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Radiolabeling with fluorine-18 of a protein, interleukin-1 receptor antagonist

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ABSTRACT

IL-1RA is a naturally occurring antagonist of the pro-inflammatory cytokine interleukin-1 (IL-1) with high therapeutic promise, but its pharmacokinetic remains poorly documented. In this report, we describe the radiolabeling of recombinant human interleukin-1 receptor antagonist (rhIL-1RA) with fluorine-18 to allow pharmacokinetic studies by positron emission tomography (PET). rhIL-1RA was labeled randomly by reductive alkylation of free amino groups (the ε-amino group of lysine residues or amino-terminal residues) using [^{18}F]fluoroacetaldehyde under mild reaction conditions. Radiosyntheses used a remotely controlled experimental rig within 100 min and the radiochemical yield was in the range 7.1–24.2% (decay corrected, based on seventeen syntheses). We showed that the produced [^{18}F]fluoroethyl-rhIL-1ra retained binding specificity by conducting an assay on rat brain sections, allowing its pharmakokinetic study using PET.

1. Introduction

The pro-inflammatory cytokine IL-1 plays an important role in inflammation, angiogenesis, and the immune response (Dinarello, 1988, 1997) and has been implicated in neurodegenerative diseases (Allan and Rothwell, 2003; Allan et al., 2005; Basu et al., 2004; Emsley et al., 2007, 2008; Lucas et al., 2006). Inhibition of IL-1 function by the naturally occurring, selective and potent antagonist IL-1RA has therapeutic promise in preventing damage in a range of inflammatory and autoimmune disorders including rheumatoid arthritis (Furst, 2004) and psoriasis.

rhIL-1RA also demonstrated benefit in preclinical studies on experimentally induced ischemic, excitotoxic, and traumatic brain insults in rodents (Pinteaux et al., 2006; Touzani et al., 1999) and potential in early clinical trials (Emsley et al., 2005). However, little is known about the biodistribution, pharmacokinetics and metabolism of rhIL-1RA so that brain penetration may be a key issue in its potential development as a therapeutic agent in brain traumatisms.

Pharmacokinetic modeling of rhIL-1RA administered intravenously in patients with subarachnoid haemorrhage (SAH) (Clark et al., 2008) reported that it crossed significantly, but slowly into the CSF. However, the CSF concentrations achieved were similar to or higher than those found to be neuroprotective in rodents (Clark et al., 2008). A potential limitation of this work, as noted by the authors, is that the concentration of proteins in the CSF of patients may not reflect the concentration in the local brain environment.

Limited pharmacokinetics, tissue distribution, and elimination studies of IL-1RA in rats using ^{35}S-IL-1RA has been reported (Kim et al., 1995). Moreover, IL-1RA, radiolabelled with iodine-123, has been evaluated as a scintigraphic imaging marker of infection in a rabbit model but showed relatively poor imaging characteristics in term of both resolution and sensitivity (Van der Laken et al., 1998). In contrast, positron emission tomography (PET) has proved useful to study the pharmacokinetics and biodistribution of labeled peptides and proteins (Grierson et al., 2004; Haubner et al., 2001; Kramer-Marek et al., 2008). This led us to develop a radiolabeling method for rhIL-1RA, based on fluorine-18, for use with PET in order to better study the pharmacokinetics of this molecule with respect to brain uptake and metabolism.

Among the approaches investigated for fluorine-18 labeling of proteins using [^{18}F]fluoride, ^{18}F-acylation using 4-[^{18}F]fluorobenzoyl moiety (Kramer-Marek et al., 2008; Okarvi, 2001) and chemoselective reactions of 4-[^{18}F]fluorobenzaldehyde ([^{18}F]FB-CHO) with peptide functionalized with a hydrazine or a aminooxy group producing respectively oxime and hydrazone have been extensively used (Cheng et al., 2008; Wester and Schottelius, 2007). The drawback of the labeling approaches using

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4-[^{18}F]fluorobenzoyl moiety is the lengthy, time-consuming multi-step preparation, involving a HPLC purification, of the ^{18}F labeling agents. Peptide labeling approaches using the highly lipophilic [^{18}F]FB-CHO require the use of an organic co-solvent such as DMSO; furthermore, preparation of [^{18}F]FB-CHO involves a step of purification using HPLC or solid phase extraction. Another labeling method for IL-1RA was therefore attempted: reductive [^{18}F]fluoroethylation of free amino groups (ε-amino group of lysine residues or amino-terminal residues) using [^{18}F]fluoroacetaldehyde (Prenant et al., 2008).

