Nuclear Medicine and Biology 41 (2014) e23–e29

Contents lists available at ScienceDirect

Nuclear Medicine and Biology

journal homepage: www.elsevier.com/locate/nucmedbio

Radioiodinated and astatinated NHC rhodium complexes: Synthesis

Holisoa Rajerison [a,*], François Guérard [a], Marie Mougin-Degraef [a], Mickael Bourgeois [a,b], Isidro Da Silva [c], Michel Chérel [a], Jacques Barbet [a,b], Alain Faivre-Chauvet [a], Jean-François Gestin [a,*]

[a] Centre de Recherche en Cancérologie Nantes/Angers, 44007 Nantes Cedex 1, France
[b] GIP ARRONAX, 44817 Saint-Herblain Cedex, France
[c] CEMHTI-CNRS UPR3079, 45071 Orléans Cedex 2, France

ARTICLE INFO

Article history:
Received 30 September 2013
Received in revised form 4 December 2013
Accepted 4 December 2013

Keywords:
Astatine-211
Iodine-125
Radioimmunotherapy
N-heterocyclic carbene (NHC)
Rhodium

ABSTRACT

Introduction: The clinical development of radioimmunotherapy with astatine-211 is limited by the lack of a stable radiolabeling method for antibody fragments. An astatinated N-heterocyclic carbene (NHC) Rhodium complex was assessed for the improvement of radiolabeling methodologies with astatine.
Methods: Wet harvested astatine-211 in diisopropyl ether was used. Astatine was first reduced with cysteine then was reacted with a chlorinated Rh-NHC precursor to allow the formation of the astatinated analogue. Reaction conditions have been optimized. Astatine and iodine reactivity were also compared. Serum stability of the astatinated complex has been evaluated.
Results: Quantitative formation of astatide was observed when cysteine amounts higher than 46.2 nmol/μl of astatine solution were added. Nucleophilic substitution kinetics showed that high radiolabeling yields were obtained within 15 min at 60°C (88%) or within 5 min at 100°C (95%). Chromatographic characteristics of this new astatinated compound have been correlated with the cold iodinated analog ones. The radioiodinated complex was also synthesized from the same precursor (5 min. at 100°C, up to 85%) using [^{125}I]NaI as a radiotracer. *In vitro* stability of the astatinated complex was controlled after 15 h incubation in human serum at 4°C and 37°C. No degradation was observed, indicating the good chemical and enzymatic stability.
Conclusion: The astatinated complex was obtained in good yield and exhibited good chemical and enzymatic stability. These preliminary results demonstrate the interest of this new radiolabeling methodology, and further functionalizations should open new possibilities in astatine chemistry.
Advances in knowledge and implications for patient care: Although there are many steps and pitfalls before clinical use for a new prosthetic group from the family of NHC complexes, this work may open a new path for astatine-211 targeting.

1. Introduction

Astatine is the heaviest member of the halogen group with the biggest atomic radius (0.45Å). More than twenty astatine isotopes are known, all of them are radioactive. Astatine-211 is regarded as particularly promising for targeted alpha-therapy of micrometastatic diseases [1, 2]. Indeed astatine-211 decays to bismuth-207 by emitting high energy alpha particles (E_{mean}: 6.4MeV, $t_{1/2}$: 7.2h.), either directly or through its short half-life (0.5 sec) daughter polonium-211. The short path length of the emitted alpha particle with a mean range in human tissues of 65 μm may limit radiotoxicity to neighboring normal tissues if the radioactivity is specifically directed to tumor cells. A monoclonal antibody fragment can ideally perform this targeting as it combines the recognition of specific or overexpressed receptors on abnormal cells to a biological half-life that matches astatine-211 physical half-life.

Astatine-211 is usually produced in a cyclotron by α-irradiation of bismuth targets via the ^{209}Bi (α, 2n) ^{211}At nuclear reaction. Purification and recovery are performed using dry distillation [3] or wet extraction in di-isopropyl ether (DIPE) [4]. Rich chemical reactivity is expected for this element since six different oxidation states (−I, 0, +I, +III, +V, +VII) have been described [5]. However, the absence of stable or long half-life astatine isotope and the small number of α-particle accelerators available for astatine-211 production considerably hamper the development of astatine chemistry.

