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Implementation of ^{89}Zr production and *in vivo* imaging of B-cells in mice with ^{89}Zr-labeled anti-B-cell antibodies by small animal PET/CT

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ABSTRACT

We examined the production, separation, and characterization of ^{89}Zr, including supplementation of a commercial Cyclone® 18/9 with a self-made Solid Target System (STS). Obtained [^{89}Zr]Zr-oxalate was used for the labeling of anti-B cell antibodies with desferrioxamine-p-SCN as a bifunctional chelator. ^{89}Zr-labeled antibodies were injected in DBA/1 mice to examine usability for detection of B cells *in vivo*. PET measurements showed binding of ^{89}Zr-labeled anti-B cell antibodies in tissues with high frequencies of B cells, i.e. in spleen and lymph nodes.

1. Introduction

The β^+-emitting radionuclide ^{89}Zr has increasingly gained attention over the past two decades. Since its first utilization for labeling of antibodies reported by Link et al. (1986) several routes for the production and purification of the radionuclide have been investigated (Eary et al., 1986; Dejesus and Nickles, 1990; Meijs et al., 1994; Verel et al., 2003a, 2003b; Kandil et al., 2007; Holland et al., 2009; Dutta et al., 2009), and numerous antibodies have been labeled and evaluated for their potential as radiotracers in positron emission tomography (Meijs et al., 1996, 1997).

Zirconium-89 is very well suited for immuno-PET, the tracking and quantification of monoclonal antibodies (mAbs) *in vivo* due to its advantageous decay characteristics (Aerts et al., 2009; Holland et al., 2009). The radionuclide's convenient half-life of 78.4 h is suitable for sufficient binding of antibodies in target tissues (Wu, 2009; van Dongen et al., 2007; Verel et al., 2005), and it possesses a considerable β^+ emission rate of 23% with low maximum β^+ energy (0.9 MeV), resulting in a short mean range in water or tissue (about 1 mm) and therefore in a good spatial resolution (Laforest and Liu, 2008). Besides the above mentioned benefits, additionally only one γ-line (909 keV) is emitted during decay, resulting in low exposure radiation dose for patients and medical staff. The target material is ^{89}Y (100% natural abundance), which requires no enrichment or time-consuming recycling and is therefore easily to handle.

Among the other positron emitters taken into consideration for the labeling of antibodies, only ^{124}I and ^{52}Mn are suitable because of their half-lives (100.2 and 134.2 h, respectively). However, ^{124}I is not capable to deliver PET images of good resolution because of its high positron energy (2.1 MeV) and a series of γ-lines (e.g. 1.69 MeV), as well as deiodinization *in vivo* (van Dongen et al., 2007; Verel et al., 2005; Zalutsky, 2006; Nayak and Brechbiel, 2009). For ^{52}Mn little to no experience has been gained with the radionuclide because of its less favorable access, less convenient decay properties, and its complex redox chemistry (Nayak and Brechbiel, 2009). Thus, ^{89}Zr appears as a radionuclide of choice for immuno-PET (Borjesson et al., 2006).

In comparison to γ-scintigraphy and single photon emission computed tomography (SPECT), PET offers the chance for tracer quantification due to attenuation correction, resolution, and sensitivity (van Dongen et al., 2007; Nayak and Brechbiel, 2009; Disselhorst et al., 2010; Verel et al., 2003a, 2003b, 2005). Immuno-PET utilizing ^{89}Zr has been particularly applied in cancer research (van Dongen et al., 2007, Verel et al., 2005). In addition to numerous animal studies (Perk et al., 2008; Dijkers et al.,

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2009), the application of ^{89}Zr-labeled antibodies to detect primary tumors in humans has been successfully demonstrated in several studies (Borjesson et al., 2006; Dijkers et al., 2009, 2010).

Another application, which has been evaluated in the last 10 years, is the role as surrogate for ^{86}Y or ^{111}In in immunoscintigraphy for dosimetry purposes. This method was developed to quantify the radiation dose delivered by β^--emitting therapy radionuclides such as ^{90}Y, ^{177}Lu, or ^{131}I, and has already been applied successfully (Borjesson et al., 2009).

Besides cancer research, the use of radionuclide labeled monoclonal antibodies (mAb) in immuno-PET is of interest for autoimmune diseases or animal models thereof (Becker et al., 1990; Malviya et al., 2007). Thereby, monitoring cell trafficking is of special interest, because major elements of the immune system relevant for autoimmune diseases are migratory cells, producing cytokines and other mediators of inflammation (Firestein, 2003; Lampropoulou et al., 2010; Toh and Miossec, 2007). The influx and presence of these cells at the site of inflammation fluctuate in the course of disease pathogenesis. For *in vivo* detection of cells, we validated the feasibility of ^{89}Zr for labeling of antibodies using anti-B220 mAb, which is frequently applied in immunological research for staining B cells.

