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Short communication

Solid-phase analysis method for (*S*)-[^{18}F]fluorocarazolol and its metabolites

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Abstract

(*S*)-[^{18}F]Fluorocarazolol is a radiopharmaceutical developed to quantitatively assess β-adrenergic receptors in vivo via positron emission tomography imaging. Since radioactive metabolites of (*S*)-[^{18}F]fluorocarazolol rapidly appear in the plasma, methods for conveniently and reliably evaluating plasma for (*S*)-[^{18}F]fluorocarazolol content are required. Here we present methods and validation of an approach using commercial extraction cartridges that is faster and more convenient than an approach using internal-surface reverse-phase chromatography but yields comparable results. © 2001 Elsevier Science B.V. All rights reserved.

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1. Introduction

(*S*)-[^{18}F]Fluorocarazolol, Fig. 1, is a high affinity β-adrenergic receptor (β-AR) antagonist with properties suitable for in vivo positron emission tomography (PET) imaging of these receptors in heart [1,2], lung [2,3], and possibly brain [4–6]. In particular, specific uptake of (*S*)-[^{18}F]fluorocarazolol uptake is substantial, saturable, stereospecific, and is competitively blocked by known β-AR ligands [1,4].

Kinetic models have been developed to analyze and interpret the PET data with the goal to estimate receptor concentration or binding potential [7–9]. This requires measurement of (*S*)-[^{18}F]fluorocarazolol content of plasma and target tissue which necessitates a means to assess the

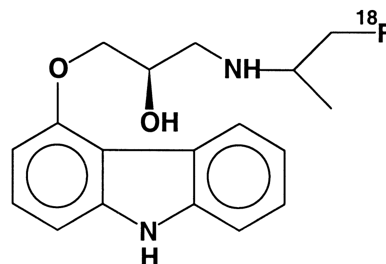

Fig. 1. (*S*)-[^{18}F]Fluorocarazolol: stereochemistry at the functionally important propanolamine center is shown. The optical center in the fluoroisopropyl group is functionally neutral and was racemic throughout this work.

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relative concentration of (*S*)-[^{18}F]fluorocarazolol separately from its radioactive metabolites.

High-performance liquid chromatography (HPLC) is the most commonly applied method for metabolite analysis in PET studies in general and is often considered as the gold-standard. Indeed, van Waarde et al. [10] have described two chromatographic approaches applicable to (*S*)-[^{18}F]fluorocarazolol. Specifically, they demonstrated good correlation between solid-phase extraction–HPLC and internal-surface reverse-phase (ISRP) HPLC methods and concluded that, of the two methods, the ISRP method is preferred, as it is faster and more convenient. We present here an extraction cartridge analysis as an alternative to ISRP HPLC that yields comparable results yet which is quicker (2 min vs. >30 min per sample), less complex, less susceptible to technical failures, and more sensitive to low levels of radioactivity.

2. Experimental

2.1. General

All chemicals used were analytical grade and acquired from Aldrich (Milwaukee, WI, USA) unless mentioned otherwise. Reverse-phase C_{18} (WA T051910) and alumina (WA T051810) extraction cartridges were obtained from Waters Millipore (Milford, MA, USA). ISRP HPLC was performed using Chromspher 5 BioMatrix columns (150×4.6 mm I.D.) and M3 guard columns purchased from Varian Chrompack (Walnut Creek, CA, USA). All radioactivity was assayed using a 1282 Compugamma gamma counter (LKB Wallac, Gaitherburg, MD, USA).

2.2. Radioligand

S-Desisopropylcarazolol, the precursor to (*S*)-[^{18}F]fluorocarazolol was prepared as reported previously [1,11]. (*S*)-[^{18}F]Fluorocarazolol was synthesized [1] by reacting the precursor with [^{18}F]fluoroacetone and purified by HPLC using an Econosil silica column (10 μm, 250×4.6 mm), eluted with chloroform containing 4.5% of a mixture of 2% triethylamine in methanol to obtain >99% enantiomerically pure product [1]. The specific activity of the radiotracer was 1500–3000 Ci/mmol. The purified radiotracer was prepared for injection by coevaporation of the solvent with ethanol and dissolution in 5% ethanol in saline.