Reductive alkylation is a well-established method for the chemical modification of amino groups in proteins with minimal perturbation in their structure and function. The method is rapid and employs mild conditions, it has little effect on the physical chemical properties, only a slight change in the *p*Ka of the ε-monoalkylamino groups which are generally slightly more basic than the corresponding primary amino groups (Means and Feeney, 1995). Means (1977) has shown that when using acetaldehyde only the corresponding monoalkylated lysine is produced. Moreover, reductive alkylation of protein amino groups with small carbonyl compounds like formaldehyde or acetaldehyde cause a minimal disturbance of existing electrostatic interactions. Larger and generally more hydrophobic carbonyl compounds should, however, increase both the bulk and hydrophobicity of the amino group and reduce its ability to form hydrogen bonds (Means, 1977).

The choice of the reducing agent is crucial, Jentoft and Dearborn (1979) and Geoghegan et al. (1981) have applied the reductive methylation to different proteins using [^{12}C], [^{13}C] and [^{14}C]formaldehyde and showed that using sodium cyanoborohydride instead of sodium borohydride prevent aldehydes or ketones reduction, inter or intramolecular bridges formation, disulfide bond reductions or labile peptide bonds cleavage. This method has been used for carbon-11 labeling with [^{11}C]formaldehyde of fibrinogen (Straatmann and Welch, 1975), and concanavalin A (Berger et al., 1984).

The mechanism for reductive [^{18}F]fluoroethylation of a free amino group of the IL-1RA involves the formation of an imine intermediate, the reduction of which yields the corresponding [^{18}F]fluoroethyllysine (Fig. 1).

Radiolabeling was carried out in a remotely controlled experimental rig. The hydrophilicity and high reactivity with amino groups of [^{18}F]fluoroacetaldehyde in reductive alkylation conditions makes it a good reagent for protein radiolabeling by targeting free amino groups producing *N*-[^{18}F]fluoroethyl derivatives. The small size of the [^{18}F]fluoroethyl substituent is expected to have little impact on the rhIL-1RA integrity. Nevertheless as one of the nine lysine moieties on IL-1RA has been identified as important for receptor interaction (Evans et al., 1995), binding properties of the [^{18}F]IL-1RA were assayed using rat brain sections and autoradiography.

2. Materials and methods

2.1. Remotely controlled radiosynthesis set-up

The remotely controlled radiosynthesis experimental rig was set up in a lead-shielded fume-cupboard (Fig. 2). The core of the radiosynthesis set-up was comprised of three remotely controlled six-port/two-position electrically activated Valco valves (Thames Restek UK Ltd., UK). [^{18}F]Fluoroacetaldehyde production was carried out in Wheaton borosilicate screw-top V-vials, capacity 3.0 ml, with open-top cap and PTFE-faced silicon septum (Sigma-Aldrich, UK) using a home-made, temperature-regulated block heater, equipped with a magnetic stirrer and cooled with compressed air via a valve manually operated from outside the fume-cupboard. Reagents were added from outside the fume-cupboard via two shut-off, low-pressure, valves from Upchurch (Sigma-Aldrich, UK). The labeling of rhIL-1RA was carried out in a 1.5 ml microtube with attached screw cap (Sigma-Alrich, UK) using a digital block heater (Jencons Scientific, UK). The produced [^{18}F]rhIL-1RA was injected through a low protein-binding Pall Acrodisc® syringe filter (13 mm diameter, 0.2 μm pore size) onto a 5 ml HiTrap™ desalting column (GE Healthcare, UK) for purification using a programmable syringe pump model ALADDIN-220 (World Precision Instruments, UK). Radioactivity in reaction vials was monitored using a Ratemeter Mini 900 (Thermo Electron Corporation, UK). [^{18}F]rhIL-1RA and other radioactive fractions collected after separation on the HiTrap column were measured on a Capintec CRC-15R.

2.2. Chemicals

Solvents were purchased from Sigma-Aldrich and were used without further purification. The rhIL-1RA, Anakinra® (Kineret®), was kindly provided by Amgen (Thousand Oaks, CA, USA). Kineret® is a recombinant, methionylated, nonglycosylated synthetic form of the human interleukin-1 receptor antagonist (IL-1RA). It was provided as a solution formulated for injection consisting of 100 mg of Anakinra, 1.29 mg of sodium citrate, 5.48 mg sodium chloride, 0.12 mg disodium EDTA, and 0.70 mg polysorbate 80 in 1 mL water for injection, USP (pH 6.5 at room temperature). [^{18}F]Fluoride was produced via the ^{18}O(p,n)^{18}F nuclear reaction by 16.4 MeV proton bombardment of an isotopically enriched [^{18}O] water target (95–97% $H_2^{18}O$ water enrichment) using a GE PETtrace cyclotron.