Direct radiolabeling of proteins with astatine was attempted on the basis of the theoretical similarity of astatine and iodine in terms of chemical reactivity. Unfortunately, rapid de-astatination occurred *in vitro* and *in vivo*. This instability was attributed to the formation of unstable S-At bonds with free thiols of the proteins, instead of the aromatic electrophilic substitution of tyrosine residue(s) as obtained with iodine [6, 7]. Indirect astatination by radiolabeling a prosthetic group and coupling to the protein in a second step has then been

Abbreviations: NHC, N-heterocyclic carbene; DIPE, Diisopropyl ether; SAB, Succinimidyl astatobenzoate; FAb, Fragment of antibody; THF, Tetrahydrofurane; COD, cyclooctadiene; FD/FI, Field desorption/field ionization; RT, Room temperature; RCY, Radiochemical yield; RCP, Radiochemical purity.

* Corresponding author at: CRCNA, IRS UN, 8 quai Moncousu, BP 70721–44007 - Nantes Cedex 1. Tel.: +33 2 28 08 02 21, +33 2 28 08 02 20; fax: +33 2 28 08 02 04.
E-mail addresses: holisoa.rajerison@inserm.fr (H. Rajerison), jfgestin@nantes.inserm.fr (J.-F. Gestin).

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http://dx.doi.org/10.1016/j.nucmedbio.2013.12.004

considered. Indirect radiolabeling mainly uses the electrophilic substitution of leaving groups attached to vinylic [8] or aromatic backbones [9–11] with At(+I). Accordingly, succinimidyl astatobenzoate (SAB), which is currently the most frequently used for proteins labeling with astatine-211, is synthesized from the halodemetalation of a stannylated aromatic precursor [12]. Radiolabeling with this prosthetic group shows some lack of stability *in vivo*, especially for small and rapidly metabolized antibody fragments. Radioactivity accumulation in lungs, spleen, stomach and thyroid was observed in biodistribution studies highlighting the *in vivo* release of astatine [13]. This instability can be correlated to the relative weakness of the aryl–astatine bond compared to the aryl–iodine one (~49 kcal/mol vs. 62 kcal/mol) [14]. *A contrario*, the B-At bond is stronger, which led Wilbur and coworkers to develop boron cages as pendant groups for proteins' radiolabeling [15]. The boronic prosthetic group can be attached to the protein and then be radiolabeled under mild conditions. No de-astatination occurs *in vivo*, but some protein pharmacokinetic modifications have been observed.

The chemistry of astatine oxidation states other than At(+I) has been clearly less explored. To the best of our knowledge, only one study reports prosthetic groups with At(+III) in their structures [16], and no example of prosthetic groups synthesized directly from higher oxidation state (> + I) species has been yet described. In addition, previously mentioned species have not been clearly identified [17]. Nevertheless, a few investigations have focused on astatide chemistry. Astatination of aromatic compounds by halide substitution [18] or from the corresponding aromatic diazonium salts [19] to form SAB type compounds has been described. Afterward, on the basis that astatide should interact as a soft ligand according to the Pearson's theory and form strong complexes with soft metal cations (such as cationic rhodium, iridium, platinum, palladium, silver, gold…), Pruszynski and coworkers conducted studies about the stability of astatide–mercury complexes [20]. They demonstrated that the astatide complex is much more stable than the iodinated analogue. More recently new rhodium (+III) and iridium (+III)-astatide complexes stabilized by the thioether ligand $16S_4$-diol have been developed, but no *in vitro* or *in vivo* stability data have yet been published [21].

Over the last decade, the N-heterocyclic carbene (NHC) ligand family has attracted considerable attention as valuable ligands in coordination chemistry and homogeneous catalysis, notably after the discovery of persistent bulky free NHC stable in the solid state [22–24]. These strongly nucleophilic ligands were found to be interesting substitutes for phosphine ligands with stronger σ-donor and weaker π-acceptor ability [25–27]. They can bind firmly to several transition metal ions under various oxidation states, and lead to complexes with low sensitivity to air, heat and moisture, highly stable especially when bulky ligands are used. In addition to electronic and steric beneficial properties, robust synthetic procedures give access to NHCs complexes bearing various tags [28, 29] including peptide and protein coupling moieties [30]. More recently, the interest of NHC complexes as pharmaceutical compounds highlighted their intrinsic promising properties as antimicrobial [31] and antitumor agents [32].