2.3. Animal studies

The Institutional Animal Care and Use Committee of Case Western Reserve University approved the protocols used for animal experiments.

2.3.1. Mice

CF-1 mice, male, 20–30 g were obtained from Charles River Labs. (Wilmington, MA, USA). Approximately 30 min before the tracer injection, the mice were anesthetized using 20–30 μl of sodium pentobarbital i.p. (1 mg/1 g of body mass). (*S*)-[^{18}F]Fluorocarazolol dissolved in 5% ethanol–saline solution (100 μl, approximately 0.5–0.8 mCi) was injected i.v. At predetermined times after the tracer injection, approximately 2 and 15 min, the mice were sacrificed by cervical dislocation and blood, 0.5–1.0 ml, was collected. The untreated blood samples were centrifuged at 14 000 *g* for 6 min using a Fisher Scientific microcentrifuge Model 235B (Pittsburgh, PA, USA) and plasma was collected in syringes. A 120-μl aliquot was reserved for HPLC analysis using the ISRP column. The remaining volume was analyzed with the extraction cartridge method described below.

2.3.2. Pigs

Male or female farm pigs, 25–37 kg, were obtained from Hog Palace (Amherst, OH, USA). Approximately 1.5 h before the tracer injection, the pig was anesthetized initially using a mixture of 2.5 mg/kg acepromazine and 7.5 mg/kg ketamine, i.m., and an i.v. was started. Subsequently thiopental sodium, 5 to 10 ml of 25 mg/ml, was administered i.v. for intubation after which the pig was ventilated with oxygen and anesthetized using 1.25% isoflurane, adjusted to effect. A catheter was then placed in the femoral artery and was used for blood sampling. Blood samples were processed in the same manner as applied in the mouse experiments. As a control, a blood sample was collected prior to tracer injection, (*S*)-[^{18}F]fluorocarazolol was added and the

sample was then processed along with the other samples.

2.4. Internal-surface reverse-phase chromatography

ISRP chromatography was performed in a system consisting of a Hewlett-Packard 1050 with quaternary pump, Rheodyne injector, 1 ml sample loop, Chromspher 5 Biomatrix (150×4.6 mm I.D.) with M3 guard column. Samples were processed as described by van Waarde et al. [10]. In brief, the mobile phase was 10 m*M* potassium phosphate (pH 7.5)–acetonitrile (90:10, v/v), flow-rate 1 ml/min. Untreated plasma samples were directly injected. Fractions were collected from 0 to at least 50 min post injection. Radioactivity in each fraction was determined using the gamma counter. Control samples, described above, were used to verify column performance and sample recovery.

2.5. Extraction cartridge analysis

Reverse-phase cartridges were conditioned by rinsing with 6 ml ethanol, 6 ml air, 6 ml deionized water and then 6 ml air. Untreated plasma samples were drawn into 10 ml syringes and diluted to 10 ml with 15% ethanol in water. The solution was then sequentially passed over the neutral alumina cartridge to remove [^{18}F]fluoride followed by the reverse-phase cartridge, which retains (*S*)-[^{18}F]fluorocarazolol. The cartridges were subsequently washed with 2×10 ml of 15% ethanol in water. The alumina cartridge, reverse-phase cartridge, and three wash volumes were separately assayed with the gamma counter.

2.6. Gamma counter efficiency correction

The 10 ml wash volumes obtained in the extraction cartridge analysis and collected in 100×16 mm test tubes were relatively large in comparison to the size of gamma counter well. Thus, due to solid angle considerations, the counting efficiency was expected to be reduced in comparison to that for the other samples. In order to assess the magnitude of this effect so that it could be taken into account, a solution of radioactivity was prepared and small (<1 ml) and large (10 ml) aliquots of the solution were assayed.