2. Experimental

2.1. Materials and methods

Yttrium-89 metal foil (> 99.9% 25 mm × 25 mm × 0.15 mm) was purchased from Goodfellow Cambridge Ltd. (Huntingdon, England). Water was deionized and purified by a Synergy® Ultrapure water system (> 18 MΩ cm^{-1}; Millipore, Billerica, MA, USA) or a Purelab Ultra system (Elga Berkefeld GmbH, Celle, Germany). Hydrochloric acid (30%), hydrogen peroxide (30%), sodium hydrogen carbonate, and 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES) were purchased from Merck KGaA (Darmstadt, Germany), acetonitrile (MeCN) and sodium chloride from Fluka Chemie GmbH (Neu-Ulm, Germany). Ammonium acetate was purchased from Riedel-de-Haën AG (Buchs, Switzerland); oxalic acid, dimethyl sulfoxide (DMSO), N-(3-dimethylaminopropyl)-N′-ethylcarbodiimide hydrochloride (EDC), 2,3,5,6-tetrafluorophenol (TFP) and diethylene triamine pentaacetic acid (DTPA) from Sigma-Aldrich (St. Lewis, MO, USA); 1-(4-isothiocyanatophenyl)-3-[6,17-dihydroxy-7,10,18,21-tetraoxo-27-[N-acetyl-hydroxylamino)-6,11,17,22-tetraazaheptaeicosane]thiourea (desferrioxamine-*p*-SCN) from Macrocyclics (Dallas, TX, USA); hydroxylamine hydrochloride from Germed (Dresden, Germany); Accell Plus CM cation exchange resin (300 Å, 0.35 mmol/g ligand density) came from Waters (Milford, MA, USA); and Na_2CO_3 came from Laborchemie Apolda (Apolda, Germany). All chemicals and solvents were of analytical grade and used as received.

All glassware (fused silica), as well as plastic ware and PTFE beakers, were rinsed with 6 M HCl (aq.) and water prior to use.

Analytical thin layer chromatography (TLC) experiments were performed on pre-coated Silica Gel 60 F_{254} and RP-18 $F_{254\ s}$ plates (Merck KGaA, Darmstadt, Germany), respectively, and quantified on a Rita Star apparatus (Raytest Isotopenmeßgeräte GmbH, Straubenhardt, Germany) utilizing Rita control 1.24 software.

Analytical radio high-performance liquid chromatography (HPLC) was carried out on a system from Sykam GmbH (Eresing, Germany) equipped with a DAD-detector (Smartline UV detector 2600) and a scintillation radio detector (γ-sensor^{PE}, Scintomics, Fürstenfeldbrück, Germany) connected in series. The system was operated with a size exclusion column (BioSep-SEC-S 3000, Phenomenex Inc., Torrance, CA, USA) at a flow rate of 1 ml/min (back pressure 29–30 bar) utilizing an isocratic phosphate buffer saline system (0.1 M, pH 6.2–7.0 see below) as the eluent.

Activimeter: Both, the Isomed 2000 and the well counter Isomed 2000 were purchased from MED (Nuklear-Medizintechnik Dresden GmbH, Dresden, Germany). For quantification of ^{89}Zr activities the analogs modality of calibration of the activimeters was used as reported (the software included ^{54}Mn mode was used, multiplying the displayed amount of activity by a factor of 0.67 (Verel et al., 2003a, 2003b)). Comparing results of this reported method with γ-ray spectroscopy measurements deviations of less than 10% were observed.

The pH-meter pH315i (WTW—Wissenschaftlich-Technische Werkstätten GmbH, Weilheim, Germany) was equipped with a micro pH electrode (Orion, ordering number 9810BN, Thermo Scientific, Waltham, MA, USA).

2.1.1. Saline/gentisic acid solution

Gentisic acid was added to 0.9% NaCl (final concentration: 5 mg/ml), and the pH was adjusted to 4.9–5.3 with 2.0 M sodium carbonate solution.

2.1.2. Phosphate buffer/saline system (pH: 6.2–7.0)

13.8 g of sodium dihydrogen phosphate monohydrate, 14.2 g of disodium hydrogen phosphate, 17.4 g of sodium chloride, and 1.3 g of sodium azide were dissolved in 2 l of deionized water and stirred thoroughly.

2.2. Cyclotron

The Institute of Radiopharmacy at the Forschungszentrum Dresden-Rossendorf (FZD) is equipped with a Cyclone® 18/9 cyclotron (IBA, Belgium, E_p=18 MeV, E_d=9 MeV).

A home made solid target system (STS) has been used in addition to liquid and gas targets. STS increases the flexibility of the Cyclone® 18/9 and enables the production of metallic PET radionuclides such as ^{61}Cu, ^{64}Cu, ^{86}Y, and ^{89}Zr for radiochemical research and applications in nuclear medicine. The STS is mounted at the 2 m long beam transport line.

The main parts of the STS are the support and the collimator units (Fig. 1). The STS is constructed for target materials on supports of 23 mm in diameter and a target area of 12 mm in diameter. During irradiation, the horizontal ion beam hits the target, which is aligned at a perpendicular angle. The support is water cooled on the rear side, and the target surface is cooled by a helium stream. After irradiation, the target on the support is unloaded into a lead container.

2.3. Target preparation and irradiation

The square yttrium foil of 12.5 mm × 12.5 mm and 150 μm in thickness was placed in the target setup consisting of an Al

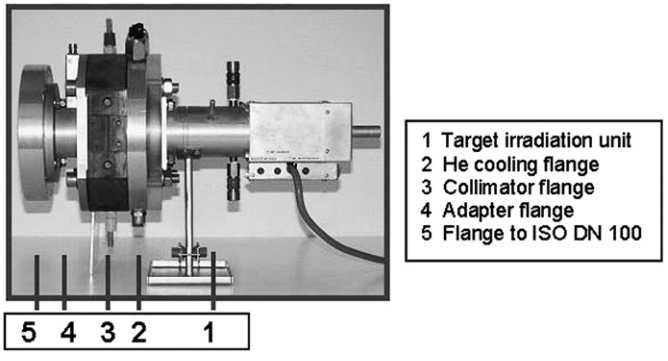

Fig. 1. Solid target system (STS) with irradiation unit ready to mount at the beam transport line.