Journal of Pharmaceutical and Biomedical Analysis 98 (2014) 140–143

Contents lists available at ScienceDirect

Journal of Pharmaceutical and Biomedical Analysis

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

Determination of plasma protein binding of positron emission tomography radioligands by high-performance frontal analysis

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ARTICLE INFO

Article history:
Received 21 February 2014
Received in revised form 14 May 2014
Accepted 16 May 2014
Available online 27 May 2014

Keywords:
Plasma protein binding
High-performance frontal analysis (HPFA)
Positron emission tomography (PET)
Radioligand
Minimum injection volume (MIV)

ABSTRACT

Positron emission tomography (PET) is an imaging technique based on the use of radioligands labeled with short lived radionuclides, such as ^{11}C ($t_{½}$ = 20.4 min) and ^{18}F ($t_{½}$ = 109.8 min), which as a consequence often requires rapid plasma protein binding analysis methods. In addition, PET radioligands can suffer from non-specific binding to the membrane when ultrafiltraion, which is the most commonly used method for measuring protein binding in PET, is employed. In this study a high-performance frontal analysis (HPFA) method based on incorporation of a gel filtration column (discovery® BIO GFC 100, 50 mm × 4.6 mm, 5 μm, 100 Å) into a radio-LC system with phosphate buffered saline (PBS, pH 7.4) at a flow rate of 3 ml/min as mobile phase was developed and investigated for four PET radioligands. The minimum injection volume (MIV) of plasma, which is a crucial factor in HPFA, was determined to be 200 μl (human), 500 μl (monkey), 700 μl (human) and 1000 μl (monkey) for these four radioligands. The MIV values increased as a higher fraction of the radioligand was present in the protein-free form. The protein binding results obtained were in good agreement with ultrafiltration and the method did not suffer from non-specific binding. The short analysis time (<12 min) allowed multiple protein binding measurements during time course of a human [^{11}C]PBR28 PET study.

1. Introduction

Positron emission tomography (PET) is a non-invasive imaging method in which a radioligand is used to study a target in a living subject. Intravenously injected radioligands, like drugs, bind to plasma proteins and establish a binding equilibrium, however only the protein-free fraction of the radioligand can reach the target of interest and be involved in pharmacokinetics and pharmacodynamics [1]. Therefore it is essential to determine the extent of radioligand binding to plasma proteins and several analytical methods have been developed for this purpose [1]. Ultrafiltration is most commonly used in PET due to its relatively short analysis time compared to other techniques [2,3]. Nevertheless, the analysis time (30–60 min) is not short enough to perform repetitive measurements during a PET scan (60–120 min) and typically only one plasma sample, taken prior to radioligand administration, can be analyzed. This is particularly challenging when a subject is treated with a drug that might compete with the radioligand for binding to proteins and the protein-free fraction of the radioligand changes over time [4]. Another limitation of the ultrafiltration method is the undesirable adsorption of radioligands, specially the highly lipophilic ones, to the membrane [5].

High-performance frontal analysis (HPFA) is a chromatographic method in which a restricted-access type column, such as Pinkerton [6] and diol-silica [7] is used. When a plasma-radioligand mixed solution is injected directly into this type of column, large proteins elute at once whereas small molecules are retained. If the injection volume is too small, the protein-bound radioligand is released from protein into the mobile phase. To generate equilibrium between the protein-free and -bound fractions in the column, as it is in the initial sample, the injection volume has to be increased. After equilibrium is reached, the protein-bound fraction is eluted first and the protein-free fraction elutes as a trapezoidal peak with a plateau region, where the plateau height and area correspond to the protein-free and total (protein-free and -bound [8,9]) radioligand concentration respectively.

In this study a HPFA method was developed for the protein binding measurement of PET radioligands in human or monkey

Abbreviations: HPFA, high-performance frontal analysis; PET, positron emission tomography; PBS, phosphate buffered saline; MIV, minimum injection volume.

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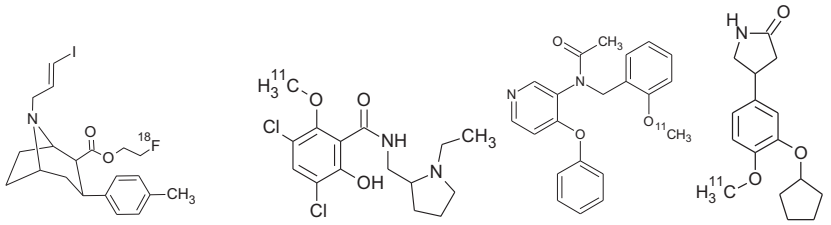
http://dx.doi.org/10.1016/j.jpba.2014.05.024
0731-7085/



[^{18}F]FE-PE2I (cLogD$_{7.4}$=4.3) [^{11}C]raclopride (cLogD$_{7.4}$=2.3) [^{11}C]PBR28 (cLogD$_{7.4}$=2.5) [^{11}C]rolipram (cLogD$_{7.4}$=1.9)

Fig. 1. Chemical structures of the PET radioligands investigated in this study and their respective cLodD$_{7.4}$ values calculated using Pallas 3.7 (CompuDrug Chemistry Ltd.).

plasma and was investigated for four radioligands: [^{18}F]FE-PE2I [10], [^{11}C]raclopride [11], [^{11}C]PBR28 [12] and [^{11}C]rolipram [13] (Fig. 1). These radioligands are used for imaging of the dopamine transporter, D_2/D_3 dopamine receptors, 18 kDa translocator protein (TSPO) and phosphodiesterase-4, respectively.

