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A simplified radiometabolite analysis procedure for PET radioligands using a solid phase extraction with micellar medium

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ABSTRACT

A solid phase extraction method has been developed for simple and high-speed direct determination of PET radioligands in plasma.

Methods: This methodology makes use of a micellar medium and a solid-phase extraction cartridge for displacement of plasma protein bound radioligand and separation of PET radioligands from their radiometabolites without significant preparation. The plasma samples taken from monkey or human during PET measurements were mixed with a micellar eluent containing an anionic surfactant sodium dodecyl sulphate and loaded onto SPE cartridges. The amount of radioactivity corresponding to parent radioligand (retained on the cartridge) and its radioactive metabolites (eluted with micellar eluent) was measured.

Results: Under the optimized conditions, excellent separation of target PET radioligands from their radiometabolites was achieved with a single elution and short run-time of 1 min. This method was successfully applied to study the metabolism for ^{11}C-labelled radioligands in human or monkey plasma. The amount of parent PET radioligands estimated by micellar solid phase extraction strongly corresponded with that determined by radio-LC. The improved throughput permitted the analysis of a large number of plasma samples (up to 13 samples per one PET study) for accurate estimation of metabolite-corrected input function during quantitative PET imaging studies.

Conclusion: Solid phase extraction together with micellar medium is fast, sensitive and easy to use, and therefore it is an attractive alternative method to determine relative composition of PET radioligands in plasma.

1. Introduction

To quantify physiological processes measured with positron emission tomography (PET), the time course of the parent radioligand concentration in arterial plasma is usually required as an input function if a PET pharmacokinetic model is employed [1–3]. In such instances, since the majority of radioligands are metabolized within the body, total radioactivity in arterial plasma needs to be corrected for the presence of radioactive metabolites to determine exact input function.

Several methods, such as reversed phase liquid chromatography and thin-layer chromatography coupled with radioactivity detection (radio-RPLC or radio-TLC), have been used to determine the relative composition of PET radioligands in plasma [4–6]. However, there are some limitations arising from the short half-lives of positron emitting radionuclides employed (*e.g.* ^{11}C: $t_{1/2} = 20.4$ min) which limit the number of samples that can be used to derive the input function as well as result in low radioactivity of the later plasma samples collected during PET studies especially for ^{11}C-labelled compounds. Moreover, certain sample preparation steps such as the protein precipitation to disrupt protein bound radioligands and to remove proteins from plasma samples are essential prior to the radio-LC or radio-TLC analysis. This step makes the analysis procedure long, tedious and often leads to binding of the radiolabelled compounds to the protein precipitate, which may introduce additional sources of error. Failure to correctly determine the unchanged PET radioligands fraction in plasma results in an under or overestimation in the input function which would in turn lead to an under or overestimation in the evaluation of quantitative PET analysis, *e.g.* binding potential. One of the other major drawbacks of radio-LC and radio-TLC is the relatively high costs for hardware. Thus, a simple method is preferable in PET metabolite analysis for accurate pharmacokinetic model of PET radioligands.

Solid phase extraction (SPE) is the most popular sample pretreatment approach nowadays for drug analyses due to the following advantages; high recovery, effective pre-concentration, ease of operation and greater possibility of automation [7–9]. SPE is

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unequivocally the leading sample preparation method used in routine bio-analytical laboratories and also has been applied for metabolite analysis of PET radioligands in plasma [10–15]. Although conventional SPE offers several advantages, it has some limitations. This especially poses difficulty for high plasma protein binding of radioligands owing to their co-elution with the matrix components.

Micellar liquid chromatography (MLC) is one of the reversed-phase liquid chromatographic modes with a mobile phase consisting of an aqueous solution of surfactant above its critical micellar concentration (CMC). [16–18]. In this condition, the stationary phase is modified with an approximately constant amount of surfactant monomers, and the solubilising capability of the mobile phase is altered by the presence of micelles, giving rise to diverse interactions (hydrophobic, ionic and steric) with major implications in retention and selectivity of analytes. One of the advantages in MLC is the possibility of direct sample injection of biological material (*e.g.* plasma, serum, urine) into the column without deproteination. Recently, we introduced MLC methods to determine PET radioligands in plasma [19,20]. These methods permit the effective and repetitive analysis of diverse compounds in untreated plasma samples by disrupting protein-bound radioligand and solubilizing plasma proteins based on the formation of micellar complex between plasma proteins and anionic surfactants. In addition to simplifying the processes the MLC analysis provides a more accurate determination of PET radioligand.

The present paper describes a simple and fast SPE method for the radiometabolite analysis of ^{11}C-labelled radioligands in plasma based on surfactant-mediated solid extraction. The method has been validated and applied to [^{11}C]PBR28 (an 18 kDa translocator protein radioligand) [21], [^{11}C]L-deprenyl-D_2 (a MAO-B radioligand) [22] and [^{11}C]ORM-13070 (an α2C-adrenoceptor radioligand) [23] (Fig. 1) metabolism studies.

2. Materials and methods

2.1. Chemicals and reagents

Oasis HLB cartridge (3 cc flangeless, 60 mg, 30 μm) was purchased from Waters. Sodium dodecyl sulphate (SDS), ammonium phosphate monobasic, ammonium phosphate dibasic, 1-butanol (1-BuOH), methanol, acetonitrile and phosphoric acid were obtained from Sigma-Aldrich. ^{11}C-labelled radiopharmaceuticals were prepared according to the published procedure [21–23].