3. Results

3.1. ISRP method

Fig. 2 shows representative ISRP chromatograms. Curves are shown for blood samples collected in a pig at 4.5, 19, and 91 min following radiotracer injection. At a flow-rate of 1 ml/min, (*S*)-[^{18}F]fluorocarazolol typically eluted between 27.5 and 37.5 min whereas metabolites had eluted at ≤25 min.

3.2. Extraction cartridge

The alumina cartridges retained 12±5% (mean±SD) of plasma radioactivity in mice and 16±7% in pigs. Presumably this fraction represents fluoride from defluorination. The reverse-phase cartridges retained a variable fraction of the plasma activity which is attributed to (*S*)-

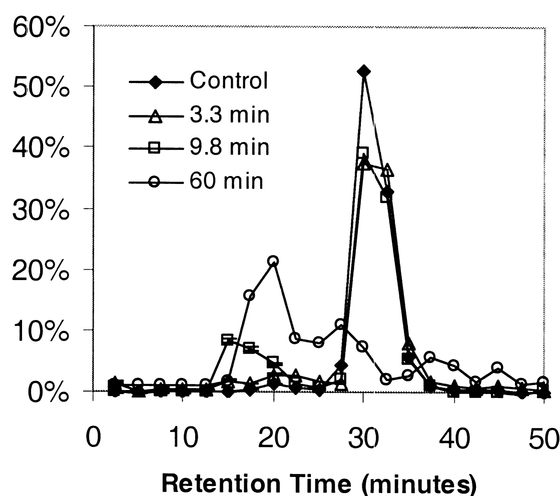

Fig. 2. ISRP HPLC results, pig data. (*S*)-[^{18}F]fluorocarazolol typically eluted between 27.5 and 37.5 min whereas metabolites had eluted at ≤25 min. Curve labels indicate sample collection time as number of minutes post injection of (*S*)-[^{18}F]fluorocarazolol. Control is from a blood sample collected prior to (*S*)-[^{18}F]fluorocarazolol administration to which (*S*)-[^{18}F]fluorocarazolol was added in vitro.

[18F]fluorocarazolol. The remainder of the plasma radioactivity was eluted in the wash and represents organic metabolites more polar than (*S*)-[18F]fluorocarazolol.

3.3. Extraction cartridge vs. ISRP HPLC

Fig. 3 shows percent of plasma radioactivity attributed to (*S*)-[18F]fluorocarazolol determined by the extraction cartridge method compared to that determined using the ISRP HPLC method. In one experiment the ISRP column failed and no data from it were available for comparison. Otherwise, the plots include all the data we have collected using both methods. Visually there appears to be excellent agreement between the analysis methods in data obtained from both mice and pigs. Regression analysis further supports the agreement with slopes (S.E.): 1.043 (0.0471) and 0.963 (0.0312), intercepts (S.E.): −0.0089 (0.0250) and 0.0362 (0.0176), and r^2 values 0.9703 and 0.9774, for mice and pigs, respectively.

The extraction cartridge method was more reliable and robust than ISRP HPLC. As noted above, the ISRP column failed on one occasion. In addition, this column has exceptional maintenance requirements and a relatively short lifetime.

3.4. Gamma counter efficiency correction

Standard dilution experiments showed that counting of a 10 ml sample placed in a 100×16 mm test tube was only 75% as efficient as counting of smaller (<1 ml) volumes. Thus, in the extraction cartridge data shown above, wash counts were divided by 0.75 in order to account for relative efficiency.

4. Discussion

We conducted preliminary experiments to determine the amount of ethanol used to wash the extraction cartridge. Initially we tried pure water. The results were in poor agreement with ISRP HPLC. This finding was consistent with the differences between blood metabolites reported previously by van Waarde et al. [10] and by Zheng et al. [1].

