ELSEVIER
Nanomedicine: Nanotechnology, Biology, and Medicine
xx (2014) xxx–xxx
nanomedicine
Nanotechnology, Biology, and Medicine
nanomedjournal.com

Gadolinium-based nanoparticles to improve the hadrontherapy performances

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Received 29 October 2013; accepted 12 May 2014

Abstract

Nanomedicine is proposed as a novel strategy to improve the performance of radiotherapy. High-Z nanoparticles are known to enhance the effects of ionising radiation. Recently, multimodal nanoparticles such as gadolinium-based nanoagents were proposed not only to amplify the effects of x-rays and γ-rays, but also to improve MRI diagnosis. For tumours sited in sensitive tissues, childhood cases and radioresistant cancers, hadrontherapy is considered superior to x-rays and γ-rays. Hadrontherapy, based on fast ion radiation, has the advantage of avoiding damage to the tissues behind the tumour; however, the damage caused in front of the tumour is its major limitation. Here, we demonstrate that multimodal gadolinium-based nanoparticles amplify cell death with fast ions used as ionising radiations. Molecular scale experiments give insights into the mechanisms underlying the amplification of radiation effects. This proof-of-concept opens up novel perspectives for multimodal nanomedicine in hadrontherapy, ultimately reducing negative radiation effects in healthy tissues in front of the tumour.

Key words: Nanomedicine; Gadolinium; Nano-sensitisation; Hadrontherapy; Theranostics

Background

Nanodrugs for cancer-therapy is a rapidly developing field of investigation, where new drug delivery vehicles, contrast agents and therapeutics are being processed with the goal of improving medical protocols.[1–3] Recently, the use of nanomaterials was proposed as a promising way to enhance the performance of radiation therapies. Indeed, the limitation of conventional radiotherapy comes from the damage induced in the healthy tissues surrounding the tumour. In 2004, it was shown that the effects of X-rays can be amplified in tumours when gold nanoparticles are present.[4] The *in vivo* study demonstrated the high potential of using tumour-targeted nanomaterials to improve radiotherapies. Other studies performed on DNA and mammalian cells confirmed the properties of high-Z nanoparticles to amplify radiation effects.[5,6]

On the other hand, fast ion-based radiation therapies (hadrontherapy and protontherapy) are considered superior approaches for the treatment of tumours located in highly sensitive tissues (brain, neck, eyes), paediatric cancers, and also tumours that are resistant to radiotherapy.[7] The advantage of ions compared to photons stems from their property to induce maximum damage at the end of the track (called the Bragg peak). In operating conditions, the beam is tuned such that the Bragg peak is spread out and the maximum of the radiation effects coincides with the total volume of the tumour (mode of spread out Bragg peak). As a result, the damage induced behind the tumour is close to zero and the healthy tissues are preserved.[8] Hence, hadrontherapy and protontherapy represent strong advances in cancer therapies. The major limitation of these techniques stems from the radiation effects that remain significant in front of the tumour (at the entrance of the track). It is thus a challenge to diminish the dose given to the patient and to enhance the biological effect of the treatment in the tumour. The use of tumour-targeted nanoparticles to amplify the radiation-effect of heavy ions in the tumour is a novel strategy, which has never been explored before.

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http://dx.doi.org/10.1016/j.nano.2014.05.005
1549-9634/

In this work we probed the effect of multimodal Gadolinium-based nanoparticles (GdBN) combined with carbon and helium ions radiations. This new type of multimodal nanoparticles, which behave not only as radiosensitisers but also as contrast agents, has been developed recently.[9,10] This multimodality is very promising as it opens the perspectives to use a unique drug to improve simultaneous tumour monitoring and targeted therapy. This double modality, named theranostics, brings new issues in personalised medicine.[11] It is already known that GdBN accumulate in tumours and present excellent properties as contrast agents in magnetic resonance imaging (MRI).[12] In addition, *in vitro* and *in vivo* experiments demonstrated that GdBN are good radiosensitisers when gamma and x-rays are used.[9,12] It is also important to mention that these nanoparticles are little toxic as demonstrated in our previous *in vitro* studies.[13,14] Finally, we found that these nanoparticles accumulate in kidneys. We used a multi-scale approach to characterise the effects of GdBN at cellular and molecular scales. The efficiency of the nanoparticles to amplify cell death was evaluated using a Chinese hamster ovary cell line (CHO) because of its well-known and simple metabolism. CHO was previously used to probe the effects of Platinum Chloro 2,2':6',2" terpiridine, a well-known radiosensitiser.[15] This model allows not only comparison of the biological impact of radiosensitisers combined with radiation, but also the avoidance of artefacts due to cell-specific biological functions. Indeed, human cell lines, which differ by their reaction to radiation (e.g. cell death pathways, radioresistance), could not be used as probes. We also quantified the yields of simple and complex (nano-size) damage using a molecular probe to distinguish and quantify the impact at the molecular level. In this perspective, pBr322 plasmid was used to quantify accurately and rapidly the induction of single strand breaks and double strand breaks that respectively correspond to simple and complex damages (see Supplementary Materials for a view of the plasmid conformations with simple and complex breaks). Plasmids and cells containing nanoparticles were irradiated with medical beams provided by the Heavy Ion Medical Accelerator Chiba (HIMAC, Japan), which is currently one of the most advanced hadrontherapy centres. In addition, the action sites of the nanoparticles in the cells were identified by two complementary methods of microscopy. Finally, this work is not only the first to highlight the amplification effects induced by multimodal nanoparticles combined with heavy ion radiation, but also the first evidence that these effects are initiated by nano and sub-nanoscale processes. We show that these processes take place in the cytoplasm, far from the nucleus.

