Automatisation and First Evaluation of [^{18}F]FE@SUPPY:2, an Alternative PET-Tracer for the Adenosine A_3 Receptor: A Comparison with [^{18}F]FE@SUPPY

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Abstract: *Introduction*: Since the Adenosine-A_3-receptor was identified in the late 1990´s, there is little data available describing its distribution *in vivo*. Recently, we introduced [^{18}F]FE@SUPPY as the first PET-tracer for this receptor. In the present investigation we translated this fluoroethyl-ester into the fluoroethyl-thioester [^{18}F]FE@SUPPY:2 (5-ethyl 2,4-diethyl-3-((2-[^{18}F]fluoroethyl) sulfanylcarbonyl)-6-phenylpyridine-5-carboxylate). Aims of the present study were the evaluation of (1) the automatized preparation of both [^{18}F]FE@SUPPY-derivatives, (2) the biodistribution of [^{18}F]FE@SUPPY:2, (3) the lipophilicity and (4) the comparison of the findings of [^{18}F]FE@SUPPY and [^{18}F]FE@SUPPY:2.

Methods: The automated preparations of both [^{18}F]FE@SUPPY-analogs were performed on a GE *TRACERlab Fx$_{FN}$* synthesizer using suitable precursors. Biodistribution experiments were performed using Sprague-Dawley rats/Him:OFA. Lipophilicity of the compounds was determined using an HPLC assay.

Results: 22 automated radiosyntheses were performed for both radiotracers. Specific radioactivity was 70 ± 26GBq/µmol for [^{18}F]FE@SUPPY and 340 ± 140GBq/µmol for [^{18}F]FE@SUPPY:2. Biodistribution experiments evinced bowels and liver as organs with highest uptake and intermediate uptake in kidney, lung and heart. LogP values of both molecules ranged from 3.99 to 4.12 at different pH.

Conclusion: From a radiopharmaceutical perspective, drastically better specific radioactivities would militate in favour of [^{18}F]FE@SUPPY:2; preclinical evaluations, so far, do not permit the decision upon the selection of the optimum [^{18}F]FE@SUPPY-derivative. With [^{18}F]FE@SUPPY:2, we are able to provide a second potential tracer that could help to further characterize the still quite unexplored Adenosine-A_3-receptor.

Keywords: Adenosine, PET, adenosine A3 receptor, fluorine-18, radioligand, SUPPY.

INTRODUCTION

Adenosine is one of the key modulators of the human body and acts through four different receptor subtypes: the A_1, A_{2A}, A_{2B}, and A_3 receptors, respectively (A1AR, A2AAR, A2BAR, and A3AR). The A3AR was identified in the late 1990´s, and, so far, there is little data available describing its distribution and density *in vivo*. The most suitable technique to collect these lacking data in the living organism would be PET (positron emission tomography). As a premise for molecular PET imaging with high quality, there is need for suitable radioligands displaying high affinity, high selectivity and low unspecific binding. Also, the absence of interfering radioactive metabolites in the target tissue is of importance. Beside these prerequisites, the most important premise for a successful PET-tracer is its widespread availability and reliable preparation. Additionally, to keep radiation burden for the operators as low as possible, a fully automated preparation would be beneficial.

Recently, we introduced [^{18}F]FE@SUPPY (5-(2-[^{18}F]fluoroethyl) 2,4-diethyl-3-(ethylsulfanylcarbonyl)-6-phenylpyridine-5-carboxylate) as the first PET-tracer for the A3AR [1, 2]. The radiosynthesis was performed in a simple

