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Influence of prosthetic radioiodination on the chemical and biological behavior of chemotactic peptides labeled at high specific activity

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Abstract

The influence of radioiodination made through prosthetic group *N*-succinimidyl-3-[^{131}I]iodo-benzoate ([^{131}I]SIB) on the behavior of small peptides was investigated using as model the chemotactic hexapeptide *N*α-for-Nle-Leu-Phe-Nle-Tyr-Lys. No carrier added labeled peptide was isolated by reverse-phase HPLC (RP-HPLC) with coupling efficiencies up to 59–75%. Biodistribution in normal and infected C57 mice showed mainly a hepatobiliary clearance, a very low thyroid uptake and the highest uptake at the infection site was within 1 h of injection. Superoxide production and competitive binding assays studies in human polymorphonuclear leukocytes showed a preserved biological activity and high-affinity specific binding. However, the results indicated that the changes observed in the receptor-binding properties with an IC_{50} almost twice than the unlabeled peptide and the increasing in the hepatobiliary excretion could be the consequence of the increased lipophicity observed due to the presence of the prosthetic group together with a strong influence of the radioisotope per se.

Keywords: Radioiodination; Labeling; Chemotactic peptides; Biological behavior; Receptor-binding

1. Introduction

Small receptor-binding peptides are the agents of choice for diagnostic imaging and radiotherapy of several diseases due to their favorable pharmacokinetics. Although molecular modification techniques allow the synthesis of a variety of bioactive peptides with modification in their structure like the addition of linkers for radiolabeling without compromising their biological properties, the radiolabeling itself could finally affect its properties. Even if site-specific labeling techniques are directed toward residues of the peptide away from the active-site, the binding of chelating groups for radioisotopes like ^{99m}Tc or ^{111}In, or prosthetic groups for $^{123/131}$I or ^{18}F, could affect chemical properties such as charge or lipophilicity, respectively. These changes could result in the modification of biochemical processes such as metabolization or membrane solubility, which would finally lead to changes on the biological behavior of the labeled peptide, even if their specific interaction with the receptor seems unmodified.

Our Institution has been involved in a Technical Cooperation Project with the International Atomic Energy Agency (IAEA) for the production of iodine-123 and its radiopharmaceuticals in the cyclotron (The Cyclotron Corporation model CP-42) at Ezeiza Atomic Center. The purpose of the present study was the evaluation of the influence of radioiodination on the biological behavior of peptides labeled with iodine-123 or iodine-131 and its possible consequences over diagnostic or treatment studies, respectively. In order to achieve this goal our work focused on the optimization of radioiodination at high specific activity; as well as quality control, in vitro, and in vivo studies of peptide-based iodine radiopharmaceuticals.

In our model the selection of the peptide was based on two main criteria besides its availability; namely the

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existence of previous publications about its labeling with different methodologies and radioisotopes and the existence of a database about its biological behavior. This background information will allow to understand the effect of the radioiodination on the peptide and to compare it not only with other known procedures, but also with other radioisotopes. The chemotactic hexapeptide *N*α-for-Nle-Leu-Phe-Nle-Tyr-Lys that binds with high affinity to human neutrophils (Niedel et al., 1979), fulfill these criteria. It is a small peptide that has been previously labeled through different techniques, which involves the use of chelates with ^{99m}Tc (Verbeke et al., 2000; Baidoo et al., 1998; Babich et al., 1993) and ^{111}In (Fischman et al., 1991), and by a similar acylation agent with ^{18}F using [^{18}F]SFB (Vaidyanathan and Zalutsky, 1995). It also contains a free lysine ε-amino group at the carboxyl-terminal portion of the molecule, which could be derivatized by reaction with the acylation agent [^{131}I]SIB, which is an active ester that modifies the lysine residues of proteins or peptides. This methodology is an indirect labeling method developed by Zalutsky and Narula (1987), which provides a very stable labeled molecule highly resistant to in vivo dehalogenation. Since the chemistry of radioiodination for iodine-123 and iodine-131 are the same, the second one was chosen to make these studies as it is produced in the nuclear reactor facility of our institute.

The influence of the derivatization on the biological activity was studied by measurement of superoxide production (SOP) and competitive binding assays to human polymorphonuclear cells (PMNs). Biodistributions studies were made in normal and infected mice.