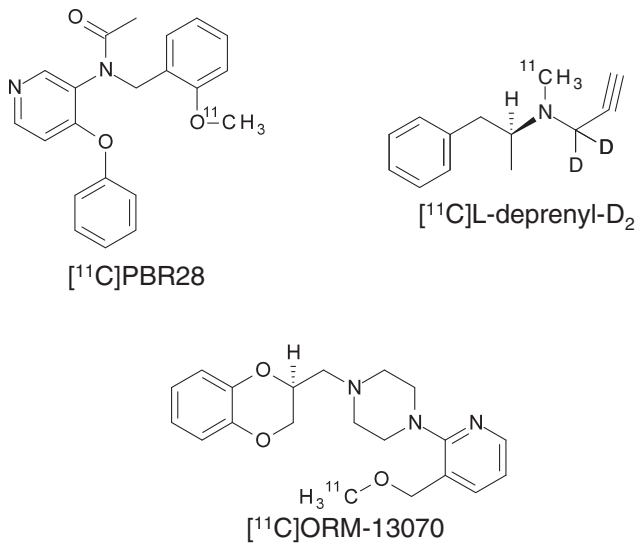

Fig. 1. PET radioligands investigated in this study.

2.2. Solid-phase extraction

SPE cartridges were preconditioned with 1 mL of methanol, 1 mL of water followed by equilibrating with 5 mL of a micellar eluent containing 40–100 mM SDS, 5%–8% (v/v) 1-BuOH and 20 mM ammonium-phosphate buffer at pH7. Micellar eluent (0.2 mL) was left above the sorbent until the use of cartridge. Plasma sample (0.2–2.0 mL) was mixed with 1 mL of micellar eluent and then loaded slowly (*ca.* 2.0 mL/20 s) onto the SPE cartridge using a 5 mL plastic syringe. The cartridge was washed with 2 mL of water and excess solvent was removed by pushing air through the cartridge. The effluents (eluted plasma mixture and washed with water) were collected into a same plastic tube. The collected solution and cartridge were counted for radioactivity on a NaI(Tl) well-counter (counting time; 10 s). The background was subtracted from the sample counts and resulting values were decay corrected to the time of radioligand administration.

The trapping efficiency of PET radioligands was determined by adding 10 μL of radioligands (*ca.* 0.1 MBq) to 0.2–2.0 mL of human or monkey plasma and extracting according to above procedure.

2.3. Radiometabolite analysis of PET radioligands in human and monkey plasma

The human and monkey PET studies were approved by the Regional Ethics Committee and by the Animal Research Ethical Committee in Stockholm. During PET measurements, whole blood samples were taken from human or monkey and collected in heparin-treated syringes at pre-specified time points after intravenous administration of radioligands. The blood samples were centrifuged at 2000 *g* for 2–4 min at room temperature to separate plasma. The plasma specimen was then collected and mixed with a micellar eluent as described in section 2.2.

2.4. Method comparison

To compare the outcomes obtained by micellar SPE described above, radiometabolite analyses were also performed by conventional radio-LC. Protein elimination from plasma was done by protein precipitation using acetonitrile. The plasma samples were mixed with 1.4 times volume of acetonitrile. The resulting denatured protein emulsion was stirred with a vortex mixer and centrifuged at 2000 *g* for 4 min. After washing the protein precipitate with 0.1 mL of acetonitrile the supernatant extract was injected into the LC system. Chromatographic separation was achieved at a flow rate of 6.0 mL/min on a semi-preparative LC column by gradient elution using following chromatographic conditions; [^{11}C]PBR28: column; XBridge OST C_{18}, Waters, 50 mm × 10 mm I.D., 2.5 μm plus guard (XBridge C_{18}, 10 mm × 10 mm I.D., 5 μm), mobile phase; 0.1% formic acid in acetonitrile/0.1% formic acid in water = 20:80 (0–2.5 min), 20:80 → 60:40 (2.5–5 min), 60:40 (5–5.5 min), 20:80 (5.5–6.5 min), [^{11}C]L-deprenyl-D_2: column; XBridge OST C_{18}, 50 mm × 10 mm I.D., 2.5 μm plus guard (XBridge C_{18}, 10 mm × 10 mm I.D., 5 μm), mobile phase; acetonitrile/20 mM ammonium phosphate (pH 7) = 40:60 → 75:25 (0–3.5 min), 75:25 (3.5–4 min), 40:60 (4–5 min), [^{11}C]ORM-13070: column; μBondapak C_{18}, Waters, 300 mm × 7.8 mm I.D., 10 μm, mobile phase; acetonitrile/10 mM phosphoric acid = 15:85 → 60:40 (0–10 min), 80:20 (10–12 min), 15:85 (12–14 min). The operating parameters of the conventional radio-LC method were as previously described [4,24]. The radioactivity of plasma and protein precipitate fraction was measured to quantify the recovery after precipitation with acetonitrile.

The radioactivity of the SPE analysis fractions (effluent and cartridge) of plasma samples from human or monkey following administration of PET radioligands was also analyzed using above described radio-LC system. The extraction of retained radioactivity in the SPE cartridge was performed by 1.5 mL of 95% acetonitrile in 0.1% phosphoric acid.