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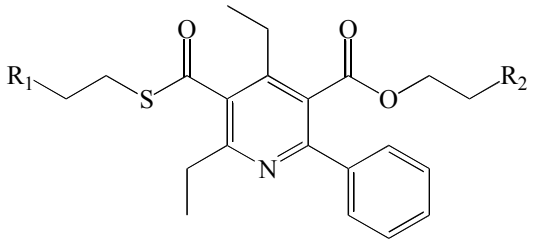
one-pot one-step reaction with good and reliable radiolabeling yields and good specific radioactivities. [^{18}F]FE@SUPPY was selected due to its favorable binding characteristics for the A3AR out of the well characterized chemical family of 3,5-diacyl-2,4-dialkylpyridines – with all derivatives showing considerable affinity for the adenosine receptor system [3]. Regarding the metabolic profile – these diacyl-derivatives all carry two ester moieties within one molecule, one carboxylic and one thiocarboxylic – enzymes derived from the family of carboxylesterases would be expected to significantly contribute to cleavage and degradation [4, 5]. To the best of our knowledge, there is no conclusive data regarding differences in stability of carboxylic and thiocarboxylic esters within one molecule. In a comparative study, Azema *et al*. [6] even presented evidence for increased stability of the thioester function compared to carboxylic esters. Hence, we translated the fluoroethyl ester [^{18}F]FE@SUPPY into the fluoroethyl-thioester [^{18}F]FE@ SUPPY:2 (5-ethyl 2,4-diethyl-3-((2-[^{18}F]fluoroethyl) sulfanylcarbonyl)-6-phenylpyridine-5-carboxylate; see Fig. **1**). Being close structural analogs, even lipophilicity of both molecules could be expected to be comparable.



Compound	R_1	R_2
[^{18}F]FE@SUPPY	-H	-^{18}F
[^{18}F]FE@SUPPY:2	-^{18}F	-H
Tos@SUPPY	-H	-OTos
OH@SUPPY	-H	-OH
Tos@SUPPY:2	-OTos	-H
OH@SUPPY:2	-OH	-H

Fig. (1). Structural differences in fluoroethylated SUPPY and SUPPY:2 compounds and their precursor molecules.

Thus, aims of the present study were

(1) The fully automated preparation of both [^{18}F]FE@SUPPY:2 and [^{18}F]FE@SUPPY.

(2) The evaluation of the biodistribution of [^{18}F]FE@SUPPY:2 in rats.

(3) The characterisation of the lipophilicity using standardized methods.

(4) The comparison of the findings of [^{18}F]FE@SUPPY and [^{18}F]FE@SUPPY:2.

MATERIALS AND METHODS

General

Mass spectra were obtained on a Shimadzu QP 1000 instrument (EI, 70 eV; Shimadzu, Kyoto, Japan). IR spectra were recorded on a Perkin-Elmer FTIR spectrum 1000 spectrometer (Perkin Elmer, Watford, UK). Elemental analyses were performed at the Microanalytical Laboratory at the University of Vienna, Austria (http://www.univie.ac.at/Mikrolabor/ind_eng.htm). ^{1}H- and ^{13}C-NMR spectra were recorded on a Bruker Avance DPX-200 spectrometer at 27°C (200.13 MHz for ^{1}H, 50.32 MHz for ^{13}C; Bruker AXS GmbH, Karlsruhe, Germany). High resolution mass spectroscopy was performed on a Finnigan MAT 8230 (EI; 70eV; Thermo Finnigan Waltham, MA, USA). Radioanalytical thin-layer chromatography (radio-TLC) was performed using silicagel 60F_{254} plates from Merck (Darmstadt, Germany). Analysis of radio-TLC plates and autoradiography were performed using a Canberra-Packard Instant Imager (Perkin Elmer, Watford, UK). Analytical high-performance liquid chromatography (HPLC) was performed using a Merck-Hitachi LaChrom system with a NaI-radiodetector from Berthold Technologies (Bad Wildbach, Germany). The semi-preparative HPLC system (as part of the GE *TRACERlab Fx $_{FN}$* synthesizer) consisted of a Sykam S1021 pump (Sykam GmbH, Eresing, Germany) and a UV detector (254 nm) and a radioactivity detector in series.

Solid phase extraction (SPE) cartridges (SepPak® C18plus) were purchased from Waters Associates (Milford, USA). All starting materials for precursor and reference standard syntheses were commercially available and used without further purification. [^{18}F]Fluoride was produced *via* the ^{18}O(p,n)^{18}F reaction in a GE PETtrace cyclotron (16.5MeV protons; GE Medical systems, Uppsala, Sweden). $H_2{}^{18}O$ (>98%) was purchased from Rotem Europe (Leipzig, Germany). Anion exchange cartridges (PS-HCO_3) for [^{18}F]fluoride fixation were obtained from Macherey-Nagel (Dueringen, Germany).