2. Experimental

2.1. Chemicals and radioligands

Sodium chloride, potassium chloride, potassium phosphate monobasic and sodium phosphate dibasic were obtained from Sigma–Aldrich. Radioligands [^{18}F]FE-PE2I [14], [^{11}C]raclopride [15], [^{11}C]PBR28 [12] and [^{11}C]rolipram [16] were prepared according to published procedures. All the experiments dealing with carbon-11 and fluorine-18 compounds were carried out in accordance with the regulations from the Swedish Radiation Protection Authority.

2.2. Protein binding measurements by HPFA

Arterial blood samples from human and monkey subjects were taken by heparin treated syringes 5–10 min prior to administration of radioligand and centrifuged at 2000 × *g* for 2–4 min at room temperature to separate plasma. Equal volumes of plasma and phosphate buffered saline (PBS, pH 7.4) solutions were mixed with a radioligand (10% v/v) and incubated at room temperature for 10 min before injection into the HPLC setup described below. Human plasma was used in the case of [^{18}F]FE-PE2I and [^{11}C]PBR28, while monkey plasma was used for [^{11}C]raclopride and [^{11}C]rolipram. The human and monkey PET studies were approved by the Regional Ethics Committee in Stockholm.

An Agilent binary pump (Agilent 1200 series) coupled to a manual injection valve (7725i, Rheodyne), 1.0 or 2.0 ml loop and a radiation detector (Oyokoken, S-2493Z) housed in a shield of 50 mm thick lead was used for protein binding measurements on a discovery® BIO GFC 100 column (50 mm × 4.6 mm, 5 μm, 100 Å, Supelco) at a flow rate of 3 ml/min. The mobile phase was PBS, pH 7.4, without addition of any organic modifier in order to preserve the PET radioligand-protein binding equilibrium. Both the mobile phase and column were heated to and kept at 37 °C during the experiments. Two alternative columns with restrictive access material stationary phases, Pinkerton GFF II (150 mm × 4.6 mm, 5 μm, Regis) [6] and LiChroCART® 100 Diol (125 mm × 4 mm, 5 μm, EMD Millipore) [7], were also investigated employing the same set up. Data collection and control of the LC system was performed using chromatographic software (ChemStation Rev. B.04.03; Agilent). The accumulation time of radiation detector was 10 s.

The protein-free fraction of the studied radioligands in plasma, *fp*, were calculated as $fp = C_{plasma}/C_{ref} \times H_{ref}/H_{plasma}$, where C_{plasma} and C_{ref} are the concentrations of radioactivity in plasma and reference solution respectively, H_{plasma} is the plateau peak height of plasma, and H_{ref} is the peak height of reference solution obtained by HPFA. All the radioactive peaks were decay-corrected to the time of injection in the LC.

2.3. Protein binding measurements by ultrafiltration

An ultrafiltration method was used to estimate the free fraction, of radioligands in plasma. Equal volumes (500 μl) of plasma and PBS solutions serving as control were mixed with a radioligand solution (50 μl, approximately 1 MBq in PBS) and incubated at room temperature for 10 min. After the incubation, 200 μl portions of the incubation mixtures were transferred into ultrafiltration tubes (Centrifree YM-30; cutoff 30,000 MW; Millipore) and centrifuged at 1500 × *g* for 15 min. Equal aliquots (20 μl) of the ultrafiltrate (C_{free}) and of the plasma (C_{total}) were counted for their radioactivity with a NaI(Tl) well counter and radio-HPLC as previously described [17]. Each determination was performed in duplicate. The free fraction of the radioligand was calculated as $f_p = C_{free}/C_{total}$ and the results were corrected for the membrane binding as measured with the control samples.

2.4. In vivo plasma protein binding measurement of [^{11}C]PBR28

To measure the plasma protein binding of [^{11}C]PBR28 *in vivo* in human, arterial blood samples were collected in heparin treated syringes at 4, 10, 20 and 30 min after intravenous administration and centrifuged at 2000 × *g* for 2–4 min at room temperature to separate plasma. Radioactivity of [^{11}C]PBR28 in human plasma was measured by a NaI(Tl) well counter and radio-HPLC as previously described. Plasma as well as reference solution, which consisted of a known amount of [^{11}C]PBR28 solution in PBS (1500–2000 Becquerel (Bq)), were injected into the described system.

3. Results and discussion

3.1. Optimization of injection volume in HPFA

The most commonly used stationary phase in HPFA is restricted access materials [18]. Two types of these columns, Pinkerton GFF II and LiChroCART® Diol, with PBS solution at physiological pH (pH 7.4) as mobile phase were investigated. However the long retention times of the short lived radioligands on these columns (>60 min) made them unsuitable for the rapid analysis required. Therefore a silica-based gel filtration column was used here, which resulted in significantly shorter retention times without addition of any organic modifier to the mobile phase.

In protein binding measurements by HPFA, the volume of the plasma-radioligand injected into the column is a crucial factor [9]