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The synthesis of a D-glucosamine contrast agent, Gd-DTPA-DG, and its application in cancer molecular imaging with MRI

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ABSTRACT

Objective: The purpose of this study is to describe the synthesis of Gadolinium-diethylenetriamine pentaacetic acid-deoxyglucosamine (Gd-DTPA-DG) which is a D-glucosamine metabolic MR imaging contrast agent. We will also discuss its use in a pilot MRI study using a xenograft mouse model of human adenocarcinoma.

Methods: This novel contrast agent was specifically studied because of its ability to "target" metabolically active tumor tissues. In this study Gd-DTPA-DG is used to investigate how tumor tissues would react to a dose of 0.2 mmol Gd/kg over a 120 min exposure in a xenograft mouse model. These experiments used athymic mice implanted with human pulmonary adenocarcinoma (A549) as demonstrated by dynamic MRI. Alternately, another contrast agent that is not specific for targeting, Gd-DTPA, was used as the control at a similar dose of gadolinium. Efficacy of the targeted contrast agent was assessed by measuring relaxation rate *in vitro* and signal intensity (*SI*) *in vivo*. Statistical differences were calculated using one-way analysis of variance.

Results: The synthesized Gd-DTPA-DG was shown to improve the contrast of tumor tissue in this model. Gd-DTPA-DG was also shown to have a similar pharmacokinetic rate but generated a higher relaxation rate in tumor tissues relative to the control contrast Gd-DTPA. In comparison to the pre-contrast imaging, the *SI* of tumor tissue in the experimental group was shown to be significantly increased at 15 min after injection of Gd-DTPA-DG ($p < 0.001$). The enhanced signal intensity spread from the edge of the tumor to the center and seemed to strengthen the idea that MRI performance would be useful in different tumor tissues.

Conclusion: This preliminary study shows that this new chelated contrast agent, Gd-DTPA-DG, can be specifically targeted to accumulation in tumor tissue as compared to normal tissues. This targeted paramagnetic contrast agent has potential for specific cancer molecular imaging with MRI.

1. Introduction

The goal of molecular imaging is to noninvasively detect and characterize nascent pathology based upon a unique presentation of biomarkers. These biomarkers should help differentiate abnormal cells from their normal cell counterparts. MRI is known to have excellent spatial resolution, is reasonably noninvasive, and is a sequentially repeatable process to help track disease changes due to its lack of ionizing radiation [1]. However, MRI that is augmented by the use of contrast agents for imaging, can help further highlight differences between tumor and normal tissues. Contrast agents can be biologically encapsulated in order to control contrast delivery to specific areas of interest using relevant targeting capabilities [2]. In this emerging field of molecular imaging, Gadolinium-diethylenetriamine pentaacetic acid is shown to be a beneficial MR contrast agent. This agent has a long history of use in MRI. Additionally, several groups have created gadolinium-containing micelles, microspheres, nanoparticles, and liposomes for the purposes of specified target delivery or for modification of biodistribution. These gadolinium-containing particles have been used to increase the site-specific concentration of gadolinium, as compared to using aqueous gadolinium or gadolinium-conjugated to targeting molecules [3–5]. We have previously reported a specifically sensitive nuclear detection agent, technetium-99m diethylenetriamine pentaacetic acid-D-glucosamine (^{99m}Tc-DTPA-DG), which showed excellent tumor targeting. These agents are promising imaging agents for clinical tumor targeting and imaging. In this study, we explore a mechanism of combining

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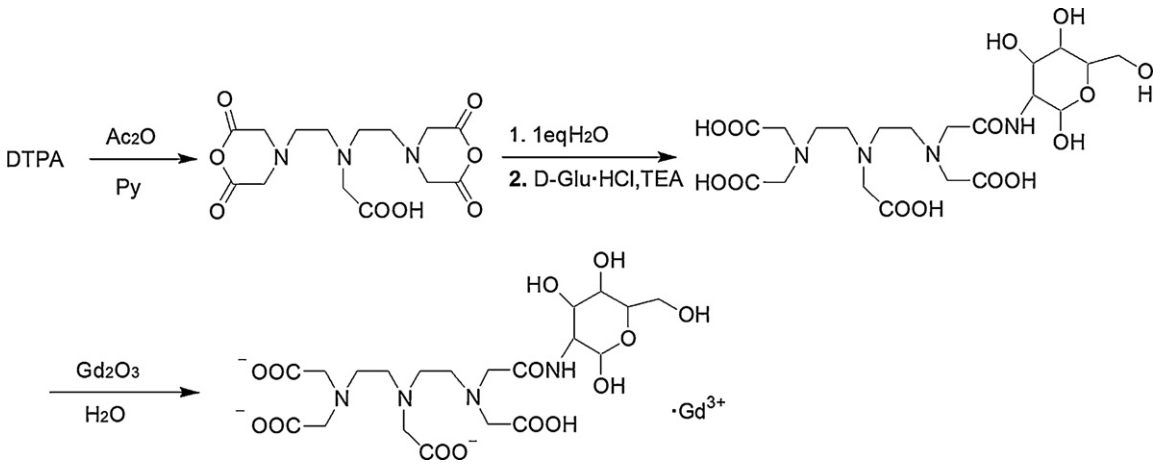

Fig. 1. The synthesis of Gd-DTPA-DG.

gadolinium with DTPA-DG to make a new specific MR contrast agent.

