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Gadolinium-loaded chitosan nanoparticles for neutron-capture therapy: Influence of micrometric properties of the nanoparticles on tumor-killing effect

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HIGHLIGHTS

- Gadolinium-loaded chitosan nanoparticles (Gd-nanoCPs) for GdNCT were applied to melanoma-bearing mice.
- GdNCT after intratumoral injection of Gd-nanoCPs showed a significant tumor-growth suppression effect.
- Micrometric properties of Gd-nanoCPs were important factors determining GdNCT effect in vivo.
- Gd-nanoCPs with a smaller particle size demonstrated an enhanced tumor-killing effect.

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ABSTRACT

As a nanoparticulate device for controlled delivery of Gd in NCT, the authors have developed gadolinium-loaded chitosan nanoparticles (Gd-nanoCPs). In the present study, influence of micrometric properties such as particle size, particle-surface charge and Gd content of Gd-nanoCPs on tumor-killing effect by Gd-NCT was investigated with Gd-nanoCPs. Two types of Gd-nanoCPs with different mean particle size, zeta potential and Gd-content (Gd-nanoCP-400; 391 nm, 28 mV, 9 wt% and Gd-nanoCP-200; 214 nm, 19 mV, 24 wt%) could be prepared by using chitosans with different molecular weights. Gd-nanoCPs incorporating 1.2 mg of natural Gd were injected intratumorally once or twice to mice subcutaneously-bearing B16F10 melanoma. Eight hours after the last administration, thermal neutron was irradiated to tumor region of the mice. Remarkable tumor-growth was observed in both hot and cold control groups. In contrast, Gd-NCT groups showed significant tumor-growth suppression effect, though their efficacy was found to depend on the micrometric properties of Gd-nanoCPs. In particular, the Gd-nanoCP-200 exhibited stronger tumor-killing effect than the Gd-nanoCP-400 at the same Gd dose and it was still similar to Gd-nanoCP-400 in tumor-growth suppressing effect even at the half of Gd dose of Gd-nanoCP-400. This significance in tumor-killing effect would be ascribed from a higher Gd retention in the tumor tissue and an improved distribution of Gd with intratumorally administered Gd-nanoCP-200. Indeed, the Gd concentration in tumor tissue at the time corresponding to the onset of thermal neutron irradiation was determined to be significantly higher in Gd-nanoCP-200, compared with Gd-nanoCP-400. These results demonstrated that appropriate modification of Gd-nanoCPs in micrometric properties would be an effective way to improve the retention of Gd in the tumor tissue after intratumoral injection, leading to the enhanced tumor-killing effect in Gd-NCT.

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1. Introduction

Gadolinium-neutron capture therapy (Gd-NCT) is a cancer therapy which utilizes the nuclear neutron-capture reaction of Gd with externally irradiated thermal neutron (Locher, 1936). Gd-NCT has the following possible advantages over boron neutron-capture therapy (BNCT) commonly used. Firstly, ^{157}Gd has the very large thermal neutron capture cross section (255,000 b), which is about 66 times larger than that of ^{10}B. Secondly, Gd-NCT may increase the possibility of hitting the target tumor cells with the long-range photons and/or a locally intensive destruction of DNA in tumor cells by Auger electrons which are short-range and high linear energy transfer (LET) electrons, compared with BNCT (Martin et al., 1988; Brugger and Shin, 1989).

One of the key issues for success in Gd-NCT is to develop a device capable of maintaining a sufficient Gd concentration in a tumor during the treatment (Sharma et al., 2007). It is well-known that free Gd^{3+} is toxic and thus must be chemically stabilized by chelation (Weinmann et al., 1984). While commercially available MRI contrast agents composed of Gd-chelating compounds may be used as a Gd source, most of them show no tumor-specific accumulation in general. From this perspective, several attempts have been made by researchers to develop a pharmaceutically engineered nanoparticulate device containing a Gd-based MRI contrast agent for achieving tumor-specific accumulative property (Ichikawa et al., 2007; Le and Cui, 2006; Watanabe et al., 2002).

As a nanoparticulate device for controlled delivery of Gd in NCT, our group has been developing gadolinium-loaded chitosan nanoparticles (Gd-nanoCPs) (Fujimoto et al., 2009; Shikata et al., 2002; Tokumitsu et al., 1999, 2000). This nanoparticulate device is composed of Gd-diethylenetriaminepentaacetic acid (Gd-DTPA) and chitosan which is a naturally abounded polysaccharide material having biodegradable, biocompatible and bioadhesive characteristics (Skjask-Braek et al., 1989). Gd-nanoCPs with mean particle size of 426 nm and Gd content of 9.3% could be prepared by our w/o emulsion-droplet coalescence technique using 100% deacetylated chitosan (Tokumitsu et al., 1999). Our previous studies demonstrated that the Gd-nanoCPs exhibited a good cell affinity and some of those were endocytosable by the tumor cells in vitro (Shikata et al., 2002). Moreover, significant tumor-growth suppression in vivo could be achieved by neutron-capture reaction after direct intratumoral (i.t.) injection of Gd-nanoCPs to tumor-bearing mice; however complete cure of the tumor could not be achieved (Tokumitsu et al., 2000). For enhancing Gd-NCT effect, several considerations must be taken. Gd-nanoCPs should be distributed extensively in tumor tissue to deliver sufficient amounts of Gd atoms to tumor cells even if they are administered by direct i.t. injection. In addition, due to the limited trajectory of the Auger electrons, the i.t. injected Gd-nanoCPs should sufficiently adhere to and be taken up by the tumor cells in order to fully utilize the potential function of high-LET Auger electrons that could cause lethal damage to the tumor cells by breaking double-stranded DNA (Salt et al., 2004).