To investigate the cause of the discrepancy, we eluted the reverse-phase cartridge using pure ethanol. ISRP HPLC analysis of the eluate revealed the presence of both (*S*)-[18F]fluorocarazolol and a non-negligible amount of metabolites. Thus the question became one of determining if there was a concentration of ethanol in water that would be sufficient to wash off metabolites without removing too

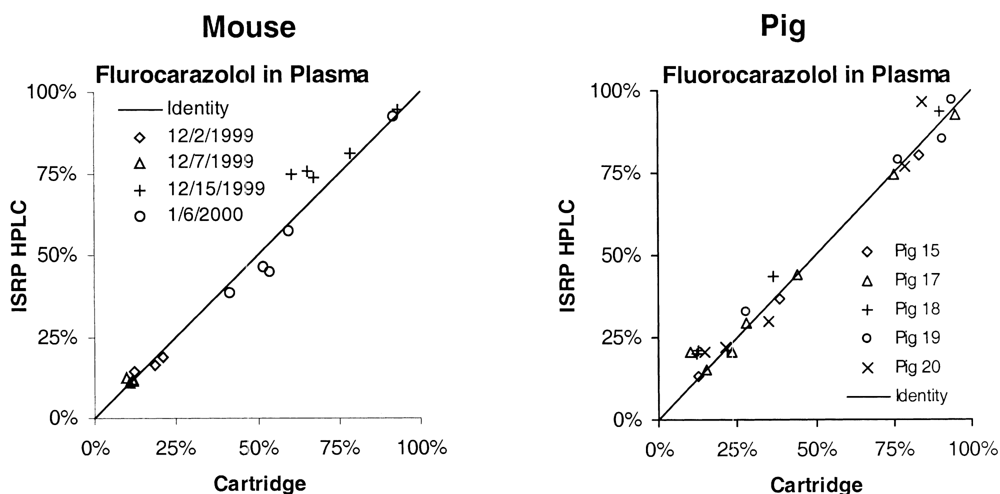

Fig. 3. Percent of plasma radioactivity attributed to (*S*)-[18F]fluorocarazolol as determined using extraction cartridge method and ISRP HPLC. Data shown are for mouse (left) and pig (right) and indicate excellent agreement between the analysis methods as indicated visually and by linear regression analysis. Regression analysis yielded slopes (S.E.): 1.043 (0.0471) and 0.963 (0.0312), intercepts (S.E.): −0.0089 (0.0250) and 0.0362 (0.0176), and r^2 values: 0.9703 and 0.9774, for mice and pigs, respectively.

much (*S*)-[^{18}F]fluorocarazolol. First we experimentally observed that the extraction of [^{18}F]fluoride by the alumina cartridge was more than 99% efficient with eluent ranging from 100% water to 100% ethanol. Next, using a plasma sample with negligible metabolite concentration {obtained by adding (*S*)-[^{18}F]fluorocarazolol to mouse blood in a test tube}, we determined experimentally that 15% ethanol was the maximum concentration that would not elute (*S*)-[^{18}F]fluorocarazolol from the reverse-phase cartridge. On this basis, we chose this ethanol concentration for the comparison to ISRP HPLC.

Our results demonstrate excellent agreement between ISRP HPLC and the extraction cartridge method using 15% ethanol. This indicated, incidentally, that the metabolites are all more polar molecules than (*S*)-[^{18}F]fluorocarazolol itself, as might be expected. The cartridge method is much quicker and easier to perform. Its speed advantage facilitates more frequent sampling thereby leading to a more accurate input function to be used in the mathematical modeling. Moreover, its ease of use and reliability are advantages for routine use. The importance of this cannot be overlooked, as our intent is to develop (*S*)-[^{18}F]fluorocarazolol for use in human studies.

5. Conclusion

We have described and validated an extraction cartridge analysis methodology for determining (*S*)-[^{18}F]fluorocarazolol content of plasma samples. We have validated the extraction cartridge methodology by demonstrating excellent agreement with the ISRP HPLC method. We now use the extraction cartridge method in our (*S*)-[^{18}F]fluorocarazolol work because it is faster and more reliable than ISRP HPLC and yields comparable results.

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