Precursor Chemistry – 5-(2-Tosyloxyethyl) 2,4-Diethyl-3-(Ethylsulfanylcarbonyl)-6-Phenylpyridine-5-Carboxylate (Tos@SUPPY)

This precursor was prepared as described previously [1, 2, 7]. Briefly, OH@SUPPY, prepared according to [8] was reacted with toluene-4-sulfonyl chloride in THF and purified by recrystallization.

5-Ethyl 2,4-Diethyl-3-((2-Tosyloxyethyl)Sulfanylcarbonyl)-6-Phenylpyridine-5-Carboxylate (Tos@SUPPY:2)

A solution of toluene-4-sulfonyl chloride (1.64g, 8.59mmol) in THF (15mL) was added to OH@SUPPY:2 [8] (1.69g, 4.36mmol) and triethylamine (1.18g, 1.6ml, 11.67mmol) in THF (40mL) at 0°C. Then the reaction mixture was refluxed and the solvent was evaporated in vacuo. The residue was purified *via* column chromatography.

Reference Standard – 5-(2-Fluoroethyl) 2,4-Diethyl-3-(Ethylsulfanylcarbonyl)-6-Phenyl-Pyridine-5-Carboxylate (FE@SUPPY)

The reference compound was obtained according to [1, 2, 7]. Briefly, OH@SUPPY [8] was treated with diethylaminosulfur trifluoride (DAST) at -78°C. Purification was performed using column chromatography.

5-Ethyl 2,4-Diethyl-3-((2-Fluoroethyl)Sulfanylcarbonyl)-6-Phenyl-Pyridine-5-Carboxylate

(FE@SUPPY:2) OH@SUPPY:2 (1.00g, 2.58mmol, [8]) was reacted with DAST (0.69g, 5.17mmol) in water-free

dichloromethane at -78°C. After hydrolysis, purification was performed using column chromatography.

Automated Radiosynthesis – [18F]FE@SUPPY and [18F]FE@SUPPY:2

The automated preparation of both fluorinated FE@SUPPY analogs was preformed on a GE *TRACERlab* Fx_{FN} synthesizer. A comprehensive overview of all components of the system is given in Fig. (**2**). The following preparation steps were performed prior to synthesis start: a C18plus SPE cartridge (360mg, SepPak® Waters) and a PS-HCO3 SPE cartridge (45mg, Macherey-Nagel) were placed on their designated spots and connected to the corresponding tubing. The vials were filled with the following solutions: vial 1 – Kryptofix K2.2.2 (4,7,13,16,21,24-hexaoxo-1,10-diaza-bicyclo[8.8.8] hexacosane; 20mg, 53.2µmol) and potassium carbonate (4.5mg, 32.6µmol) in a mixture of 700µL acetonitrile and 300µL water; vial 2 – 1mL acetonitrile; vial 3 – 0.5mL acetonitrile; vial 4 – 1mL of precursor solution (i.e. 10mg Tos@SUPPY (18.5µmol) in acetonitrile for [18F]FE@SUPPY and 15mg Tos@SUPPY:2 (27.8 µmol) in acetonitrile for [18F]FE@SUPPY:2, respectively); vial 5 – 0.5mL water; vial 7 – 15mL physiological saline; vial 8 – 2mL ethanol and vial 9 – 10mL water. The round bottom flask was filled with 80mL water and the reactor was filled with 1mL acetonitrile.

No-carrier-added aqueous [18F]fluoride was produced *via* the $^{18}O(p,n)^{18}F$ reaction in a GE Gen2-silver target filled with 2.4mL [18O]water (>98%) and delivered to a 5mL v-vial. This vial was placed on the designated spot of the synthesizer (left hand side, see Fig. **2**) and connected. Then, vacuum was applied and [18F]fluoride was sucked over the anion exchange cartridge on-line (*via* V10 and V11) and separated from excess water. [18F]fluoride was eluted with a solution containing Kryptofix 2.2.2. and potassium carbonate in acetonitrile/water into the reactor (V1, V10, V11, V13). The resulting solution was heated for 2 minutes at 60°C and acetonitrile was added (V2). Heating was continued for another 3min at 60°C and then for 5min at 120°C in a stream of helium (V20) while adding the final portion of acetonitrile (V3) to complete azeotropic drying. The dried [18F]fluoride-aminopolyether complex was cooled to 65°C, precursor was added (V4) and the mixture was heated to 75°C for 18min. After cooling to 35°C, the crude reaction mixture was quenched with water (V5) and transferred (V14) to the injec-