2.3. Radiochemistry

2.3.1. Production of [^{18}F]potassium fluoride

The three electrovalves in the experimental rig (Fig. 2) were set in position A at initial time of the synthesis, aqueous [^{18}F]fluoride solution (2 ml, 1.73–3.42 GBq) was sent from the cyclotron target onto a QMA cartridge, in the carbonate form. Electrovalve 1 was subsequently switched to position B and [^{18}F]KF was eluted from the QMA with 1.1 mg (8 μmol) potassium carbonate and 10 mg (26.5 μmol) of 4,7,13,16,21,24-hexaoxa-1,10-diazabicyclo[8,8,8]-hexacosane (Kryptofix 222) in 0.5 ml water, added from shut-off valve 1, into the reactor. Electrovalve 1 was subsequently switched back to position A and 1 ml of acetonitrile was added from shut-off valve 1. Temperature of the heater was set to 110 °C and the mixture was then dried under nitrogen for 5 mins. This drying step was repeated twice with 0.8 ml of acetonitrile after what the heater was cooled to 30 °C.

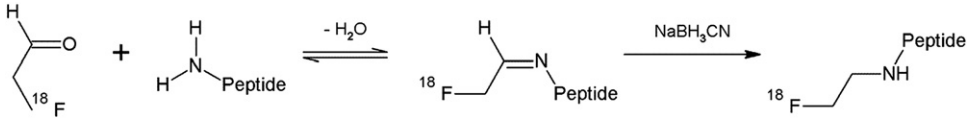

Fig. 1. Reductive [^{18}F]fluoroethylation of the IL-1RA with [^{18}F]fluoroacetaldehyde.

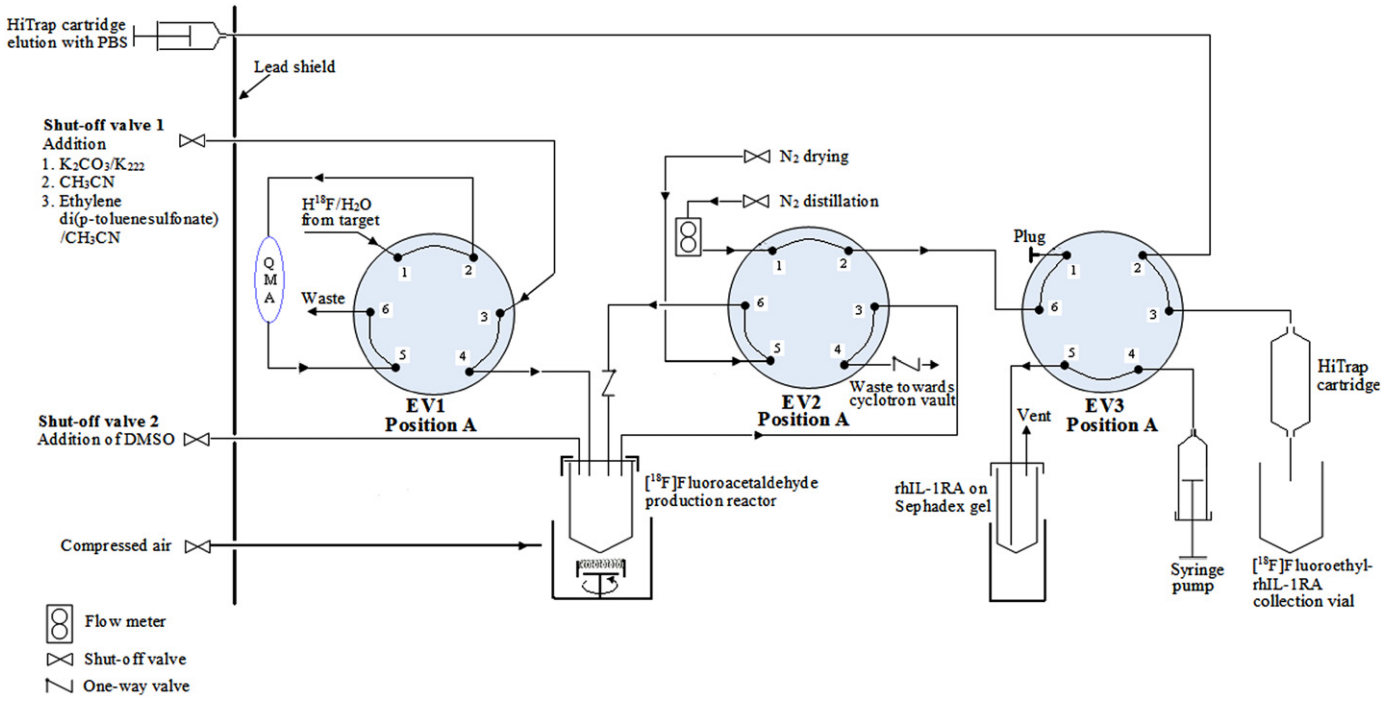

Fig. 2. Remotely controlled radiosynthesis experimental rig for [^{18}F]rhIL-1RA production.

