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Preliminary evaluation of two radioiodinated maleimide derivatives targeting peripheral and membrane sulfhydryl groups for in vitro cell labeling

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ABSTRACT

A factor impeding the advancement of cell mediated therapy is the inability to track these cells in vivo by noninvasive techniques. It has been shown that cells express high levels of sulfhydryl groups. We sought to explore these groups to covalently label cells with radiolabeled maleimide derivatives. Two maleimide derivatives; N-[2-(2,5-dioxoazolinyl)ethyl](5-iodo(3-pyridyl))carboxamide and N-[2-(2,5-dioxoazolinyl)ethyl](3-iodophenyl)carboxamide (**[^{125}I]-4** and **[^{125}I]-8**) were synthesized and radioiodinated. These compounds were evaluated for in vitro binding to neutrophils, endothelial and mesenchymal stem cells, and biodistribution of the radiolabeled stem cells in nude mice. These radiotracers were obtained in moderate to high radiochemical yields. Binding to cells were moderate (20–60%/10^6 cells) and the label was retained, although washout (an average of 18–55%) was observed depending on the cell type and the tracer used. The labeled cells initially localized in well perfused organs and at a later time showed a general distribution as expected. The novel tracers labeled several cell types and shown that the stability of the label and viability of the cells were maintained in vitro and in vivo for a reasonable period and warrant further in vivo investigation.

1. Introduction

A key factor impeding the progress in therapeutic application of cells is the inability to track noninvasively the distribution of these cells upon in vivo delivery.

^{111}Indium-oxine (^{111}In-oxine) labeled leukocytes have been the gold standard for the localization of occult infection and inflammation (Thakur and Gottschalk, 1980; Rennen et al., 2001). Lately, technetium-99m hexamethyl propylenamine oxime (^{99m}Tc-HmPAO) has been used frequently for cell labeling (Becker et al., 1988; Arndt et al., 1993; Amartey et al., 1997). These two radiopharmaceuticals have also been used to label stem cells and studied in vivo (Zhou et al., 2005; Beeres et al., 2007; Barbash et al., 2003). There have been reports on investigations using Cu-64 and F-18 radiotracers to label cells. Amongst these 2-[^{18}F] fluorodeoxyglucose (FDG), the key radiopharmaceutical for positron emission tomography (PET) studies, has been used to label cells for clinical application. The radiopharmaceutical is carried mainly by the glucose transporters (GLUTs) into cells. The mechanism of cell labeling with FDG is well understood (Zhuang and Alavi, 2002; Tamura et al., 2004). The main drawback of FDG application for effective cell labeling is the extensive washout of the tracer (Pellegrino et al., 2005). Moreover for cell labeling with FDG, these cells must express sufficient levels of GLUTs. Another radiofluorinated compound, N-succinimidyl-4-[^{18}F]-fluorobenzoate (SFB) has been utilized for cell labeling (Olasz et al., 2002). Although good labeling was reported, the washout of the tracer was very high. It was anticipated that with SFB the cell labeling mechanism might involve a covalent linkage to cellular components by the fluorinated ester. The apparent fast washout implies that this was not entirely the mechanism. The excellent properties of ^{18}F compared with other common positron emitting radionuclides makes it more attractive to search for cell labeling agents based on ^{18}F, especially for short term tracking studies. Hence recently Ma et al., reported the use of hexadecyl-4-[^{18}F]-fluorobenzoate ([^{18}F-HFB]), a long chain benzoic ester to label rat mesenchymal stem cells (Ma et al., 2005). A modest 25% uptake was reported, however the washout was also faster than expected.

