles dispersed in solvent was characterized by dynamic light scattering (DLS) using a Nanosizer (Zetasizer Nano ZS, Malvern, U.K.).

2.3.2. Verification of NOTA–OA–IONP by FT-IR spectroscopy

The FT-IR spectra of the nanoparticles were obtained with a microscopic FT-IR/Raman Spectrometer (Vertex 80V, Bruker, Germany). The powder samples (OA and NOTA–OA–IONP) were transformed into a thin plate with KBr pellet.

2.3.3. Measurement of T_2 relaxation properties

All MRI experiments were performed with a 4.7T MRI (Bruker Biospec, Germany) equipped with a 72-mm inner diameter quadrature RF coil at the Korea Basic Science Institute in Ochang, and elemental analysis of iron in the hydrophilic nanoparticle samples was performed using an inductive coupled plasma-atomic emission spectrometer (ICP-AES; Optima 4300 DV; PerkinElmer, USA) at the Korea Basic Science Institute in Gwangju. T_2 relaxation maps of OA–IONP and NOTA–OA–IONP were measured using the MSME sequence (TR/TE = 1000/8 ms, flip angle = 180) with a FOV of 5 × 5 cm and a slice thickness of 2 mm. The r_2 values were calculated by fitting a curve to $1/T_2$ relaxation time (s^{-1}) versus the iron ion concentration (mM).

2.4. Proliferation of nanoparticles in colon cancer

2.4.1. Cancer cell line

Colon cancer (HT-29) cells were obtained from a human cell line (KCLB®). The cells were cultured in RPMI 1640 (Gibco, Grand Island, New York, USA) supplemented with 10% heat-inactivated fetal bovine serum (FBS, Gibco) and penicillin (100 units/mL). The culture was maintained at 37 °C in 5% CO_2.