2.3.2. [^{18}F]Fluoroethyltosylate

A solution of ethylene di-*p*-toluenesulphonate, 5 mg (13.5 μmol) in 0.3 ml acetonitrile was added to the dried [^{18}F]potassium fluoride/Kryptofix complex through shut-off valve 1. Electrovalve 2 was subsequently switched to position B, the heater was set to 90 °C, and the mixture was heated for 8 min.

2.3.3. [^{18}F]Fluoroacetaldehyde

The heater was subsequently cooled to 60 °C, electrovalve 2 switched to position A and acetonitrile was evaporated under nitrogen. Evaporation was completed within 3 min after which electrovalves 2 and 3 were switched to position B, anhydrous DMSO (0.2 ml) was added through shut-off valve 2 and the temperature of the heater was set to 150 °C. Around 1 min after the heater had reached this temperature [^{18}F]fluoroacetaldehyde started to distil conveyed by nitrogen (7–8 ml/min).

2.3.4. [^{18}F]rhIL-1ra

Distilled [^{18}F]fluoroacetaldehyde was trapped into a microtube containing the rhIL-1RA in suspension in a Sephadex® gel to avoid foaming of the protein solution. The gel was made by addition of 20 μl (2 mg) of a solution of the rhIL-1RA formulated for injection (100 mg/ml) and 40 μl of a 0.25 M solution of sodium cyanoborohydride in citrate buffer (citric acid, ~0.060 M, sodium hydroxide, ~0.16 M, pH=6), onto dry Sephadex® G-50 (6 mg). After 8 min all the [^{18}F]fluoroacetaldehyde was distilled, as monitored by a radioactivity detector. The microtube was subsequently heated at 38 °C for 45 min after which PBS (1 ml, pH 7.2) was added to the gel via the syringe pump. This addition was followed by the withdrawing of suspension via a programmed step on the syringe pump, after which electrovalve 3 was switched to position A. The reaction mixture was then injected by the syringe pump onto a HiTrap® column via a low-protein-binding filter for purification. After injection into the column electrovalve 3 was switched back to position A and the HiTrap® was eluted with PBS using a syringe from outside the fume-cupboard. The first 0.5 ml were sent to waste, [^{18}F]rhIL-1RA eluted within the following 2 ml fraction which was collected, weighted, the radioactivity measured and a sample (20 μl) analyzed for QC.

[^{18}F]rhIL-1RA was prepared within 100 min, from the time of the addition of the $^{18}F^-$, with 7.1–24.2% radiochemical yield (mean value 11.4 ± 4.1%, based on seventeen syntheses and decay corrected). [^{18}F]rhIL-1ra, 60.31–310.8 MBq (uncorrected for decay) was thus collected as a 2 mL fraction. The specific activity ranged from 913.15 to 5040.20 MBq/μmol at the end of synthesis. From 2 mg of initial rhIL-1RA, 1.1–1.9 mg was recovered as a mixture of labeled and unlabelled protein.

2.4. Quality control

2.4.1. SE-HPLC

QC analyses of rhIL-1RA and [^{18}F]rhIL-1RA were made by size exclusion high-performance liquid chromatography (SE-HPLC) performed on a HPLC Shimadzu prominence system operated using a LabLogic software Laura 3 and configured with a CBM-20 A controller, an LC-20AB solvent delivery system and a SPD-20 A dual wavelength absorbance detector set at 254 and 235 nm. The system was equipped with a Superdex® Gel Filtration Column Peptide HR10/300 GL (GE Healthcare, UK) eluted with PBS at a 1 ml/min flow-rate. Radioactivity was monitored with a radio-HPLC Bioscan Flowcount B-FC 3100 detector.

