Radiotherapy and Oncology xxx (2013) xxx–xxx

Contents lists available at SciVerse ScienceDirect

Radiotherapy and Oncology

journal homepage: www.thegreenjournal.com

Original article

The radiosensitization effect of titanate nanotubes as a new tool in radiation therapy for glioblastoma: A proof-of-concept ☆

C. Mirjolet [a,1], A.L. Papa [b,1], G. Créhange [a], O. Raguin [c], C. Seignez [d], C. Paul [d], G. Truc [a], P. Maingon [a,*], N. Millot [b,*]

[a] Radiotherapy Department, Centre Georges-François Leclerc, Dijon; [b] Laboratoire Interdisciplinaire Carnot de Bourgogne, U.M.R. 6303 C.N.R.S./Université de Bourgogne; [c] Oncodesign® Biotechnology, Dijon Cedex; and [d] EPHE-INSERM-Université de Bourgogne, Cancer Immunology Laboratory, CRI U866 « Lipides, Nutrition, Cancer », Dijon, France

ARTICLE INFO

Article history:
Received 25 June 2012
Received in revised form 18 February 2013
Accepted 2 April 2013
Available online xxxx

Keywords:
Titanate nanotubes
Glioblastoma
Radiosensitization
Internalization
Cytotoxicity

ABSTRACT

Background and purpose: One of the new challenges to improve radiotherapy is to increase the ionizing effect by using nanoparticles. The interest of titanate nanotubes (TiONts) associated with radiotherapy was evaluated in two human glioblastoma cell lines (SNB-19 and U87MG).

Materials and methods: Titanate nanotubes were synthetized by the hydrothermal treatment of titanium dioxide powder in a strongly basic NaOH solution. The cytotoxicity of TiONts was evaluated on SNB-19 and U87MG cell lines by cell proliferation assay. The internalization of TiONts was studied using Transmission Electron Microscopy (TEM). Finally, the effect of TiONts on cell radiosensitivity was evaluated using clonogenic assay. Cell cycle distribution was evaluated by flow cytometry after DNA labeling. DNA double-stranded breaks were evaluated using γH2AX labeling.

Results: Cells internalized TiONts through the possible combination of endocytosis and diffusion with no cytotoxicity. Clonogenic assays showed that cell lines incubated with TiONts were radiosensitized with a decrease in the SF_2 parameter for both SNB-19 and U87MG cells. TiONts decreased DNA repair efficiency after irradiation and amplified G_2/M cell-cycle arrest.

Conclusion: Our results indicated that further development of TiONts might provide a new useful tool for research and clinical therapy in the field of oncology.

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Glioblastoma (GBM) is the most frequent primary brain tumor in adults. Despite a multimodal approach that combines surgery, radiotherapy and chemotherapy, this tumor almost invariably recurs at its initial site. The combination of radiotherapy and chemotherapy is the standard approach for the treatment of malignant glioma. The median survival time with conventional therapy for patients with multiform glioblastoma is 10–12 months with a three-year survival rate invariably ranging between 6% and 8%. New treatments, including temozolomide, an alkylating agent, in combination with radiotherapy have shown a statistically significant and clinically meaningful survival benefit for patients suffering from GBM and treated with combined chemoradiation [1].

Improving survival in this disease is long overdue. New techniques are needed to improve the results of conventional radiation therapy so as to improve clinical outcomes. In an attempt to enhance efficacy, it is necessary to radiosensitize or radiopotentiate tumor cells. To decrease the side effects of the systemic drugs, delivering them directly to the tumor using specific nanocarriers is a promising approach. Nanoparticles can accumulate specifically in cancer cells by two mechanisms. First, it is possible to target passive cancer tissue by the extravasation of nanoparticles through the increased permeability of endothelial cell junctions in the tumor (Enhanced Permeability Retention effect). Secondly, active cellular targeting can be achieved by functionalizing the surface of the nanoparticles with ligands (such as peptides [2], or antibodies [3]...) which specifically bind to receptors that are overexpressed on the surface of tumor cells. However, there are no known targets for many tumor cells. Consequently, nanocarriers, which are injected directly into the tumor, constitute a new approach [4–6]. This technique also precludes chemo-induced toxicity.