Copper (II)—[^{64}Cu] pyruvaldehyde-bis-(N^4-methylthiosemicarbazone) (PTSM) is a copper complex that has also been used for cell labeling (Adonai et al., 2002). This complex was originally developed for hypoxia imaging of tumors (Shelton et al., 1990;

Abbreviations: DCM, dichloromethane; ACN, acetonitrile; TEA, triethylamine; Prep-TLC-SG, preparative silica gel chromatographic plates

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Padhani et al., 2007). The complex enters cells by passive diffusion, and within the cell it undergoes a reduction–oxidation (redox) reaction. This process effectively produces a labile Cu (I) complex, which releases copper and is subsequently taken up by intracellular macromolecules (Bass et al., 2000; Dearling and Packard, 2010). Nonetheless, this bioreductive process has been reported to vary with cell types, and so labeling yield and washout may differ (Blower et al., 1996).

To effectively advance the application of labeled cells into the clinic, especially in the areas of cell mediated therapy new methods of labeling are being sought. Recently, Vaidyanathan et al. reported the use of N-formylmethyl-5-iodopyridine-3-carboximide (^{125}I-FMIC) and 4-diethylamino-3-iodobenzaldehyde (^{125}I-DEIBA) to target aldehyde dehydrogenase, an enzyme highly expressed in stem cells as an agent for labeling these cells (Vaidyanathan et al., 2009). Although the final outcome was not as expected, the authors were able to prove the principle behind the approach.

It has been shown that cells including neutrophils express a high level of reactive sulfhydryl groups (Schwartz and Harlan, 1989; Dinkova-Kostova et al., 2001). Maleimide is a known thiol scavenger (Berndt et al., 2007). We hypothesize that this reaction between thiol groups and radiolabeled maleimide derivatives of pyridine and phenyl carboxylates can be explored for cell labeling in vitro. We report here the synthesis, radioiodination and in vitro evaluation of two aminoethylmaleimide derivatives of benzoic and pyridine carboxylic acids to target sulfhydryl groups of cells. We have tested these compounds with thiol containing molecules and in several cell types in vitro, and performed initial biodistribution of radiolabeled human stem cells in nude mice to assess their potential clinical application.

2. Materials and methods

2.1. General

The chemicals used were all purchased from Sigma-Aldrich (St. Louis, MO, USA) and were used without further purification, except where stated. Acetonitrile was kept over molecular sieves. The [^{125}I]-NaI was purchased from The Institute of Isotopes (Budapest, Hungary). [^{123}I]-NaI was prepared in house with the CS-30 Cyclotron (CTI, USA), using the ^{124}Te (p, $2n$) ^{123}I reaction. Sep-Pak cartridges were purchased from Waters-Millipore (Milford, MA, USA). Iodogen was purchased from Pierce Chemical Company (Rockford, IL, USA). Instant thin layer (ITLC-SG) and thin layer chromatographic (TLC) sheets were purchased from Gelman Sciences Inc. (Ann Arbor, USA). The mobile phases used were ethyl acetate:methanol 9:1 v/v (system A); ethyl acetate: hexane 7:3 v/v (system B) and ethyl acetate: hexane 1:1 v/v (system C). Radio-TLC strips were scanned with an InstantImager (Packard, Meriden, CT, USA). The High Pressure Liquid Chromatography (HPLC) analysis was carried out on Econosil C-18, 10 μ columns (semi-preparative, 250 × 10 mm^2 or analytical, 250 × 4.6 mm^2). A JASCO (Tokyo, Japan) chromatographic system equipped with a variable wavelength ultraviolet monitor and in tandem with a Canberra flow through radioactivity detector was used. Ultraviolet absorption was monitored at 254 nm. Chromatograms were acquired and analyzed, using the BORWIN® software. The gradient program used was as follows: solvent A=0.1% TFA in water and solvent B=0.1% TFA in ACN; time=0–2 min, 5% B; 2–15 min, 80% B; 15–20 min, 80% B, 20–25 min, 40% B and 25–30 min, 5% B at a flow-rate of 1.2 mL/min. Mass spectrometry was run on electrospray mass spectrometer (ES-MS, Waters Micromass, Quattro, Manchester, UK). NMR was run on JEOL, 400 MHz spectrometer. Chemical shifts are reported in parts per million (ppm) downfield relative to TMS (δ=0). Cobra® Meriden, CT, USA) gamma counter was used to count biodistribution and other radioactive samples. Human umbilical vein endothelial cells and human mesenchymal stem cells (HUVEC and HMSC) were purchased from Lonza Walkersville Inc. (Walkersville, MD, USA).