The unique stabilization properties of NHC ligands and the possibility of tailored structural changes led us to consider the introduction of astatine in an NHC complex structure. Herein, as a proof of this original concept, we choose to attach astatide to rhodium(I) by ligand exchange; this metallic nucleus is stabilized by an NHC ligand. Ultimately, this complex could be subsequently conjugated to biomolecules with a proper selection of ligand functionalization. This choice has been made according to three distinct ideas: first, we have hypothesized that rhodium(I) soft cation and astatide soft ligand should form strong bonds. In a second time, we have considered that the chloride anion of a rhodium-halide structure should be easily displaced by astatide having regards to their respective nucleophilic characters. Finally, we have assumed that electronic and steric stabilizing properties of bulky NHC should increase the stability of the radiolabeled complexes *in vitro* and *in vivo*. In this paper, we thus describe the synthesis and radiolabeling of a new prosthetic group, the (1-benzyl-3-nitrophenylimidazolidene)rhodium(I) chloride complex with iodide and astatide. The *in vitro* stability of astatinated complexes under physiological conditions has also been evaluated.

2. Materials and methods

2.1. General

2.1.1. Reagents

Chemicals purchased from commercial sources were reagent grade or better and were used without further purification. All solvents were obtained as HPLC grade or better. Solvents for HPLC analysis were degassed before use.

2.1.2. Spectral analysis

^{1}H and $^{13}C\{H\}$ NMR spectra were obtained on a Bruker AC (400MHz). Proton chemical shifts are expressed as parts per million (ppm) using solvent peak as internal reference. High resolution mass spectra measurements were recorded on Waters-Micromass GCT Premier spectrometers.

2.1.3. Radioactive materials

All radioactive materials were handled according to the approved protocols at the Centre de Recherche en Cancérologie de Nantes-Angers. Astatine-211 was produced at the CEMHTI (Orléans, France) using the $^{209}Bi(\alpha, 2n)^{211}At$ reaction by bombarding a 240 μm thick natural bismuth layer on copper target with a 1.95–2.15 μA beam of 28MeV α-particle during 2 h. Wet extraction of astatine in DIPE was previously described [33]. The harvested activity was determined in an ACAD 2000 ionization chamber (Lemer Pax, Carquefou, France). Iodine-125 was purchased (Perkin Elmer, Courtaboeuf, France) as [^{125}I]NaI in 0.048 M NaOH. For radioactive experimentation, each reaction condition was duplicated and performed using at least two different astatine or iodine batches.

2.1.4. Chromatography

Non radioactive compounds: Silica gel chromatography was conducted with 2–25 μm 60 A silica gel (Carlo Erba SDS, Val de Reuil, France). TLC was carried out on precoated silica gel $60F_{254}$ TLC plastic sheets (Merck, Darmstadt, Germany), and reaction products were detected by UV light (254 nm). HPLC analysis was performed using a Waters HPLC System equipped with a Waters 486 Tuneable Absorbance. A Waters Prep NovaPack HR Silica column was used with a gradient elution of heptane (A) and ethyl acetate (B) (0–3 min: A, 3.01–9.5 min: 70/30 (A/B), 9.51–15 min: 60/40 (A/B), 15.01–20 min: A) at 2 ml/min. All data were analyzed using Waters Empower data acquisition and analysis software.

Radioactive compounds: Silica gel chromatography was conducted with SepPack silica gel cartridges (Oasis, France). Radio-TLC was carried out on precoated silica gel $60F_{254}$ TLC plastic sheets (Merck, Darmstadt, Germany), was eluted with heptane/acetone 3:2 and was examined using a Typhoon 9410 Variable Mode Imager (GE Healthcare Bioscience). HPLC analysis was performed using a Waters HPLC System equipped with a Waters 486 Tuneable Absorbance Detector and a Packard Bioscience Flow Scintillation Analyser 150 TR (Meriden, CT, US). A Waters Prep NovaPack HR Silica column was used with a gradient elution of heptane (A) and ethyl acetate (B) (0–3 min: A, 3.01–9.5 min: 70/30 (A/B), 9.51–15 min: 60/40 (A/B), 15.01–20 min.: A) at 2 ml/min. All data were analyzed using Waters Empower data acquisition and analysis software.