Nanotechnologies devoted to health have shown considerable growth in terms of innovation and the development of new thera-

☆ Oral presentation in 9th Japan-France Workshop on Nanomaterials, Toulouse, November 2010 (by N. Millot), UK Colloids, London, July 2011 (by N. Millot), ESTRO Meeting about Novel targeting drugs in radiotherapy, Toulouse, September 2012 (by C. Mirjolet) and in XXVIIth GTRV Scientific Meeting, Paris, December 2012 (by C. Mirjolet).

* Corresponding authors. Addresses: Radiotherapy Department, Centre Georges-François Leclerc, 1 rue Pr Marion, 21079 Dijon, France (P. Maingon), Laboratoire Interdisciplinaire Carnot de Bourgogne, 9 av. A. Savary, BP 47 870, 21078 Dijon Cedex, France (N. Millot).

E-mail addresses: pmaingon@cgfl.fr (P. Maingon), nmillot@u-bourgogne.fr (N. Millot).

[1] These authors have contributed equally to this study.

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http://dx.doi.org/10.1016/j.radonc.2013.04.004

peutic approaches. Nanomaterials and nanohybrids are being developed to target tumors and to deliver therapies (Myocet®, Caelyx®, Abraxane®). Other nanocarriers have been tested in animal models [7,8], and even the intrinsic properties of nanoparticles (hyperthermia via magnetic effects [9], photo-sensitization [10] and radio-sensitization [11,12]) can also be used to treat tumors. Iron oxide nanoparticles are clinically used as contrast agents for MRI (Endorem®, by Guerbet, Cliavist®, Resovist® by Bayer Schering...), and gold nanoparticles are being studied for their ability to improve radio-sensitization [13]. Several reports have shown that the in vivo efficacy of X-rays and γ-rays was enhanced by gold nanoparticles [14–19].

Titanium dioxides are used for disinfection and to kill cancer cells using photocatalytic chemistry [20]. Titanate nanotubes have shown a significant potential for the detection of dopamine [21,22], for enhanced bone growth [23] or for dental composites [24]. Magrez et al. published a study on the cytotoxicity of titanium oxide-based nanoparticles according to their morphology and chemical composition [25]. Several cytotoxic studies showed that the impact of nanoparticles depends on cell type, particle morphology, functionalization, etc. [26]. Consequently, all biological studies require their own evaluation of cytotoxicity. The shape effect of nanoparticles on biodistribution is a new parameter highlighted in the literature [27]. Elongated organic nanoparticles internalize into cells more effectively than do their spherical counterparts of a similar volume [28]. Carbon nanotubes have already been studied to deliver drugs or to detect molecules [29,30] and they are the main competitor of TiONts with regard to the shape. However, they have several drawbacks: they are insoluble in most solvents [31] and contrary to TiONts, have closed ends [32].

The aim of this study was to validate the use of titanate nanotubes to radiosensitize tumor cells in patients with GBM. Most preclinical studies involving nanoparticles in oncology involve nanospheres. Due to their particular shape, TiONts are internalized better than their spherical counterparts TiO_2 [33]. The interest of these nanoparticles associated with radiotherapy was evaluated on two models of glioblastoma (SNB-19 and U87MG cells): the internalization of titanate nanotubes, their cytotoxicity and the surviving fraction of cells after different doses of radiotherapy were studied. The potential reason for the observed radiosensitization effect was subsequently investigated by the evaluation of ROS (Reactive Oxygen Species) production, apoptosis, the induction of DNA double-stranded breaks and repair efficacy (formation of γH2AX foci in the nucleus), as well as cell cycle analyses.

Materials and methods

TiONts synthesis

Titanium dioxide rutile precursor (Tioxide) powders (440 mg) were added to a solution of NaOH (10 mol/L, 110 mL). The mixture was transferred into a sealed Teflon reactor under magnetic stirring and the temperature was kept at 150 °C for 36 h. The resulting white TiONts product was isolated by centrifugation and washed with distilled water until the pH was neutral. Finally the powder was freeze-dried [34].

