Applied Radiation and Isotopes 67 (2009) 88–94

Contents lists available at ScienceDirect

Applied Radiation and Isotopes

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

Studies into radiolytic decomposition of fluorine-18 labeled radiopharmaceuticals for positron emission tomography

Peter J.H. Scott [a], Brian G. Hockley [a], Hank F. Kung [b], Rajesh Manchanda [c], Wei Zhang [c], Michael R. Kilbourn [a,*]

[a] Department of Radiology, University of Michigan Medical School, Ann Arbor, MI, USA
[b] Department of Radiology, University of Pennsylvania, Philadelphia, PA, USA
[c] Avid Radiopharmaceuticals, Inc., Philadelphia, PA, USA

ARTICLE INFO

Article history:
Received 4 December 2007
Received in revised form
21 August 2008
Accepted 26 August 2008

Keywords:
Tomography
Emission computed
Radiolysis
Stability
Anti-oxidants

ABSTRACT

Radiolytic decomposition of high specific concentration radiopharmaceuticals is an undesired side-effect that can hamper development of novel PET tracers. This was particularly evident in a series of carbon-11 and fluorine-18 labeled mono- and dimethyl-substituted aryl amines, where rapid decomposition was observed in isolation and formulation steps. We tested a number of additives that inhibit radiolysis and can be safely added to the synthesis procedures (purification and isolation) and reformulation steps to provide suitable clinical formulations. Ethanol and sodium ascorbate are established anti-oxidant stabilizers that completely inhibit radiolytic decomposition and are amenable to human use. Herein, we also demonstrate for the first time that nitrones are non-toxic radical scavengers that are capable of inhibiting radiolysis.

1. Introduction

Positron emission tomography (PET) imaging is a rapidly growing field of research in which molecules labeled with short-lived radionuclides such as carbon-11 or fluorine-18 are utilized to non-invasively examine biochemistry in living human subjects. As part of a broad program to deliver not only established PET radiopharmaceuticals for clinical care (2-deoxy-2-[^{18}F]fluoro-D-glucose, [^{18}F]FDG) and for clinical research (carbon-11 and fluorine-18 labeled radiotracers for brain, heart, pancreas and tumor imaging) we have a research program dedicated to the development and clinical implementation of new PET radiopharmaceuticals. In particular, we and others have significant interest in the development of new fluorine-18 labeled PET imaging agents for neurodegenerative diseases such as Alzheimer's disease (AD) and Parkinson's disease (PD). The application of fluorine-18, with a convenient 110 min half-life, will facilitate the more widespread distribution and use of such radiopharmaceuticals in clinical populations. During the development of radiochemical syntheses of such new fluorine-18 labeled radiopharmaceuticals, including the validation of methods to prepare suitably high amounts for potential distribution, we observed a distressing occurrence of decomposition of the final formulated products. As we suspected this might be due to radiolysis, and that it was particularly acute for the types of structures (*N*-methylanilines) common in our recently developed radiotracers, we undertook and report here a limited study of the factors involved in this decomposition process, examining what chemical structures seem particularly susceptible and what steps can be taken to effectively prevent decomposition. Radiolysis in radiopharmaceutical preparations is certainly not an unknown phenomenon, having been implicated originally in the decomposition of carbon-14 and hydrogen-3 labeled species (Bayly and Evans, 1966) and more recently of carbon-11 labeled species (Suzuki et al., 1990; Bogni et al., 2003; Fukumura et al., 2003, 2004a, b). These studies suggest that the degree of radiolysis of labeled compounds depends on the level of radioactivity, the level of specific activity, the structure of the radiopharmaceutical and the position of the radiolabel. These hypotheses are all supported by the results of the current study.

In contrast to the above studies, radiolytic decomposition has not been extensively studied for fluorine-18 labeled compounds, although many of the mechanisms are expected to be similar to those discussed by Fukumura et al., (2004a, b). One example, a recent study of the stability of [^{18}F]fluorodeoxyglucose (FDG), (Fawdry, 2007) reported a slow but steady decomposition of the radiopharmaceutical to release free [^{18}F]fluoride ion. Similarly, MacGregor reported on the decomposition of [^{18}F]-*N*-methylspiroperidol,

* Corresponding author.
E-mail address: mkilbour@umich.edu (M.R. Kilbourn).

0969-8043/$ - see front matter © 2008 Elsevier Ltd. All rights reserved.
doi:10.1016/j.apradiso.2008.08.015

in which it was shown that rate of radiolysis was proportional to specific activity (MacGregor et al., 1987). With the more widespread availability of medical cyclotrons capable of producing high levels of [^{18}F]fluoride ion coupled with the development and implementation of automated synthesis modules, high level preparations of fluorine-18 compounds are becoming increasingly common. Therefore, the problem of radiolytic decomposition of fluorine-18 labeled radiopharmaceuticals needs to be recognized and addressed as a part of the overall radiopharmaceutical development program.