Materials and Methods

Gadolinium-based nanoparticles (GdBN)

The Gadolinium-based nanoparticles consist of a polysiloxane core surrounded by gadolinium chelates that are covalently grafted on the inorganic matrix.[16] The procedure of synthesis and the characteristics of these nanoparticles are detailed elsewhere.[16] Briefly, their size is 3.0 ± 1.0 nm diameter and their mass is about 8.5 ± 1 kDa. These nanoparticles, highly stable, can be lyophilised and are stored at 4 °C. They are found to be biocompatible and to efficiently enrich tumours.[9]

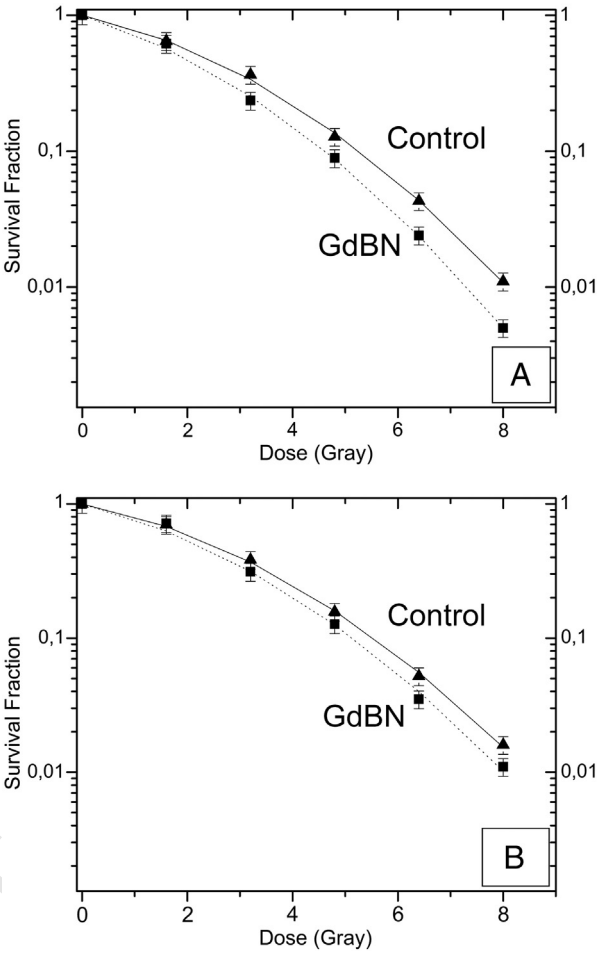

Figure 1. Survival fractions of CHO cells irradiated by C^{6+} **(A)** and He^{2+} **(B)** in the presence of GdBN (dot lines) and free of GdBN (plain lines).

Cell culture

CHO cells grew in Minimum Essential Medium-alpha (MEM-a) supplemented with 10% foetal bovine serum, penicillin (100 mg/mL) and streptomycin (100 mg/mL).[15] Exponentially growing cells (1.56×10^5 cells) were plated in flasks (Nunc Slide Flask 170920, 25 cm^3) at least 12 h before irradiation. Cells were maintained in 5% CO_2 incubator at 37 °C. GdBN was added to the cell medium 6 h before irradiation at a concentration of 1 mmol L^{-1} in gadolinium. At this concentration, the nanoparticles are not toxic.[9,17] The cells were irradiated under atmospheric conditions, at room temperature. The combined effect of radiation and nanoparticles on cells was quantified by clonogenic assay. After irradiation, cells were trypsinised and plated into 60 mm Petri dishes (Falcon 3002) at a density of 100 surviving cells per dish. The plating efficiency was close to 85%. After ten days of incubation, the colonies were treated with 10% formalin and stained with 1% methylene blue. The colonies were counted to determine the surviving fractions.