2.2. Syntheses

2.2.1. 5-Iodopyridine-3-carboxylic acid (2)

The reference compounds were synthesized using the scheme 1; Fig. 1. The ethyl-(1,1-dimethylstannaethyl)phenyl-3-carboxylate, compound (**1**) is available to us from the previous work. The compound (**1**) 200 mg (0.64 mmol) was dissolved in DCM (4 mL) together with iodogen 140 mg (0.32 mmol) in a reaction vial. The solvent was evaporated to dryness with a stream of argon. Potassium iodide, 110 mg (0.64 mmol), was dissolved in water (150 μL) and added to the film. This was followed by 0.1% acetic acid/methanol (500 μL). The reactants were incubated at an ambient temperature for 30 min. The reaction was terminated with sodium thiosulfate solution (1%, 200 μL). The product was extracted into ethyl acetate. The organic phase was dried over anhydrous sodium sulfate and evaporated to dryness to obtain a chromagraphically pure product, and was used without further purification. The ester was hydrolyzed by addition of LiOH, 96 mg (4 mmol) in ethanol/water 50/50 v/v mixture (2 mL). The reactants were refluxed for 30 min. The solvent was evaporated under reduced pressure, and the residue was dissolved in 500 μL each of water and methanolic-HCl and the product extracted with diethyl ether (25 mL × 2). The ether layer was evaporated to obtain a light yellow solid (60 mg, 38%). ES-MS; $[M+1]^+$=250 (100%).

2.2.2. N-[2-(2,5-dioxoazolinyl)ethyl](5-iodo(3-pyridyl)) carboxamide (4)

The title compound was prepared using Fig. 1; scheme 1 below. Compound (**2**) 30 mg (0.12 mmol) was suspended in DCM (2 mL). Thionyl chloride (0.3 mL) was added and refluxed for 60 min. The volatile materials were evaporated under reduced pressure to obtain a paste. The residue was redissolved and dried repeatedly to remove as much acidic residues as possible. Finally, the paste was dissolved in fresh DCM (2 mL) and placed in a dropping funnel. The 2-aminoethylmaleimide (**3**) 32 mg (0.12 mmol) was dissolved in DCM (2 mL) and basified with TEA (100 μL, 0.7 mmol). The acid chloride was added dropwise to the amine. The reaction mixture was heated at 60 °C for 30 min. The product was isolated by extraction into DCM, evaporated to obtain the product (**4**) 32 mg (65%).

2.2.3. [5-(1,1-dimethyl-1-stannaethyl)(3-pyridyl)]-N-[2-(2,5-dioxoazolinyl)ethyl]carboxamide (6)

Fig. 1; scheme 2 was followed for the synthesis of (**6**). 2,5-Dioxoaxolidinyl 5-(1,1-dimethyl-1-stannaethyl)pyridine-3-carboxylate (**5**) was prepared using the method published previously (Amartey et al., 2005). Compound (**5**) 86 mg (0.23 mmol) was dissolved in ACN (2 mL) and TEA 70 μL (0.5 mmol) was added. To this compound (**3**), 68 mg (0.26 mmol), dissolved in ACN (5 mL) was added and stirred at an ambient temperature for 60 min. The product was isolated by prep-TLC using ethyl acetate:methanol 9:1 as the mobile phase. The compound (**6**) was obtained as a solid (60 mg, 65%).

2.2.4. N-[2-(2,5-dioxoazolinyl)ethyl](3-iodophenyl)carboxamide (8)

Meta-iodobenzoic acid (**7**) is available to us from our previous investigations (Al-Jammaz et al., 2002). Fig. 2; scheme 3 was used to prepare the title compound. The (**7**) 43 mg, (0.16 mmol) was