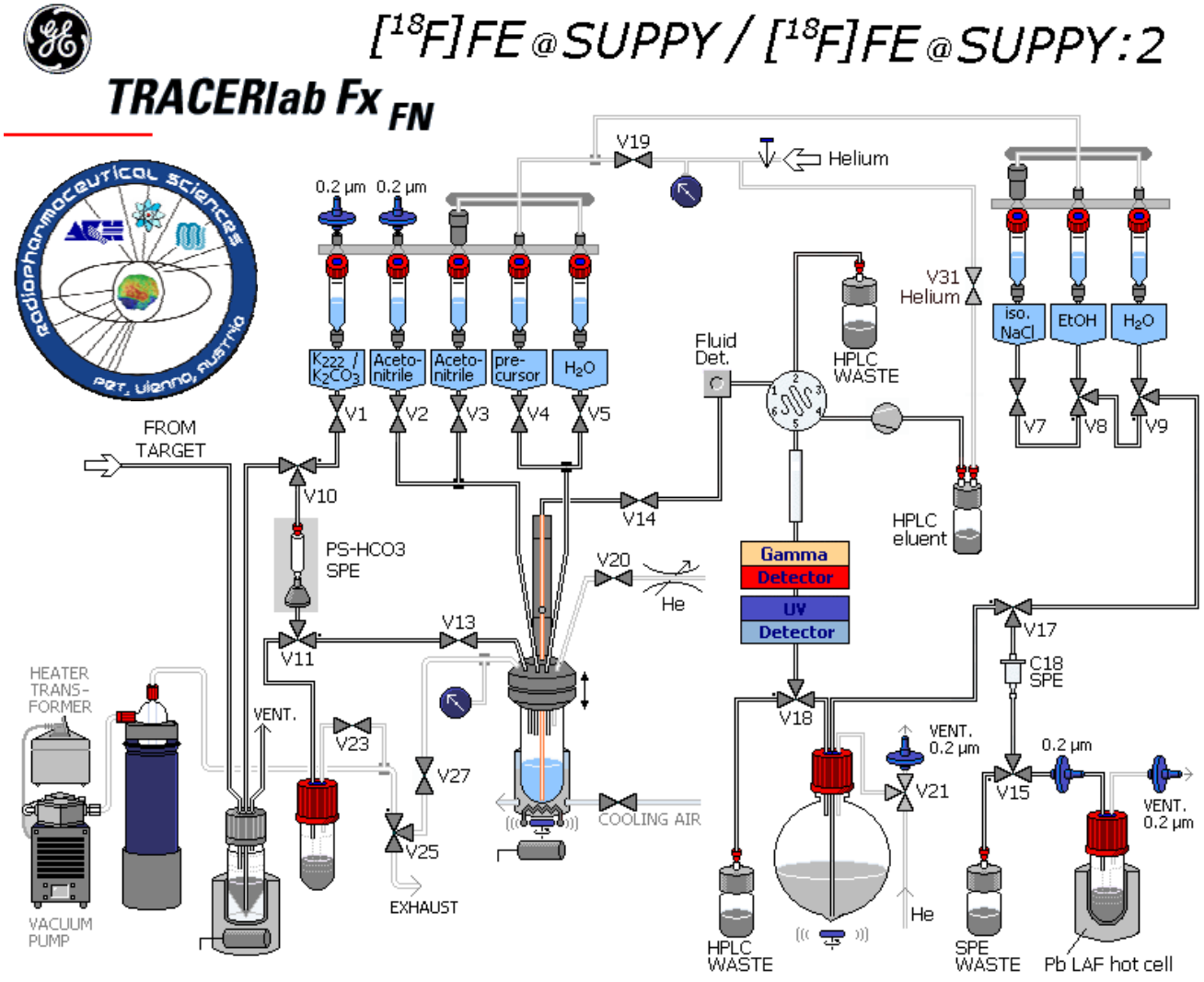


Fig. (2). Graphical illustration of the automated set-up of the commercially available TRACERlab Fx_{FN} module for [18F]FE@SUPPY and [18F]FE@SUPPY:2 synthesis.