2.4.2. SDS PAGE

Fifteen percent acrylamide gels were cast with a 4% acrylamide stacking gel using a Mini Protean III system (Bio-Rad). 5 μl Prestained Precision Plus Protein Standards (BioRad, UK, Catalogue number 161-0375) were loaded into lane one and a sample of ca 50 ng of mixed radiolabelled and unlabelled protein from the 1.1–1.9 mg recovered fraction of rhIL-1RA diluted in 2X Laemlli Buffer (4% sodium dodecyl sulphate (SDS), 20% glycerol, 120 mM Tris. Cl pH 6.8, 0.01% Bromophenol blue, 10% 2-mercaptoethanol) was loaded into lane 2. They were run at 30 mA until the dye

appeared at the bottom at which point they were removed and fixed by immersion in 45% deionised water, 45% methanol and 10% acetic acid. Gels of radiolabelled rhIL-1RA were then photographed, sealed in clingfilm and placed into an imaging cassette. Autoradiography was performed using a Fujifilm Bio-Imaging Analyzer BAS-1800II (FujiFilm UK Ltd., UK) and data were analyzed using Advanced Image Data Analyzer software (Raytest, Straubenhardt, Germany). Radiolabelled protein was sized by overlaying the photograph onto the autoradiogram. Gels of unlabelled rhIL-1RA were stained with Coomassie Brilliant Blue (PhastGel™ Blue R, GE Healthcare, UK).

2.5. *In vitro* binding of [^{18}F]rhIL-1RA to IL-1 receptor

An *in vitro* assay was performed to investigate the ability of the produced [^{18}F]rhIL-1RA to bind to the IL-1 receptors. The assay was conducted using rat brain slices ($n=3$; 9–11 brain sections per rat). Binding of the labeled protein was detected by autoradiography.

2.5.1. *Brain sample preparation*

Adult, male, Sprague-Dawley rats (250–300 g body-weight) were used to perform autoradiography experiments. The animals were housed in a controlled environment of 12 h light/dark cycle (08:00/20:00 h) at 22 °C. Transient focal cerebral ischemia (60 min) was induced in rats by the intraluminal thread method, as described previously (Longa et al., 1989). 7 days post-MCAO, rats were killed by anesthetic overdose with isoflurane and decapitated. Brains were removed quickly and frozen in cooled (−40 °C) isopentane. Coronal brain sections were cut serially (20 μm at 1.2 mm intervals between each level) on frozen brains by cryostat. All procedures were performed in accordance with UK legislation under the 1986 Animals (Scientific Procedures) Act and by approved protocols (Home Office Project License Number 40/3076).

2.5.2. *Binding assay*

Specific binding of [^{18}F]rhIL-1RA was determined by comparing the level of total binding (specific and non-specific binding) and the level of non-specific binding in matched rat brain sections. To determine total binding, slices of healthy rat brains were pre-incubated in 10 mM phosphate buffer saline (PBS) at 4 °C for 5 min, then incubated in solutions of different concentrations of the [^{18}F]rhIL-1RA (10, 20, and 40 nM in PBS, 10 mM at room temperature). After 30 min incubation, the slices were washed three times 3 min in PBS to remove unbound radiolabelled protein, then quickly rinsed in cold distilled water, before exposition to PhosphorImager plates overnight. To determine non-specific binding, a second set of brain sections were treated as described above but in a solutions containing [^{18}F]rhIL-1RA (10, 20, and 40 nM) and one thousand-fold excess (i.e. 10–40 μM) of non-radiolabelled rhIL-1RA. Radiolabelled rhIL-1RA that was not displaced by non-radiolabelled rhIL-1RA accounted for the non-specifically bound. Specific binding was calculated by subtracting the non-specific from total binding of [^{18}F]rhIL-1RA.

3. Result and discussion

Although targeting ε-amino groups of lysine moieties randomly could be considered as the drawback of this labeling approach, with [^{18}F]fluoroacetaldehyde the size of the added [^{18}F]fluoroethyl substituent is small and should have little effect on the physical chemical properties and the structure of the protein allowing PET pharmacokinetic studies. In addition to its small size [^{18}F]fluoroacetaldehyde presents following advantages: first it is easily produced via a fast and simple procedure in a two-step, one-pot reaction using oxidation with DMSO of [^{18}F]fluoroethyltosylate; second it is simply extracted continuously by distillation from the reaction mixture sparing a purification step using HPLC or solid phase extraction and finally the highly hydrophilic aldehyde is efficiently trapped in a small volume of water (<100 μl). Furthermore the mild labeling conditions, citrate buffer pH 6 and low temperature (38 °C), make the purification of the radiolabelled protein straightforward. Purification of the [^{18}F]rhIL1-ra was thus simply made by eluting the reaction mixture through a HiTrap desalting column. Analysis of the crude synthesis mixture by SE-HPLC using the radioactivity detector before purification on the HiTrap cartridge showed that it contained [^{18}F]rhIL-1RA ($R_t=12.2$ min), unreacted [^{18}F]fluoroacetaldehyde ($R_t=20$ min) and a minor unidentified radioactive impurity ($R_t=24.2$ min) (Fig. 3). [^{18}F]rhIL-1RA accounted for 28–35% of the total radioactivity of the analyte of the synthesis mixture (yield based on three analyses). Identification of the second peak as [^{18}F]fluoroacetaldehyde was made by comparing the retention time with standard fluoracetaldehyde prepared as previously described (Prenant et al., 2008). The unidentified radioactive impurity may have resulted from