Cancer cell lines and culture conditions

The SNB-19 human glioma cell line was established from the surgical resection of a left parieto-occipital glioblastoma in a 47-year-old man, and was purchased from DSMZ (Germany). The culture medium for these cells was DMEM (Dulbecco's Modified Eagle Medium) supplemented with 10% FBS (Fetal Bovine Serum).

The U87MG human cell line was initiated by Ponten from a grade IV glioblastoma from a 44-year-old Caucasian man, and was purchased from ATCC (Manassas, USA). The culture medium for U87MG cells was RPMI-1640 supplemented with 10% FBS.

The SNB-19 and the U87MG tumor cells were immediately grown as adherent monolayers at 37 °C in a humidified atmosphere (5% CO_2, 95% air).

Transmission electron microscopy (TEM)

Titanate nanotubes have previously been investigated on a JEOL JEM-2100 LaB_6 microscope operating at 200 kV and equipped with a high-tilt pole-piece achieving a point-to-point resolution of 0.25 nm.

The tumor cells were seeded and incubated at 37 °C in the culture medium for 24 h before being treated with TiONts for 10 days.

The cells were fixed for 30 min in a solution containing 4% paraformaldehyde and 1.5% glutaraldehyde in Sorensen phosphate buffer (0.1 mol/L, pH 7.4), then washed in Sorensen phosphate buffer, post-fixed in 1% osmium tetroxide diluted in Sorensen phosphate buffer (0.1 mol/L, pH 7.4) for 1 h, and rinsed in Sorensen phosphate buffer (0.1 mol/L, pH 7.4). The preparations were then dehydrated in graded ethanol solutions and embedded in Epon. Ultra-thin sections were cut with an ultramicrotome, contrasted with uranyl acetate and lead citrate, and examined under an H7500 electron microscope (Hitachi).

Cytotoxicity of TiONts on SNB-19 and U87MG cell assessed by MTS assay

The tumor cells were seeded in 96-well flat-bottom microtitration plates, at a concentration of 5000 cells/well for U87MG and 2000 cells/well for SNB-19, and incubated at 37 °C in 190 μL of drug-free culture medium for 24 h before treatment. At this time, the cells were at the beginning of the exponential growth phase. The cytotoxicity assays were performed twice (two independent experiments) with quadruplicate samples at each concentration of TiONts or Cisplatin (CDDP), used as the positive control. The tumor cells were incubated for 72 h with a range of concentrations of TiONts (from 0.1 ng/mL to 20 μg/mL) or CDDP. After 72 h of incubation, the cytotoxicity of TiONts and CDDP were evaluated using an MTS (3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium) assay according to Mirjolet et al. [35]. Results were expressed as relative absorption compared with the untreated control. CDDP concentrations yielding 50% growth inhibition (IC_{50}) were calculated using the medium-effect algorithm [36].

Determination of the cytotoxicity of TiONts combined with irradiation against SNB-19 and U87MG tumor cell lines using clonogenic assay

The SNB-19 cell lines were plated in 6-well flat-bottom microtitration plates and incubated at 37 °C for 24 h before treatment in 2 mL of drug-free culture medium. They were then incubated without (control) or with 1 μg/mL of TiONts and were irradiated 24 h later at 0.5, 1, 2, 5 and 10 Gy by a linear photon accelerator (clinac600, Varian).

The radiosensitivity of the U87MG cell line was determined by using soft agar clonogenic assays according to the method previously described [37]. Briefly, 6-well flat-bottom microtitration plates containing 1 mL of culture medium with 0.5% of soft agar were prepared before the experiment. Cells were seeded into these plates in 1 mL of 0.3% soft agar (Bacto Agar, Difco, Detroit) mixed with culture medium with (1 μg/mL TiONts) or without TiONts (Control). The plates were irradiated 24 h later at 0.5, 1, 2, 5 and 10 Gy by a linear photon accelerator (clinac600, Varian).

After 10 days of incubation, the number of colonies was evaluated as follows: colonies were fixed with glutaraldehyde (6.0% v/v),

Please cite this article in press as: Mirjolet C et al. The radiosensitization effect of titanate nanotubes as a new tool in radiation therapy for glioblastoma: A proof-of-concept. Radiother Oncol (2013), http://dx.doi.org/10.1016/j.radonc.2013.04.004