tor of the semi-preparative reversed-phase HPLC system (column: Merck Chromolith® SemiPrep RP-18e, 100x10mm, mobile phase: acetonitrile/water/acetic acid (60/38.8/1.2 v/v/v; 2.5 g/L ammonium acetate; pH 3.2); flow: 10 mL/min). Triggered by the fluid detector, the injector automatically changed from the "load" to the "inject" position and the chromatographic plotting started. Using the presettings from our evaluation runs, the desired peak was cut automatically and collected in the round bottom flask (V18). The diluted solution was transferred over a C18plus SepPak® in a stream of helium (V17, V21). After washing with water (V9), the purified product was eluted with ethanol (V8) directly into a sterile vial within a laminar air-flow hot-cell (V15) and on-line sterile filtered (0.22µm). The product was diluted and formulated using physiological saline (V7).

Biodistribution Experiments

The experiments were approved by the Austrian law on animal experiments and the procedure followed the protocol established in various previous studies of our group. Male Sprague-Dawley rats/Him:OFA (n=20, 271-346 g) were injected with 2.17–7.52 MBq (180-225µL) through a tail vein. Subsequently, individuals were sacrificed by exsanguination from the abdominal aorta in ether anaesthesia after 5, 15, 30, 60 and 120 minutes (n=4 each). Organs were removed, dry weighed and counted. Results are expressed as percent injected dose per gram tissue (% I.D./g).

Lipophilicity

The lipophilicity of the title compounds was determined using an HPLC assay based on Donovan and Pescatore [9]. Briefly, a short octadecyl-poly(vinyl alcohol) HPLC column (Supelco ODP-50; 4.6 x 20mm) was eluted with a flow rate of 2mL/min. A linear gradient from 10 to 100% methanol (organic phase) within 7 min was applied and buffers for pH 2, 7.4 and 10 (aqueous phase) were prepared; UV-detection was done at 270 nm. Toluene and triphenylene (dissolved in methanol) were used as internal standards and the injection volume was 20µL. Between consecutive HPLC runs, 5min for re-equilibration of the HPLC column were allowed. The $_{\mathrm{HPLC}}$logP of a compound was then determined using the following equation:

$$ {}_{\mathrm{HPLC}}\log P_x = \frac{\left(\log P_{tol} - \log P_{tri}\right)\cdot t_{R,x} + t_{R,tol}\log P_{tri} - t_{R,tri}\log P_{tol}}{t_{R,tol} - t_{R,tri}} $$

where $logP_{tol}$ and $logP_{tri}$ are the logP values for toluene and triphenylene, respectively, from the literature [10] and $t_{R,tol}$ and $t_{R,tri}$ are the retention times for toluene and triphenylene, respectively, determined in the HPLC run. $t_{R,x}$ is the retention time of the compound.

$_{c}$logP-values were calculated based on the structural formulas of the compounds using the logP add-on within the ACD/chemsketch software (ACD labs; version 11.01; October 2007).

RESULTS

Precursor Chemistry

1.7g Tos@SUPPY was obtained as white crystalline powder and fully characterized as presented recently [1, 2].

2.10g purified Tos@SUPPY:2 was obtained (89%) as yellowish oil. For NMR-analysis, the solvent signal was used as an internal standard which was related to TMS with δ = 7.26ppm (^{1}H in $CDCl_3$) and δ = 77.0ppm (^{13}C in $CDCl_3$), respectively.

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm) 7.82 (m, 2H), 7.58 (m, 2H), 7.38 (m, 4H), 4.26 (t, 2H, *J* = 6.30 Hz), 4.09 (q, 2H, *J* = 7.18 Hz), 3.39 (t, 2H, *J* = 6.20 Hz), 2.77 (q, 2H, *J* = 7.44 Hz), 2.64 (q, 2H, *J* = 7.82 Hz), 2.44 (s, 3H), 1.28 (t, 3H, *J* = 7.56 Hz), 1.17 (t, 3H, *J* = 6.94 Hz), 0.98 (t, 3H, *J* = 7.06 Hz).

