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Validation of an amino-acid-based radionuclide therapy plus external beam radiotherapy in heterotopic glioblastoma models ☆

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Abstract

Background and purpose: Malignant gliomas represent a major therapeutic challenge because no efficient treatment is currently available. *p*-[^{131}I]iodo-L-phenylalanine ([^{131}I]IPA) is a glioma avid radiopharmaceutical that demonstrated antiproliferative and tumoricidal effects in gliomas. The present study validated the therapeutic efficiency of [^{131}I]IPA combined with external beam radiotherapy in experimental gliomas.
Materials and methods: Glioma cells derived from the primary human A1207, T5135, Tx3868 and M059K glioblastoma cell lines or rat F98 glioma cell line were treated with various doses of [^{131}I]IPA, external photon irradiation (RT) or combined [^{131}I]IPA/RT treatment. Responsiveness of glioma cells to the different therapy modalities was investigated at 24, 48 and 72 h after treatments by trypan blue, WST-1 assay, propidium iodide and bisbenzimide staining as well as by clonogenic assay. In addition, the therapy-induced DNA damage and repair were evaluated using phosphorylated histone H2AX (γ-H2AX). In vivo, the effectiveness of the combination treatment was validated in human Tx3868 and A1207 glioblastoma xenografts in CD1 nu/nu mice and RNU rats.
Results: In vitro, the combination treatment resulted in a greater than additive increase in cytotoxic effect in glioma cell lines. Cell survival rate following a treatment with 1.0 μCi (37 kBq) of [^{131}I]IPA amounted to 70%±15% and 60%±10% after 48 and 72 h, respectively, and decreased under 20% after additional RT with 5 Gy. At higher RT doses, cell survival rate decreased below 5%. As a measure of DNA double-strand break, nuclear γ-H2AX foci were determined as a function of time. Within 24 h, the number of γ-H2AX foci per cell was significantly greater after combined modality compared with the individual treatments. In vivo, when combined with RT, the radionuclide therapy with [^{131}I]IPA resulted in an extended tumor growth delay, a reduction of the initial tumor volume and an enhanced radiosensitivity in Tx3868 and A1207 glioblastoma xenografts in CD1 nu/nu mice and RNU rats. On day 90 after monotherapy with [^{131}I]IPA (20 MBq) or RT (20 Gy), 35%–50% of the treated rats were still alive. In comparison, up to 70%–80% survival rates were registered after combined [^{131}I]IPA/RT treatment on day 100 for all animal models.
Conclusions: These preclinical data convincingly demonstrated that [^{131}I]IPA plus external beam photon radiotherapy is a safe and highly effective treatment for experimental gliomas, which may merit a clinical trial to ascertain its potential as a therapeutic approach in patients. As only a low [^{131}I]IPA activity and a low RT dose were applied, further optimization strategies should be pursued experimentally, including application of higher radiation doses and conventional fractionated regimens or use of methods aiming to increase target doses and maximize dose effects.

Keywords: Amino acid transport; Radionuclide therapy; External radiotherapy; Malignant gliomas; γ-H2AX foci

1. Introduction

Despite advances in surgery, radiotherapy and chemotherapy, the prognosis for patients with malignant glioma remains poor. The overall survival generally ranges from a few months to about 1 year for glioblastoma multiforme, the most common malignant glioma [1,2]. To overcome these dismal prospects, various experimental therapies have been administered, among them gene therapy, antisense treatment,

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methods for sensitizing glioma cells to the induction of apoptosis, boron neutron capture, implantation of iodine-125 seeds, radioimmunotherapy and photodynamic therapy with 5-aminolevulinic acid, or aim at different targets like the coagulation system, to name only some [3–10]. Unfortunately, all these efforts have failed to appreciably increase the median overall survival of patients with gliomas.