2. Materials and methods

2.1. Radioiodination of the peptide with iodine-131 labeled *N*-succinimidyl-3-iodo-benzoate ([^{131}I]SIB)

All reagents used were analytical grade and was used as purchased. 3-Iodobenzoic acid and *N*, *N*'-dicyclohexylcarbodiimide (DCC) were obtained from Aldrich. The Na[^{131}I] was obtained from our nuclear reactor facility as a solution in 0.05 M NaOH (11 GBq/mL) and from Nordion Canada (NEZ035H 65 GBq/mL, specific activity: 679 MBq/mg); and the chemotactic peptide *N*-formyl-Nle-Leu-Phe-Nle-Tyr-Lys (fNleLFNleYK) was purchased from Sigma. High-performance liquid chromatography was conducted with an HPLC Waters Millennium model equipped with a model 600 pump and UV diode array detector. A NaI(Tl) scintillation detector, with a 200 μL sample loop, coupled to a single channel analyzer connected via an analog-digital interface was used for monitoring the radioactivity in the column eluate.

2.2. Synthesis of the radiodinated agent [^{131}I]SIB

The precursor for radioiodination *N*-succinimidyl 3-tri-*n*-butylstannylbenzoate (STB) was prepared as we described in a report of the International Atomic Energy Agency (Pozzi et al., 2002). The radioiodination of STB was made according to previously reported methods (Zalutsky and Narula, 1987; Garg et al., 1996) with some changes (Pozzi et al., 2002). Briefly, different amounts of STB and the oxidant tert-butylhydroperoxide (TBPH) were tested from 0.1 to 0.6 and 0.2 to 15 μmol, respectively. The condition finally used was: in a 500 μL conical vial 15 μL ethyl-acetate (anhydrous), 20 μL of 2% acetic acid/chloroform (anhydrous), 10 μL of STB/$CHCl_3$ (anhydrous) 10 mM (0, 1 μmol), and 20 μL of TBPH/$CHCl_3$ (anhydrous) 20 mM (0.4 μmol) were added followed by sodium [^{131}I]-iodide (⩽5 μL). After 15–20 min of reaction at room temperature the [^{131}I]SIB was isolated by normal phase HPLC using a Hypersilica Silica Hewlett–Packard 10 μm, 150 × 4.6 mm using ethyl-acetate/hexane/acetic acid (30/70/0.12) at a flow rate of 1 mL/min as eluent. The fraction containing the [^{131}I]SIB (t_R = 13–15 min) was evaporated under a stream of nitrogen, when the volume was ⩽50 μL the activity was transferred to a conical vial (Wheaton, 500 μL) and evaporated to dryness with a stream of nitrogen.

2.3. Synthesis of fNleLFNleYK-IBA

This compound was synthesized as a reference standard in the HPLC analysis and to be used in the in vitro competitive binding assays and superoxide production assay.

The *N*-succinimidyl-3-iodo-benzoate (non-radioactive SIB) was prepared as described by Garg et al. (1995) by reaction of 3-iodobenzoic acid (IBA) (1 g, 4 mmol) in 50 mL of anhydrous tetrahydrofuran (THF) with DCC (1.03 g, 5 mmol) followed by *N*-hydroxysuccinimide (NHS) (0.575 g, 5 mmol). The cold SIB was obtained as a white crystalline compound (mp. 153–154 °C). This compound was used as reference standard in HPLC studies and for the synthesis of the fNleLFNleYK-IBA.

The hexapeptide *N*-formyl-Nle-Leu-Phe-Nle-Tyr-Lys was derivatized by the reaction of a solution of the peptide in dimethylformamide (DMF) (60 mM, 20 μL, 1.2 μmol) with SIB in dimethylformamide (DMF) (120 mM, 20 μL, 2.4 μmol) and triethylamine (TEA) 4 μL, see reaction scheme on Fig. 1. The peptide was solubilized by the addition of small crystals of LiCl. The reaction mixture was incubated at room temperature overnight. The desired product was isolated from the reaction mixture by HPLC with a reverse-phase column (Vydac 218TP5415, 150× 4.6 mm, 5 μm; sample capacity: at optimum resolution 1–200 μg, at practical range 0.2–10 mg). The elution was made at a flow rate of 1 mL/min solvent with 100% of A [acetonitrile/water (25/75) containing 0.1% TFA] over 10 min followed by 0–100% linear gradient of solvent B [acetonitrile/water (45/55) containing 0.1% trifluoroacetic acid (TFA)] in solvent A over 20 min, followed by 100% solvent B. The HPLC chromatogram is shown in Fig. 2A, the retention times for IBA, SIB, fNleLFNleYK, and