^{13}C-NMR (50 MHz, $CDCl_3$): δ (ppm) 194.2, 168.2, 159.2, 157.3, 148.0, 145.1, 139.6, 132.6, 132.3, 129.9, 128.9, 128.3, 127.9, 126.6, 67.7, 61.5, 29.1, 28.8, 24.1, 21.6, 15.6, 14.0, 13.5.

IR (KBr): ν (cm^{-1}) 2978, 2938, 2878, 1724, 1678, 1598, 1556, 1495, 1463, 1447, 1403, 1362, 1278, 1250.

MS: m/z (%) 543 (M^+ + 2, 1), 542 (1), 311 (24), 310 (100), 282 (17), 264 (4), 236 (3), 155 (3), 91 (9).

Elemental analysis: calculated for $C_{28}H_{31}NO_6S_2$: C, 62.08; H, 5.77; N, 2.59. found: C, 61.92; H, 5.77; N, 2.57.

Reference Standards

136.8mg FE@SUPPY was obtained as yellowish oil and fully characterized as presented recently [1, 2].

0.37g FE@SUPPY:2 was obtained after purification as yellowish oil.

^{1}H-NMR (200 MHz, $CDCl_3$): δ (ppm) 7.60 (m, 2H), 7.42 (m, 3H), 4.76 (t, 1H, *J* = 5.92 Hz), 4.53 (t, 1H, *J* = 5.94 Hz), 4.10 (q, 2H, *J* = 7.06 Hz), 3.50 (t, 1H, *J* = 5.94 Hz), 3.39 (t, 1H, *J* = 5.92 Hz), 2.86 (q, 2H, *J* = 7.44 Hz), 2.72 (q, 2H, *J* = 7.58 Hz), 1.34 (t, 3H, *J* = 7.46 Hz), 1.23 (t, 3H, *J* = 7.44 Hz), 0.98 (t, 3H, *J* = 7.06 Hz).

^{13}C-NMR (50 MHz, $CDCl_3$): δ (ppm) 194.4, 168.2, 159.2, 157.2, 148.0, 139.6, 132.6, 126.6, 81.3 (d, *J* = 170.5 Hz), 61.5, 30.0 (d, *J* = 21.8 Hz), 29.1, 24.1, 15.6, 14.0, 13.5.

IR (KBr): ν (cm^{-1}) 3061, 2978, 2939, 2902, 2879, 1726, 1678, 1557, 1465, 1448, 1405, 1380, 1279, 1251, 1175, 1144, 1091, 1076, 1014, 973.

MS: m/z (%) 389 (M^+, 3), 343 (4), 311 (20), 310 (100), 282 (14), 236 (7),105 (20), 77 (12).

High-resolution mass spectroscopy (HRMS): m/z calculated for $C_{21}H_{24}NO_3SF$: 389.1461; found: 389.1473.

Automated Radiosynthesis – [^{18}F]FE@SUPPY and [^{18}F]FE@SUPPY:2

The described automated preparation of both fluorinated FE@SUPPY analogs was set up using standard PET radiochemistry equipment. The whole preparation including radiosynthesis, purification, sterile filtration and formulation was completed within 70-80min. Semi-preparative HPLC revealed consistent retention patterns for [^{18}F]FE@SUPPY and [^{18}F]FE@SUPPY:2 (retention times: 4.2-4.9min; k': 2.8-3.5). SPE purification led to a recovery of more than 90% using 2.0mL of ethanol.

So far, 15 complete high-scale radiosyntheses were performed for [^{18}F]FE@SUPPY and 7 preparations were conducted for [^{18}F]FE@SUPPY:2. Starting from 51 ± 25 GBq of

[18F]fluoride, 9.4 ± 3.6 GBq of formulated [18F]FE@SUPPY and 5.1 ± 4.2 GBq of formulated [18F]FE@SUPPY:2 were achieved.

Radiochemical purity as determined using radio-TLC and radio-HPLC always exceeded 97%. The only radioactive contaminant was found to be [18F]fluoride. Specific radioactivity was determined *via* HPLC and found to be 70 ± 26 GBq/μmol for [18F]FE@SUPPY and 340 ± 140 GBq/μmol for [18F]FE@SUPPY:2, respectively, at the end of synthesis (EOS).