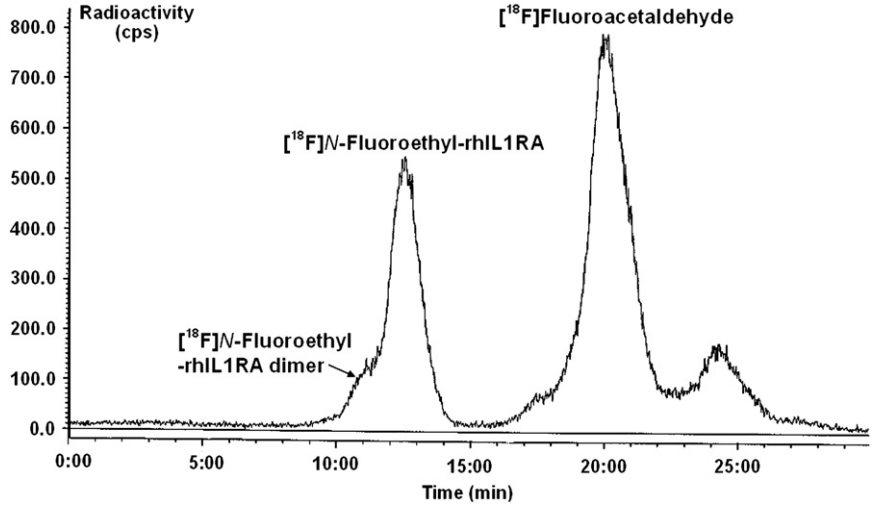

Fig. 3. Radio-SE-HPLC analysis of the crude synthesis mixture. [^{18}F]rhIL-1RA ($R_t=12.2$ min), [^{18}F]fluoroacetaldehyde ($R_t=20$ min), unidentified radioactive impurity ($R_t=24.6$ min).

[^{18}F]fluoroacetaldehyde reduction to [^{18}F]fluoroethanol or the reaction of free cyanide ion released from sodium cyanoborohydride with [^{18}F]fluoroacetaldehyde to produce the cyanohydrin, lactonitrile (Gidley and Sanders, 1982). Both small molecules were sufficiently retained on the HiTrap column to allow the protein to be eluted as a pure compound under monomeric and dimeric form with a radiochemical purity, for the mixte two forms, over 95% in a small volume (2 ml). QC SE-HPLC analysis of the [^{18}F]rhIL-1RA, UV absorbance trace as well as the radioactivity trace (Fig. 4) showed two poorly resolved peaks. On the UV absorbance trace, the small peak at retention time 10.0 min preceding a larger one at retention time 11.8 min, suggested the presence of a dimer. SDS-PAGE analysis of the labeled rhIL-1RA (Fig. 5) confirmed the presence of a minor dimeric fraction (faint band at approximately 35 kD), but most of the radioactivity migrated to a position consistent with a 17 kD protein. Similarly, the SE-HPLC chromatogram of the reference rhIL-1RA showed two peaks (data not shown) nevertheless only a monomeric molecular weight was seen by SDS-PAGE (data not shown).

At relatively high concentrations (i.e. 100 mg/ml), rhIL-1RA exists in a monomer–dimer equilibrium (Chang et al., 1996; Roy et al., 2005; Alford et al., 2008a, 2008b). Chang et al. (1996) have also shown that an irreversible dimer formed, that amounted to 8% of the total soluble protein, during storage for 2 months at 30 °C and this irreversible dimer was mostly dissociated during SDS-PAGE. Our radiolabeling method was applied to other proteins without producing dimerization (data not shown); thus, we cannot exclude the possibility that production of the dimer was caused during storage of the rhIL-1RA solution and the non-visualisation of unlabelled rhIL-1RA dimer by SDS-PAGE/Coomassie staining was due to a lack of sensitivity of this technique.

