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Isostructural folate conjugates radiolabeled with the matched pair ^{99m}Tc/^{188}Re: a potential strategy for diagnosis and therapy of folate receptor-positive tumors ☆

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Abstract

^{99m}Tc-technetium (^{99m}Tc) and ^{188}Re-rhenium (^{188}Re) represent an interesting pair of radionuclides for diagnosis and therapy. The aim of this study was to synthesize and characterize in vitro/in vivo the first ^{188}Re-folate derivative [^{188}Re(CO)$_3$–picolylamine monoacetic acid 188Re-PAMA-folate (**2**)] for potential targeted radionuclide therapy of FR-positive tumors. The data were compared with those of the isostructural ^{99m}Tc-analog [^{99m}Tc–PAMA folate (**1**)] reported previously.

Methods: In vitro stability of compound **2** was tested in phosphate-buffered saline and human plasma. Cell binding experiments were performed with FR-positive human KB cells. Biodistribution was assessed in female nude mice, bearing KB tumor xenografts.

Results: Cell binding experiments showed high and FR-specific uptake. In vivo, compound **2** accumulated specifically in the FR-positive tumors with maximal values 4 h post injection (p.i.) [**2**: 1.87±0.04 percent injected dose per gram of weight tissue (% ID/g) vs. **1**: 2.33±0.36% ID/g]. Unfavorably high retention of radioactivity was found in FR-positive kidneys (12.04±0.62% ID/g; 4 h p.i.). Tumor-to-blood ratio of radioactivity (**2**: 14.5±1.32, 4 h p.i.) was lower than for compound **1** (58.0±12.2, 4 h p.i.), whereas tumor-to-kidney ratios were in the same range (**2**: 0.15±0.01 vs. **1**: 0.13±0.02, 4 h p.i.). Preadministration of the antifolate pemetrexed significantly improved the tumor-to-kidney ratio (**2**: 1.59±0.30, 4 h p.i.).

Conclusions: The isostructural radiofolates **1** and **2** displayed almost identical pharmacokinetic profiles and accumulated both specifically in FR-positive tumors. However, only the coapplication of the antifolate pemetrexed improved the biodistribution of the radiotracers in such ways that a potential therapeutic application of compound **2** can be envisaged in the future.

Keywords: Tc(I); Re(I); Folic acid; Folate receptor; Antifolates; Pemetrexed

1. Introduction

^{99m}Tc-technetium (^{99m}Tc) and ^{188}Re-rhenium (^{188}Re) represent an attractive pair of radionuclides for biomedical use because of their favorable decay properties for diagnosis (^{99m}Tc: 6 h half-life, 140-keV γ-radiation) and therapy (^{188}Re: 17 h half-life, 2.12-MeV β^-_{max}-radiation) and because of their onsite availability, thanks to corresponding ^{99}Mo/^{99m}Tc- and ^{188}W/^{188}Re generator systems. Furthermore, the elements technetium and rhenium reveal similar chemical properties since they are localized in the second and third transition metal row (group VII B) of the periodic table. This circumstance offers the possibility to simultaneously develop diagnostic and therapeutic tracers with almost identical chemical and, presumably, also pharmacokinetic characteristics.

The [M(CO)$_3$] core (M=^{99m}Tc, ^{188}Re) possesses a high in vivo stability due to the kinetic inertness of the low-valent, organometallic metal core [1]. First data of in vivo studies with tumor affine peptides and proteins labeled with both the [99mTc(CO)$_3$]- and the [188Re(CO)$_3$]-core have shown promising results [2–4]. Therefore, we reasoned to radiolabel folate derivatives with ^{99m}Tc/^{188}Re using the same strategy.

The high-affinity folate receptor (FR) is frequently overexpressed on a variety of tumors but highly limited in

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most normal human tissues [5,6]. Thus, the vitamin folic acid can be used to carry diagnostic or therapeutic probes into tumor cells in an unperceived manner. To date, preclinical and clinical studies with diverse folate conjugates with, e.g., radionuclides, chemotherapeutics, immunotherapeutic agents, proteins and protein toxins etc. have been described in the literature [7–19]. Recently, we reported the syntheses and characterization of several new $^{99m}Tc(CO)_3$-folate conjugates suitable for imaging of FR-positive cancer cells [20–22]. Among these compounds, the 99mTc-PAMA-folate (**1**) with a tridentate picolylamine monoacetic acid chelator (Fig. 1) proved to be the most favorable candidate with regard to accumulation in FR-positive tumors [21,23]. We reasoned that using the same folate derivatives labeled with the particle emitting radionuclide ^{188}Re could potentially be interesting for targeted tumor therapy.

We herein describe the preparation and in vitro/in vivo characteristics of the ^{188}Re–PAMA folate (**2**). Labeling efficiency, in vitro stability as well as FR-binding capacity and in vivo tissue distribution of the ^{188}Re radiofolate **2** were compared with the results of the ^{99m}Tc derivative **1**. In addition, we investigated if preadministration of the antifolate pemetrexed resulted in an improved tumor-to-kidney ratio of the ^{188}Re radiotracer **2**, as previously observed for the ^{99m}Tc match **1** [24].