Biodistribution Experiments

All values are shown in Table **1**. The organ with the lowest uptake was fat showing 0.05 ± 0.02% I.D./g after 5 minutes, followed by lung (0.07 ± 0.05% I.D./g), spleen (0.07 ± 0.03% I.D./g) and brain (0.07 ± 0.01% I.D./g, all values after 120 minutes). Organs with highest uptake were bowels with 1.45 ± 0.97% I.D./g after 15 minutes followed by the liver showing 1.08 ± 0.39% I.D/g after 5 minutes. Other organs with pronounced uptake were kidney and heart. Blood activity was 0.12-0.18% I.D./g throughout the whole experiment. Remaining activity in the carcass was 0.15-0.39% I.D./g.

Lipophilicity

We found retention times of 4.95-5.15min for toluene and 8.05-8.15min for triphenylene. Both [18F]FE@SUPPY and [18F]FE@SUPPY:2 eluted at 6.48-6.67min. Using 2.74 as $logP_{tol}$ and 5.49 as $logP_{tri}$ [10], the $_{HPLC}$logP values of [18F]FE@SUPPY and [18F]FE@SUPPY:2 were calculated according to the equation given in the methods section. These $_{HPLC}$logP values at three different pH values and the calculated $_{c}$logP of [18F]FE@SUPPY and [18F]FE@SUPPY:2 are presented in Table **2**. All HPLC-experiments were repeated at least 4-times; given values are arithmetic means.

DISCUSSION

General

Collectively, adenosine receptors are widespread on virtually every organ and tissue and represent promising drug targets for pharmacological intervention in many pathophysiological conditions such as asthma, neurodegenerative disorders, chronic inflammatory diseases, and cancer. The A3AR is the most recently identified adenosine receptor and its involvement in tumors has recently been shown: the A3ARs are highly expressed on the cell surface of tumor cells [11-16] and in human enteric neurons [17] but not in the majority of normal tissues [15]. In a very comprehensive study, A3AR mRNA expression in various tumor tissues was tested using reverse transcription-PCR analysis and A3AR protein expression was studied in fresh tumors and was correlated with that of the adjacent normal tissue. The authors conclude that primary and metastatic tumor tissues highly express A3AR indicating that high receptor expression is a characteristic of solid tumors. These findings suggest the A3AR as a potential target for tumor growth intervention or imaging [18]. The A3ARs are also known to be involved in many other diseases, such as cardiac [19] and cerebral ischemia [20], glaucoma [21], stroke [22] and epilepsy [23].

Li *et al.* [3] published a series of chemical structures, with most of them displaying reasonable affinities for the A3AR. Amongst all the investigated structures, FE@SUPPY (Fig. **1**) was the most affine compound for the A3AR (K_i 4.22 nM) and thus was selected and developed as [18F]FE@SUPPY, the first PET-tracer [1, 2]. Hence, having developed a series of fluoroethyl-esters over the last five years [24-29], it was obvious for us to develop [18F]FE@SUPPY:2.

Table 1. Biodistribution Values of [18F]FE@SUPPY:2 in Rats at Different Time Points

Tissue	5 min	15 min	30 min	60 min	120 min
blood	0.18 ± 0.05	0.15 ± 0.05	0.18 ± 0.05	0.14 ± 0.02	0.12 ± 0.03
liver	1.08 ± 0.39	0.76 ± 0.34	0.79 ± 0.24	0.40 ± 0.06	0.21 ± 0.04
femur	0.27 ± 0.10	0.20 ± 0.10	0.31 ± 0.10	0.48 ± 0.08	0.67 ± 0.10
lung	0.48 ± 0.14	0.31 ± 0.11	0.25 ± 0.06	0.12 ± 0.04	0.07 ± 0.05
heart	0.85 ± 0.24	0.40 ± 0.07	0.31 ± 0.08	0.15 ± 0.03	0.10 ± 0.03
thyroid	0.41 ± 0.13	0.24 ± 0.09	0.30 ± 0.13	0.21 ± 0.03	0.14 ± 0.09
kidney	1.06 ± 0.37	0.56 ± 0.16	0.61 ± 0.13	0.43 ± 0.11	0.26 ± 0.05
testes	0.10 ± 0.04	0.08 ± 0.04	0.13 ± 0.05	0.11 ± 0.02	0.09 ± 0.03
fat	0.05 ± 0.02	0.08 ± 0.04	0.18 ± 0.17	0.19 ± 0.03	0.25 ± 0.08
muscle	0.26 ± 0.10	0.15 ± 0.09	0.18 ± 0.08	0.12 ± 0.04	0.08 ± 0.04
colon	0.19 ± 0.09	0.21 ± 0.15	0.66 ± 0.84	0.69 ± 1.00	0.16 ± 0.11
ileum/jejunum	0.78 ± 0.41	1.45 ± 0.97	0.50 ± 0.32	0.45 ± 0.28	0.21 ± 0.11
spleen	0.33 ± 0.13	0.18 ± 0.05	0.17 ± 0.05	0.09 ± 0.02	0.07 ± 0.03
brain	0.34 ± 0.13	0.18 ± 0.02	0.21 ± 0.06	0.09 ± 0.02	0.07 ± 0.01
carcass	0.31 ± 0.09	0.39 ± 0.13	0.33 ± 0.13	0.26 ± 0.05	0.15 ± 0.05

