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Improved synthesis of no-carrier-added *p*-[^{124}I]iodo-L-phenylalanine and *p*-[^{131}I]iodo-L-phenylalanine for nuclear medicine applications in malignant gliomas

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Abstract

This work describes the synthesis and the tumor affinity testing of no-carrier-added (n.c.a.) *p*-[^{124}I]iodo-L-phenyalanine ([^{124}I]IPA) and n.c.a. *p*-[^{131}I]iodo-L-phenylalanine ([^{131}I]IPA) as radiopharmaceuticals for imaging brain tumors with PET and for radionuclid-based therapy, respectively. Parameters for labeling were optimized with regard to the amount of precursor, temperature and time. Thereafter, n.c.a. [^{124}I]IPA and n.c.a. [^{131}I]IPA were investigated in rat F98 glioma and in primary human A1207 and HOM-T3868 glioblastoma cells in vitro, followed by an in vivo evaluation in CD1 nu/nu mice engrafted with human glioblastoma.

No-carrier-added [^{124}I]IPA and n.c.a. [^{131}I]IPA were obtained in $90\pm6\%$ radiochemical yield and $>99\%$ radiochemical purity by iododestannylation of *N*-Boc-4-(tri-*n*-butylstannyl)-L-phenylalanine methylester in the presence of chloramine-T, followed by hydrolysis of the protecting groups. The total synthesis time, including the HPLC separation and pharmacological formulation, was less than 60 min and compatible with a clinical routine production. Both amino acid tracers accumulated intensively in rat and in human glioma cells. The radioactivity incorporation in tumor cells following a 15-min incubation at 37 °C/pH 7.4 varied from 25% to 42% of the total loaded activity per 10^6 tumor cells (296–540 cpm/1000 cells). Inhibition experiments confirmed that n.c.a. [^{124}I]IPA and n.c.a. [^{131}I]IPA were taken up into tumor by the sodium-independent L- and ASC-type transporters.

Biodistribution and whole-body imaging by a gamma-camera and a PET scanner demonstrated a high targeting level and a prolonged retention of n.c.a. [^{124}I]IPA and n.c.a. [^{131}I]IPA within the xenotransplanted human glioblastoma and a primarily renal excretion. However, an accurate delineation of the tumors in mice was not possible by our imaging systems. Radioactivity accumulation in the thyroid and in the stomach as a secondary indication of deiodination was less than 1% of the injected dose at 24 h p.i., confirming the high in vivo stability of the radiopharmaceuticals.

In conclusion, n.c.a. [^{124}I]IPA and n.c.a. [^{131}I]IPA are new promising radiopharmaceuticals, which can now be prepared in high radiochemical yields and high purity for widespread clinical applications. The specific and high-level targeting of n.c.a. [^{124}I]IPA and n.c.a. [^{131}I]IPA to glioma cells in vitro and to glioblastoma engrafts in vivo encourages further in vivo validations to ascertain their clinical potential as agent for imaging and quantitation of gliomas with PET, and for radionuclid-based therapy, respectively.

Keywords: Amino acids; Iododestannylation; ^{124}I- and ^{131}I-labeled radiopharmaceuticals; PET imaging; Radionuclide therapy

1. Introduction

Despite aggressive treatment protocols, including surgery followed by radiotherapy, additional brachytherapy or stereotactic radiosurgery and chemotherapy, patients with malignant gliomas still experience poor outcome (DeAngelis, 2001; Nieder et al., 2004; DeAngelis et al., 1998). In order to overcome these dismal prospects, various experimental therapies have been administered, among them are gene therapy with the herpes simplex thymidine kinase gene, methods for sensitizing glioma cells to the induction of apoptosis, implantation of iodine-125 seeds,

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locoregional radioimmunotherapy, photodynamic therapy with 5-aminolevulinic acid, or aim at different targets like the coagulation system, to name only some (Bowers et al., 2003; Riva et al., 2000; McDermott et al., 1998; Olzowy et al., 2002; Perkins et al., 2003; Stummer et al., 1998; Zalutsky, 2005; Stupp et al., 2006). Unfortunately, in spite of all these efforts the prognosis of malignant gliomas remains poor, and no significant improvement in median survival has been demonstrated.