2. Materials and methods

2.1. General considerations

Fluorine-18 ([^{18}F]fluoride in H_2[^{18}O]O) and carbon-11 ([^{11}C]CO_2 in nitrogen gas) radionuclides were generated using a General Electric Medical Systems (GEMS) PETtrace cyclotron. Fluorine-18 labeled radiopharmaceuticals were prepared in a GEMS TRACERlab FX F-N synthesis module and carbon-11 tracers were synthesized using a GEMS TRACERlab FX C-Pro synthesis module or Bioscan Loop synthesis system. *N,N*-Dimethylaniline, *N*-methylaniline, 4-aminophenol and *N*-tert-butyl-α-phenylnitrone (PBN) were purchased from Sigma-Aldrich and used as received. Sodium ascorbate (SA) (Cenolate® Ascorbic Acid (as SA) Injection, USP, 500 mg/mL) was purchased from Hospira Inc. and used as received. Ethanol (sterile dehydrated alcohol injection, USP) was supplied by American Regent Inc. HPLC analysis of radiochemical purity was conducted using a Shimadzu LC-2010A_{HT} Liquid Chromatograph fitted with UV and Bioscan γ-detectors. HPLC peaks corresponding to products were identified by co-injection with unlabeled reference standards. Gas chromatography (GC) analysis used to determine residual levels of organic solvents was performed on a Shimadzu GC-2010 Gas Chromatograph.

2.2. Typical fluorine-18 radiolabeling procedure

[^{18}F]Fluoride in [^{18}O]H_2O (37–74 GBq) was delivered to the TRACERlab FX F-N synthesis module and collected on a QMA-light Sep-Pak. The [^{18}F]fluoride was then eluted from the QMA cartridge using aqueous potassium carbonate (3.5 mg in 0.5 mL) and transferred into the reactor vessel. Kryptofix-2.2.2 in acetonitrile (15 mg in 1 mL) was added and the water–acetonitrile azeotrope was evaporated (60 °C for 7 min under vacuum with argon stream followed by 120 °C for 5 min under vacuum). Precursors (1–1.5 mg) in anhydrous dimethylsulfoxide (DMSO, 1 mL) were added and the reaction was heated to 120 °C for 10 min. Following labeling, the reactor was cooled to 50 °C and the reaction mixture was diluted with HPLC solvent (3 mL). The crude mixture was passed through an alumina-light Sep-Pak and purified by semi-preparative HPLC. Those compounds purified using HPLC solvent systems suitable for injection (e.g., ethanol and water) were diluted with saline and transferred into a sterile 10 mL dose vial through a Millex 0.22 μm sterile filter.

For those compounds purified using solvent systems unsuitable for injection (e.g., acetonitrile–water), HPLC fractions containing desired products were collected, diluted with sterile water (50 mL), and the resulting solution was passed through a C18-light Sep-Pak. Radiopharmaceuticals remained bound to the Sep-Pak whilst residual HPLC solvent was washed away with further sterile water (10 mL). Products were then eluted with USP ethanol (0.5 mL) and diluted with 0.9% sterile saline (9.5 mL). The resulting isotonic solution was passed into a sterile 10 mL dose vial through a Millex 0.22 μm sterile filter.

2.3. Typical carbon-11 radiolabeling procedure

[^{11}C]CO_2 (100 GBq) in a nitrogen stream was delivered to the TRACERlab FX C-Pro and trapped on molecular sieves. Heating (350 °C) under an atmosphere of hydrogen over a nickel catalyst reduced the [^{11}C]CO_2 to [^{11}C]CH_4 and subsequent reaction with iodine vapor at 720 °C provided [^{11}C]CH_3I. [^{11}C]CH_3I was then converted to [^{11}C]CH_3OTf by passing over silver triflate. Labeling reactions were carried out by bubbling methyl triflate through a solution of precursor either in the TRACERlab FX C-Pro reactor or in a Bioscan Autoloop methylation system. In both cases, purification by semi-preparative HPLC or Sep-Pak provided ^{11}C-labeled radiopharmaceuticals as isotonic solutions suitable for injection that were released for QC analysis.

2.4. Modified radiopharmaceutical preparation procedures incorporating anti-oxidant stabilizers

Radiopharmaceuticals subject to radiolytic decomposition were prepared following modified versions of the general procedures described above to allow for incorporation of anti-oxidant stabilizers into the manufacturing processes. HPLC solvent systems were prepared containing 0.5% w/v (5 g/L) sodium ascorbate (SA) (or ascorbic acid depending upon the pH of the buffer in question). HPLC fractions were simultaneously collected and diluted into aqueous SA (0.5% w/v, 0.25 g in 50 mL sterile water). The resulting solution was passed through a C18-light Sep-Pak that trapped the product and residual solvents were washed away with additional aqueous SA (50 mg in 10 mL sterile water). Products were eluted with ethanol (USP, 0.5 mL) and diluted with SA (USP, 500 mg/mL, 0.1 mL) in 0.9% saline (USP, 9.4 mL). This provided formulations (5% v/v ethanol in saline containing 0.5% w/v SA) suitable for injection that were transferred into sterile 10 mL dose vials through Millex 0.22 μm sterile filters and released for QC analysis.

2.5. Quality control

All radiopharmaceuticals prepared for human use undergo extensive quality control according to the USP guidelines before they are released for use. Formulations are analyzed for chemical and radiochemical purity (HPLC); specific activity (HPLC); pyrogenicity (Charles Rivers); residual Kryptofix-2.2.2 (TLC); residual organic solvents (GC); formulation pH; radionuclidic half-life; and sterile filter integrity (bubble-point test). Every batch must pass all of these tests before release to the clinic is approved.

2.6. Determination of rates of radiolytic decomposition

The radiolytic decomposition of fluorine-18 and carbon-11 labeled products was monitored as a function of radiochemical purity through repeated injections of formulated products on the analytical HPLC. Decomposition of unlabeled standard compounds placed either next to or into a vial containing high concentrations of [^{18}F]fluoride ion was monitored by HPLC coupled with UV detection of the mass peak.

3. Results and discussion

3.1. Potential PET ligands for imaging AD pathophysiology

We have been involved in two research projects investigating new tracers to image AD pathophysiology. The disease is