2. Materials and methods

2.1. Materials

Chemicals were used without further purification unless stated. DTPA was purchased from Sigma–Aldrich (Shanghai, China), Gd_2O_3 was obtained from Rare-Chem. Hi-Tech Co., Ltd. (Huizhou, China). Human adenocarcinoma (A549) cells were purchased from the Key Laboratory of Biotherapy of Human Disease of the Ministry of Education, West China Hospital, Sichuan University (Chengdu, Sichuan, China). RPMI-1640 culture solution, 10% fetal calf serum, parenzyme and penicillin-streptomycin were purchased from GIBCO/BRL Corporation (GIBCO Grand Island, NY). Nude mice were purchased from the Animal Experimental Center of Sichuan University. Gd-DTPA (Magnevist) was purchased from Schering Plough Corp. (Schering-Plough Corp., Guangzhou, China).

2.2. Methods

2.2.1. Tumor model

Human pulmonary adenocarcinoma cells were harvested from patients with trypsin/EDTA, washed, and suspended in normal saline at a concentration of 1×10^7 cells/ml. A 0.1 ml dose of suspended cells (1×10^6 cells) were injected subcutaneously in the right dorsal scapular region of mice using a 25-gauge needle. Animals were visually inspected every other day for changes in general appearance, behavior, and tumor growth. MRI studies were conducted when the tumor volume reached 10 mm × 10 mm × 10 mm, as measured by caliper.

2.2.2. Preparation of Gd-DTPA-DG

2.2.2.1. The synthesis of DTPA-bis(anhydride). A mixture of DTPA (7.8 g, 0.02 mol) was added to a 50 ml flask, acetic anhydride (7.6 ml, 0.08 mol) and dry pyridine (10 ml, 0.12 mmol) were added and heated at 65 °C for 24 h. After cooling to room temperature, the resulting mixture was filtered and the solid portion was washed with diethyl ether. After drying 15 min under a vacuum, the residue (6.5 g) was used for the next step in the reaction without extra purification.

2.2.2.2. The synthesis of DTPA-DG. To prepare monosubstituted derivatives, DTPA-bis (anhydride) (3.57 g, 10 mmol) was dissolved in 80 ml DMSO by heating at 80 °C. Once dissolved, water (0.18 ml, 10 mmol) was added to the solution and kept at 80 °C. After stirring for 2 h, the solution was cooled to room temperature. D-Glucosamine hydrochloride (2.13 g, 10 mmol) and triethylamine (1.01 g, 10 mmol) was added and the resulting mixture was stirred for 24 h at room temperature. 80 ml CH_2Cl_2 was added to the reaction mixture to initiate separation, the supernatant was then decanted. This *pellet* crude product was washed with dichloromethane (50 ml, 3 times) to remove the remaining DMSO. This product was then purified by running over a sephadex G-15 column and the white solid (2.3 g) was obtained. This monosubstituted derivative will be used for the next step in the synthesis reaction.

2.2.2.3. The synthesis of Gd-DTPA-DG. The above mentioned monosubstituted derivatives (0.554 g, 1 mmol) were dissolved in 8 ml water and Gd_2O_3 (0.363 g, 1 mmol) was added. After stirring for 6 h at 80 °C, the mixture was filtered to remove excess Gd_2O_3. Acetone (25 ml) was added to the filtrate and the suspension was filtered to leave a white product. The crude product was washed with acetone and diethyl ether and after washing yielded 0.612 g of product. A diagram of the synthesis of Gd-DTPA-DG is shown in Fig. 1.

2.2.3. In vitro MRI studies

Consideration of the role of paramagnetically chelated Gd-DTPA was calculated for only T_1, because the T_2 values were not statistically different at the tested Gd-DTPA particle concentrations, as was expected. We therefore only determined T_1 relaxivities *in vitro*. Gd-DTPA and Gd-DTPA-DG were dissolved in distilled water and samples were read using a 1.5-T scanner (Excite-II, Echospeed; GE medical system) accompanied by an 8-channel neurovascular array coil. Suspended particles containing Gd-DTPA from 0 to 0.5 mM were scanned and compared to the same concentrations of unencapsulated Gd-DTPA. For T_1 measurements, a fast spin echo sequence inversion recovery (FSE IR) sequence was used with the following imaging parameters: repetition time (TR): 3000 ms; echo time (TE): 7.8 ms; TI: 50, 75, 100,150, 200, 250, 300, 400, 500, 600, 700, 800, 900 and 1000 ms; band width: 15.6 kHz; FOV: 180 mm × 90 mm; slice thickness: 3.0 mm; matrix: 256 × 128; flip angle: 90°; and NEX: 1.

2.2.4. In vivo MRI studies

Twenty, Specific Pathogen Free (SPF) female athymic nude mice (5–6 weeks old, 20–25 g) were randomly assigned to two groups: the experimental group ($n = 10$) and the control group ($n = 10$). Mice were given vena caudalis injections, where Gd-DTPA-DG was injected in the experimental group and Gd-DTPA was used in the