Micrometrical properties including particle size, particle-surface charge and Gd-content are considered to be crucial factors affecting Gd-NCT effect of Gd-nacoCPs because they would change intratumoral behavior of Gd-nanoCPs. Especially particle size reduction of Gd-nanoCPs can lead to enhancement of diffusion of the particles as expected from the Stokes–Einstein law, giving rise to the improved distribution of Gd-nanoCPs in tumor tissue. Additionally, elevation of Gd-content would allow Gd-nanoCPs to deliver the increased amount of Gd atoms to tumor. In the present study, therefore, an attempt was made to modify the micrometric properties of Gd-nanoCPs, particularly particle size reduction, by using low molecular weight chitosan. Then, influence of micrometrical properties of Gd-nanoCPs on tumor-killing effect by Gd-NCT in vivo was investigated.

2. Materials and methods

2.1. Materials

Chitosan with nominal average molecular weight of 10,000 (10 k, 100D EL, deacetylation degree of higher than 98%, Dainichiseika Color & Chemicals Mfg. Co., Ltd., Japan) or 950,000 (950 k, 10B, deacetylation degree of higher than 98%, Katokichi Co., Ltd., Japan) was used. Gadopentetic acid (Gd-DTPA) was purchased from Sigma-Aldrich. All other materials were at least of special reagent grades.

2.2. Animal and tumor

All animal experiments were performed according to the regulations of the Animal Care and Use Committee of Kobe Gakuin University. Six-week-old male C57BL/6 mice (body weight, 18–23 g) were purchased from Nihon SLC, Japan. The B16F10 malignant melanoma cell was employed for obtaining a model solid tumor bearing mouse and purchased from RIKEN BioResource Center, Japan.

2.3. Preparation of Gd-nanoCPs with two different particle sizes

Gd-nanoCPs with two different average particle sizes (400 and 200 nm) were prepared by using Gd-DTPA and chitosan with molecular weight of 950 k or 10 k through the w/o emulsion-droplet coalescence technique previously developed (Tokumitsu et al., 1999). Briefly, chitosan dissolved in a 10% w/v Gd-DTPA aqueous solution was added to paraffin liquid containing 5% v/v Arlacel C. This mixture was emulsified by a high-speed homogenizer (Physcotron®) for 3 min to form water-in-oil (w/o, water-to-oil volume ratio was 23:77) emulsion. Similarly, w/o emulsion consisting of 3 M NaOH solution (water-to-oil volume ratio was 13:87) were prepared. Then, these emulsions were mixed to form Gd-nanoCPs through coalescence of the emulsion droplets and thereby neutralization. The resultant products were washed with solvents, i.e., toluene, ethanol and water sequentially in this order, and separated from the contaminated solvents by centrifugation. This washing process was repeated twice per each solvent.

2.4. Residual amount of gadolinium in tumor tissue

For the experiment, 0.1 mL of a cell suspension containing 7.0×10^6 melanoma cells was inoculated subcutaneously (s.c.) into the left buttocks of C57BL/6 mice under light ether anesthesia. When the B16F10 melanoma in the mice grew to about 10 mm in diameter (approximately 7–10 days after tumor inoculation), Gd-nanoCPs with two different particle sizes were injected once or twice by intratumoral (i.t.) administration with multi-injection at a dose of 1.2 mg Gd before 8 and 24 h from the assay of Gd. After that, the mice were sacrificed, the blood samples were collected and the tumor mass was excised. Then, the excised tumor mass and the collected blood samples were incinerated completely in the presence of 30 w/w% H_2O_2 (1.2 mL) and 60 w/w% $HClO_4$ (0.6 mL) at 75 °C for 48 h. The samples were diluted with ultrapure water to be 5 mL in total volume and then the Gd concentration in the samples was determined by inductively coupled plasma atomic emission spectrometry (ICP-AES, SPS3100, Hitachi High-Tech Science Corporation, Japan).

2.5. In vivo Gd-NCT trial

The experimental protocol of the Gd-NCT trial is shown in Fig. 1. The B16F10 melanoma-bearing mice were obtained by the same manner described in Section 2.4 and divided into five groups