The finding that malignant gliomas accumulate amino acids more avidly than healthy brain has led to the development of amino-acid-based radiopharmaceuticals for detecting gliomas using positron emission tomography (PET) or single photon emission computed tomography (SPECT) [11–14]. *p*-[^{123}I]iodo-L-phenylalanine (IPA) is a new radioiodinated amino acid, which accumulates specifically in gliomas [15,16]. IPA actively crosses the blood–brain barrier after intravenous administration and is taken up by gliomas, presumably via the L and ASC amino acid transporters for neutral amino acids, which are overexpressed in gliomas [15,17,18,19]. A specific accumulation and prolonged retention of IPA were demonstrated clinically for low-grade and high-grade gliomas alike. At the same time, the radioactivity uptake into normal brain parenchyma and nonneoplastic lesions remained moderate [18,20,21]. Therefore, we postulated that when administrated systemically, L-phenylalanine that was conjugated to an α- or β-emitting radionuclide such as astatine-211 or iodine-131 could selectively deliver an effective radiation dose to glioma cells. Consequently, we developed *p*-[^{131}I]iodo-L-phenylalanine ([^{131}I]IPA), which — when used as a single agent — was tumoricidal to glioma cells in vitro and led to improved survival of rats with cerebral C6-gliomas [22]. Besides the therapeutic β-emission, [^{131}I]IPA improved the therapeutic efficacy of external radiation therapy significantly [23]. However, besides the controversial discussion related to the usefulness of the rat C6-glioma model in preclinical neurooncology [24], a major drawback of our previous studies in the above-mentioned intracranial glioma models was the limitation in the assessment of tumor formation in vivo and at follow-up after therapy. In some cases, magnetic resonance tomography (MRT) and PET failed in identifying tumors, which were clearly confirmed immunohistopathologically after autopsy. For these reasons, we decided to perform confirmatory investigations in animal model with heterotopically transplanted and therefore well-visible and palpable human gliomas. In this work, we test the therapeutic efficacy of [^{131}I]IPA combined with external radiotherapy on different glioma cell lines and on human glioblastomas xenografts in CD1 nu/nu mice and RNU rats.

2. Materials and methods

2.1. Materials

Iodine-131 for radiolabeling was purchased from GE Healthcare (Braunschweig, Germany). The fluorescent dyes propidium iodide (PI) and bisbenzimide H33342 were from Sigma-Aldrich (Taufkirchen, Germany). RPMI 1640, Dulbecco modified Eagle medium (DMEM), penicillin/streptomycin and fetal bovine serum (FBS), phosphate-buffered saline (PBS), collagenase II and trypsin/EDTA were purchased by PAA (Pasching, Austria) or Gibco Invitrogen (Karlruhe, Germany). The water-soluble tetrazolium salt (WST-1) for the determination of cell viability was from Roche Diagnostics (Mannheim, Germany). Unless otherwise stated, all other solvents and chemicals were of analytical or clinical grade and were either obtained from VWR International (Darmstadt, Germany) or purchased via the local university hospital pharmacy.

2.2. Preparation of [^{131}I]IPA

p-[^{131}I]iodo-L-phenylalanine used for radionuclide therapy in the present study was prepared by [^{131}I]iododestannylation of the high-purity tributyltin precursor as described previously [25]. The n.c.a. radiopharmaceutical was obtained with a radiochemical yield of 90%±6%. The radiochemical purity as determined by analytical high-performance liquid chromatography was greater than 99%, and the specific activity was >1600 GBq/μmol. [^{131}I]IPA was buffered with PBS (pH 7.0) and sterile-filtered through a 0.22-μm filter (Millipore, Eschborn, Germany) into a sterile vial for in vitro investigations on glioma cell lines and for animal studies.

2.3. External irradiation

Cells and tumor xenografts were irradiated at room temperature using 6-MV photons generated by a Siemens Mevatron MXE linear accelerator (Siemens, Erlangen, Germany) at dose rate of 2 Gy/min. The focus-isocenter distance was 100 cm with field sizes of 5×5 cm at the isocenter for irradiation of well plates or glioma xenografts in mice and rats, respectively.

2.4. Cell lines and cell experiments

The Tx3868 and T5135 glioma cell lines both derived from primary human glioblastoma multiforme were provided by Prof. Dr. Eckard Meese of the Institute of Human Genetics, University of Saarland (Homburg, Germany). The primary human A1207 glioblastoma cell line was provided by Dr. R. Class (Symbiotec GmbH, Saarbrücken, Germany), while the human M059K glioblastoma cell line and the rat F98 glioma cells were from the American Type Culture Collection (ATCC, Rockville, MD, USA). Cells were cultivated in RPMI-1640 (A1207) or in DMEM (T5135, Tx3868, M059K and F98) supplemented with glucose (4.5 g/L), L-glutamine (300 mg/L), 10% (vol/vol) heat-inactivated FBS and 1% penicillin (100 U/ml)/streptomycin (100 μg/ml). The cells were maintained in a humidified 5% CO_2 incubator at 37°C and passaged routinely. Before experiments, subconfluent cell cultures were trypsinized with a solution of 0.05% trypsin in PBS containing 0.02% EDTA. Cells were washed with the medium and concentrated in PBS shortly