Since expression of IL-1 receptors has been reported before in a rat and mouse brains (Parnet et al., 1994; French et al., 1999; Friedman, 2001), we choose to use autoradiography on rat brain sections to test whether we could observe any specific binding (displaceable) of our [^{18}F] radiolabelled IL-1RA. Specificity of binding was determined by displacement of the bound [^{18}F]rhIL-1RA by an excess of unlabelled rhIL-1RA on rat brain sections as measured by autoradiography (Fig. 6a). Comparison of the total binding (Tot) and non-specific binding (NS) indicated that specific binding (Spe) of the radiolabelled [^{18}F]rhIL-1RA to IL-1 receptors accounted for 24–29% of total binding (Fig. 6b). Aware of the high concentrations of [^{18}F]rhIL-1RA (mixed with unlabelled) used for autoradiography when compared to the reported K_d of IL-1RA for the IL-1 receptors, we have tried to assess specific binding with lower concentrations of [^{18}F]rhIL-1RA (1 nM) using autoradiography and binding on cell membranes preparation; unfortunately, due to the relatively short half-life of fluorine-18 (when compared with iodine-125 for example), we could not detect any reliable signal on the PhophorImager plates or by γ-counting. Clearly, fluorine-18 labeling of protein would not be the most convenient and appropriate technique to assess *in vitro* binding of a tracer to receptors expressed at very low levels such as the IL-1 receptors. In this study, exemplifying this new radiochemistry, the main aim of labeling rhIL-1RA was more to develop a tool to assess the

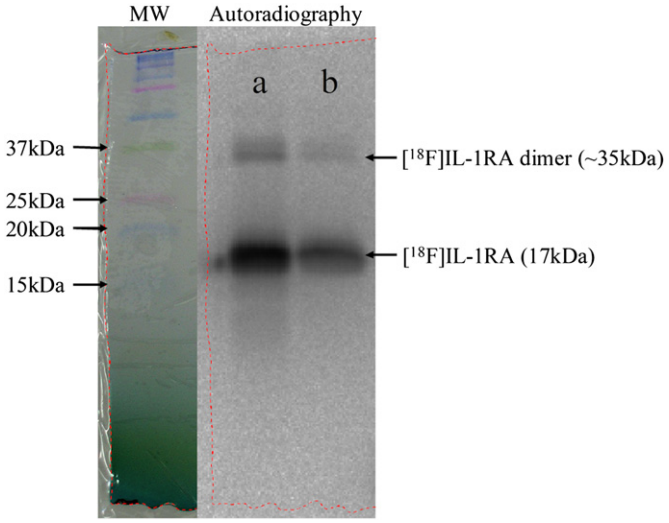

Fig. 5. PAGE analysis of the purified [^{18}F]rhIL-1RA (MW: molecular weight references loaded in the same lane than [^{18}F]rhIL-1RA (a)) and autoradiography (a: 1.1 μg of rhIL-1RA=17.8 kBq in 20 μl; b: 0.1 μg of rhIL-1RA=2.2 kBq in 20 μl).

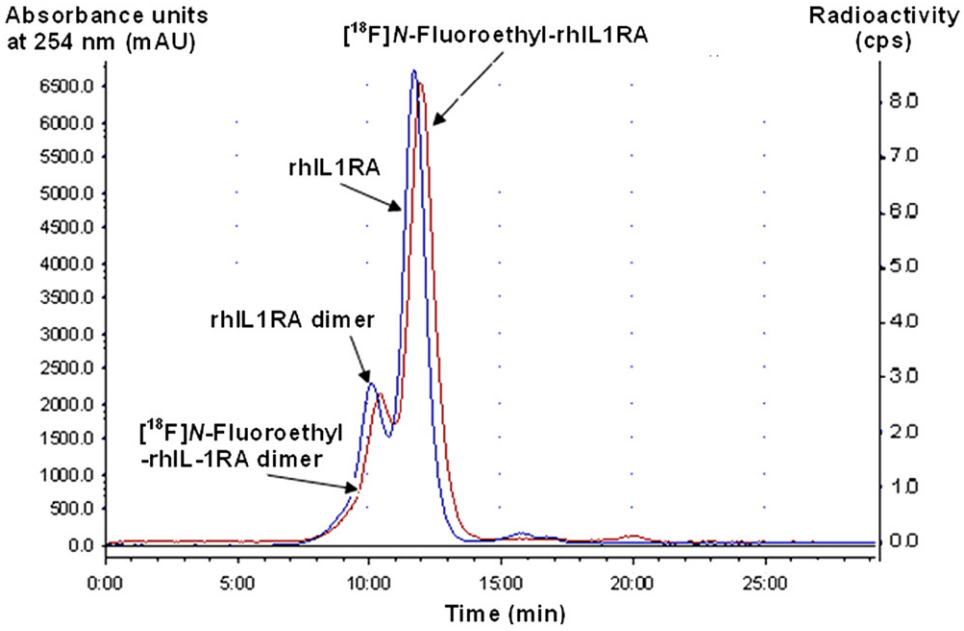

Fig. 4. QC of the purified [^{18}F]rhIL-1RA, SE-HPLC uv-trace and radio-trace.