2. Materials and methods

2.1. General

The PAMA folate was synthesized as previously reported [20]. Precursor $[^{99m}Tc(OH_2)_3(CO)_3]^+$ was prepared using the Isolink kit (Mallinckrodt-Tyco, Petten, The Netherlands). $[Na][^{99m}TcO_4]$ was eluted from a $^{99}Mo/^{99m}Tc$ generator (Mallinckrodt-Tyco, Petten, The Netherlands) with a 0.9% saline solution. The $[Na][^{188}ReO_4]$ was eluted from a $^{188}W/^{188}Re$ generator (Oak Ridge National Laboratories, Oak Ridge, TN, USA). Pemetrexed (400 μg/100 μl; Alimta; Lilly, Bad Homburg, Germany) was diluted with NaCl 0.9% according to the instruction of the manufacturer. KB cells (CCL-17) were purchased from American Type Culture Collection (Manassas, VA, USA). FFRPMI cell culture medium (modified RPMI medium without folic acid, vitamin B_{12}, phenol red) was purchased from Cell Culture Technologies, Gravesano/Lugano, Switzerland. High-performance liquid chromatography (HPLC) analyses were performed on a Merck-Hitachi L-6200A system, equipped with an L-3000 tunable absorption detector, a Berthold LB 508 radiometric detector and an Xterra (Waters) MS C-18 reversed phase column (5 μm, 15 cm×4.6 mm) [HPLC solvents: Aqueous 0.05 M triethylammonium phosphate buffer, pH 7.0 (solvent A), methanol (solvent B)]. The HPLC system started with 100% A with a linear gradient to 20% A and 80% B over 15 min, followed by 5 min of 100% A with a flow rate of 1 ml/min. For cell experiments and biodistribution studies, radioactivity (γ-radiation of ^{99m}Tc and ^{188}Re, respectively) was measured with a γ-counter (Cobra II, Model B 5003, Packard). Protein concentrations for the in vitro experiments were measured with a microplate reader (Bio-Rad, Model 550), using a Micro BCA Protein Assay kit (Prod #23235) (Socochim).

2.2. Preparation of complex *2*

The precursor $[^{188}Re(OH_2)_3(CO)_3]^+$ was synthesized as described by Schibli et al. [1]. The precursor was used without further purification for the subsequent labeling step. Compound **2** was formed by addition of a stock solution of

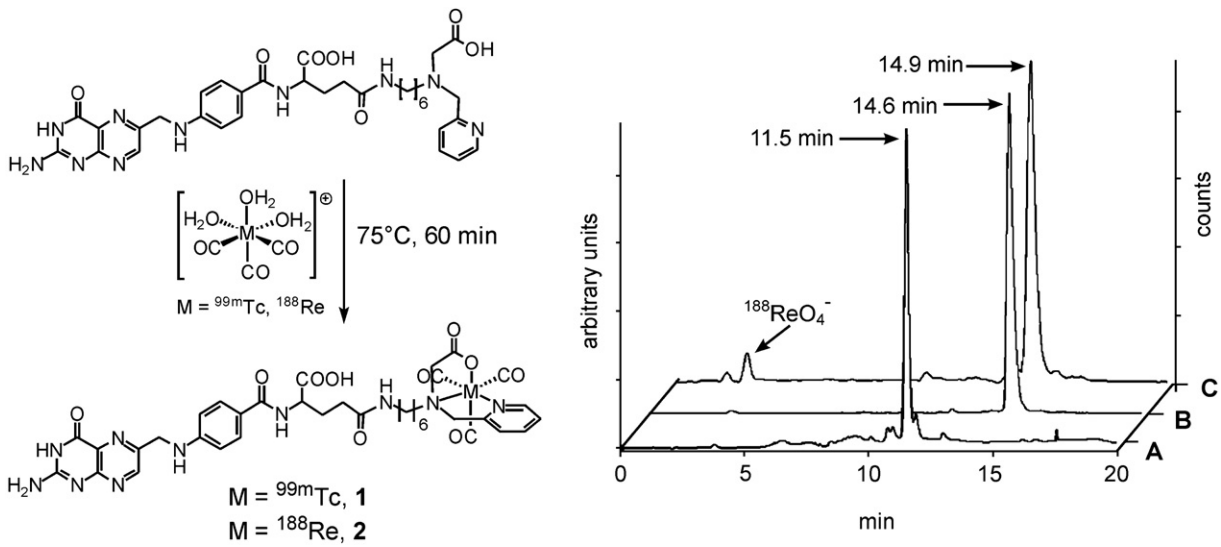

Fig. 1. (Left) Radiolabeling of the PAMA-folate ligand with the precursor $[M(CO)_3(OH_2)_3]^+$ (M=^{99m}Tc, ^{188}Re) and structure of the radioconjugates **1** and **2**. (Right) Representative HPLC traces: (A) unlabeled PAMA folate, (B) ^{99m}Tc-radiofolate **1**; (C) ^{188}Re-radiofolate **2**.