In recent studies, we demonstrated the effectiveness and safety of single-photon emission computed tomography (SPECT) with *p*-[^{123}I]iodo-L-phenylalanine ([^{123}I]IPA) for imaging brain tumors (Samnick et al., 2002 b; Hellwig et al., 2005a, 2007). [^{123}I]IPA actively crosses the blood–brain barrier after intravenous administration and is highly taken up by glioma cells, presumably via the amino acid transporters L and ASC, which have been described to be overexpressed in glioma cells (Fuchs and Bode, 2005; Miyagawa et al., 1998; Samnick et al., 2001, 2004b; Lahoutte et al., 2004). Moreover, a specific uptake and prolonged retention of [^{123}I]IPA were clinically demonstrated for low-grade and high-grade gliomas alike. In contrast, accumulation of [^{123}I]IPA in normal cerebral parenchyma and non-neoplastic lesions was moderate and/or decreases rapidly with time (Samnick et al., 2002b; Hellwig et al., 2005a). This preferential uptake and prolonged retention of [^{123}I]IPA by gliomas, compared to non-neoplastic brain tissue provides the rationale to consider an iodine-131 labeled analog of [^{123}I]IPA as a potential agent for targeting gliomas. Importantly, *p*-[^{131}I]iodo-L-phenylalanine ([^{131}I]IPA) was demonstrated to be tumoricidal to glioma cells in vitro and in vivo (Romeike et al., 2004; Samnick et al., 2004a). [^{131}I]IPA administered as a monotherapy to rats bearing C6-gliomas improved the survival significantly compared to control animals (Romeike et al., 2004). In addition to the cytostatic effect, [^{131}I]IPA seems to act synergistically tumoricidal in connection with external field radiation, which forms part of most current standard therapy protocols for malignant gliomas (Samnick et al., 2005). The uniquely synergistic properties of [^{131}I]IPA, which combines a high, specific and sustained tumor uptake and an intrinsic cytostatic activity as well as an intrinsic radiosensitizer effect make it highly attractive as a therapeutic agent for malignant gliomas. So far, [^{131}I]IPA has not been administered to human patients. Introducing a radiopharmaceutical into clinical use requires a preparation method suitable for clinical applications. This prompted us to develop a radiolabeling procedure, which should result to [^{131}I]IPA in high radiochemical yield and high purity under routine clinical conditions. Besides the therapeutic beta-emission, development of the iodine-124 labeled analog [^{124}I]IPA would provide a new long-lived radiotracer for both imaging and therapy quantitation with positron emission tomography (PET) in patients with malignant gliomas. This work describes the radiosynthesis of no-carrier-added (n.c.a.) *p*-[^{124}I]iodo-L-phenylalanine and n.c.a. *p*-[^{131}I]iodo-L-phenylalanine for routine clinical applications, and investigates both radiopharmaceuticals in primary human glioblastoma and in rat glioma cells as well as in a murine xenograft model to test their potential as radiopharmaceutical for PET imaging and for radionuclid-based therapy.

2. Materials and methods

2.1. General

[^{124}I]Iodide for radiolabeling was produced on a CV28 cyclotron (PET center, Department of Nuclear Medicine, University Hospital Essen, Germany) by a [^{124}Te]tellurium (d,2n) [^{124}I]iodine reaction as described previously (Knust et al., 2000). In details, a platinium–iridium target plated with 99.8% [^{124}Te]tellurium dioxide was irradiated with 14-MeV deuterons at 15 μA. Subsequently, the [^{124}I]iodine was distilled at 745 °C in a quartz apparatus and the [^{124}I]iodide was inserted in 0.2 ml of 0.02 N NaOH for radiolabeling. Carrier-free sodium [^{131}I]iodide was purchased commercially from GE Healthcare Buchler (Braunschweig, Germany). The authentic *p*-iodo-L-phenylalanine used as reference for high-pressure liquid chromatography (HPLC) analysis and to assess the integrity of the radioiodinated analogs after radiosynthesis, as well as bis(tributyltin), chloramine-T, arginine, L-phenylalanine, L-tyrosine, L-serine, L-cysteine, L-alanine, 2-amino-2-norbornane-carboxylic acid (BCH) and ethylamino-iso-butyric acid (MeAIB) were from Sigma-Aldrich (Deisenhofen, Germany). *N*-Boc-4-iodo-L-phenylalanine was from Fluka (Buchs, Switzerland). *N*-Boc-4-bromo-L-phenylalanine, trifluoroacetic acid and dimethylsulfoxide-d6 with 0.03% tetramethylsilane (TMS) were purchased from ACROS Organics (Geel, Belgium). Silica gel 60 (0.2–0.5 mm) for column chromatography, silica gel aluminum sheets for thin-layer chromatography (TLC), tetrakis(triphenylphosphine)-palladium(0) and dimethylsulfate were obtained from Merck (Darmstadt, Germany). RPMI 1640 and DMEM with high glucose supplemented with L-glutamine, 100 U/ml penicillin, 100 μg/ml streptomycin and 5% fetal bovine serum as well as phosphate buffered saline (PBS), collagenase II and trypsin/EDTA were purchased from PAA (Pasching, Austria) or Gibco Invitrogen cell culture (Karlruhe, Germany), respectively. Unless otherwise stated, all other solvents and chemicals were of analytical or clinical grade and were obtained either from Merck or purchased via the local university hospital pharmacy.

^{1}H-NMR was recorded on a Brucker DRX-500 NMR-Spectrometer (Brucker, Karlruhe, Germany). NMR data are referenced to TMS as an internal standard ($\delta = 0.0$ ppm) and are reported in ppm (δ) downfield. Mass spectra were recorded on a TRIO 2000 spectrometer (VG Organic, UK), using positive ion mode with electrospray as interface (ES^+). Samples were dissolved in MeOH/0.01 M aqueous acetic acid (50:50, v/v) for MS measurements.