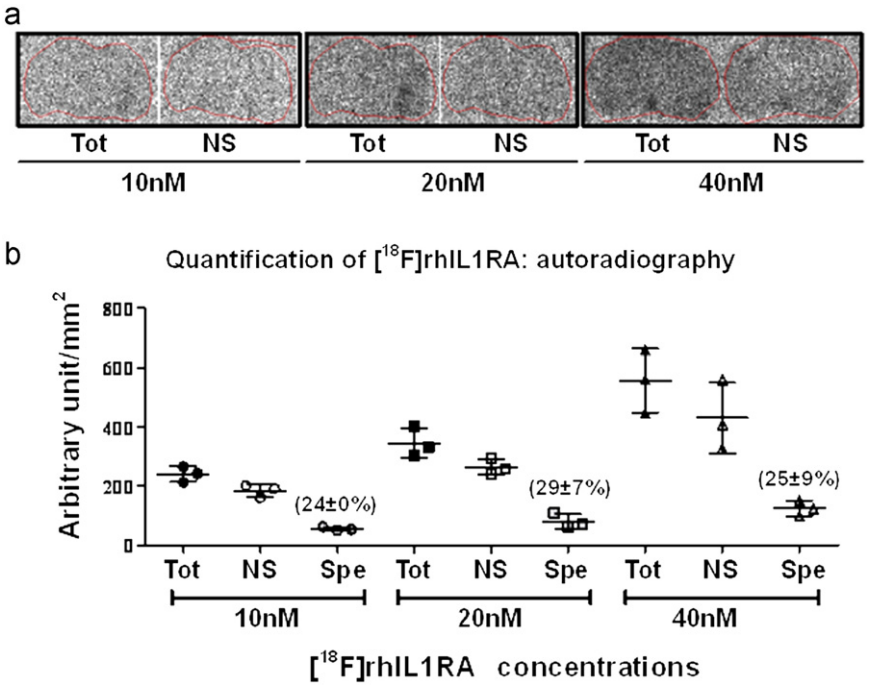

Fig. 6. (a) Representative examples of autoradiography of rat brain slices by incubation with 10, 20, and 40 nM of [^{18}F]rhIL-1RA (mixture of [^{18}Frh]IL-1RA, [^{19}F]rhIL-1RA and precursor rhIL-1RA. Non-specific binding (NS) was assessed by incubating the brain sections with [^{18}F]rhIL-1RA (10, 20, and 40 nM) together with an excess of unlabelled rhIL-1RA (10, 20, and 40 μM). (b) Specific binding (indicated as percentage of total binding in brackets, mean ± SD) of various concentrations of [^{18}F]rhIL-1RA on rat brain sections.

biodistribution and metabolisms of IL-1RA than its *in vitro* binding, especially when considering the high level of non-specific binding observed here.

4. Conclusion

We describe here a simple method for radiolabeling of rhIL-1RA using [^{18}F]fluoroacetaldehyde. Radiosynthesis and purification, using a HiTrap® desalting column, were carried out in a remotely controlled experimental rig within 100 min. The radiochemical yield was in the range 7.1–24.2% (mean value 11.4 ± 4.1%, decay-corrected, based on seventeen syntheses). Starting from 1.73 to 3.42 GBq (64–126 mCi) of fluorine-18, 60.31–310.80 MBq (1.63–8.40 mCi) of [^{18}F]rhIL-1RA were produced. Moreover, we showed that [^{18}F]rhIL-1RA retained binding potency in a rat brain binding assay. Therefore, this method allowed us to produce [^{18}F]rhIL-1RA on a small scale with sufficient radiochemical purity and specific activity to enable pharmacokinetic studies in small animals using PET and the investigation of the relevance of rhIL-1ra as a therapeutic agent in brain traumatisms. Moreover, this radiolabeling technique can be applied to any peptide and protein with lysine moieties available for reaction with [^{18}F]fluoroacetaldehyde, thus providing another method for the radiolabeling of proteins of interest/therapeutic agents, for the assessment of both their pharmacokinetics and biodistribution or their use as imaging biomarkers.

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