Values represent percentage of injected dose per gram of tissue (% I.D./g; arithmetic means ± standard deviation).

Table 2. Experimentally Determined $_{HPLC}$logP and Calculated $_{c}$logP Values of [^{18}F]FE@SUPPY and [^{18}F]FE@SUPPY:2

	$_{HPLC}$logP			$_{c}$logP
	pH 2	*pH 7.4*	*pH 10*	
[^{18}F]FE@SUPPY	3.99	4.04	4.06	5.81
[^{18}F]FE@SUPPY:2	4.12	4.05	4.10	5.81

Precursor and Reference Standard

Organic chemistry of precursor and reference molecules were accomplished in straightforward procedures. Preparations and purifications were performed with good yields; unexpectedly the tosylation reactions for both precursors were time-consuming.

Automated Radiosynthesis – [^{18}F]FE@SUPPY and [^{18}F]FE@SUPPY:2

Automation of the radiosyntheses of [^{18}F]FE@SUPPY and [^{18}F]FE@SUPPY:2 was straight forward using the GE *TRACERlab* Fx_{FN} synthesizer with minor modifications (shortcuts). No problems were encountered when implementing the "manual" radiosynthesis [1, 2] on this synthesizer platform and, so far, not a single failed preparation was observed.

For the satisfying synthesis of [^{18}F]FE@SUPPY:2, a higher amount of precursor was needed (+50%) in comparison to [^{18}F]FE@SUPPY. Nevertheless, radiochemical yields were drastically lower for [^{18}F]FE@SUPPY:2 – 16.4 ± 9.7 % compared to 32.3 ± 12.4 % for [^{18}F]FE@SUPPY (values based on [^{18}F]fluoride, corrected for decay). Nucleophilic substitution of the tosyl-leaving group on a thiocarboxylic ester moiety therefore seems to be slower than on a carboxylic ester. Interestingly, the found specific radioactivities were dramatically higher for [^{18}F]FE@SUPPY:2.

Biodistribution Experiments

As presented in Table **1**, organs displaying highest uptake were the bowels, followed by liver. Initially, uptake in the kidneys was high, too, yet decreasing significantly after 15 minutes. Since our group has demonstrated recently that [^{18}F]fluoroethyl esters are primarily metabolized by carboxylic esterases, high kidney uptake could be explained by the renal excretion route of the major expected metabolite, [^{18}F]fluoroethanol [30, 31]. High liver uptake could be explained by metabolism taking place in the cytochrome P-450-rich hepatosomes.

Comparing maximum uptake values over time of [^{18}F]FE@SUPPY and [^{18}F]FE@SUPPY:2, we found good correlations in brain, muscle and testes; all other organs showed pronounced uptake variations. Comparing mean uptake values over time, only muscle and testes showed some degree of correlation. Fig. (**3**) shows a comparison of the six most expedient organs and tissues: liver, fat, colon, ileum/jejunum, femur and brain. Interestingly - in contrast to [^{18}F]FE@SUPPY - uptake of [^{18}F]FE@SUPPY:2 increased over time in fat and femur. Increasing uptake in fat could be explained by the relatively pronounced lipophilicity of [^{18}F]FE@SUPPY:2 (Table **2**), whereas usually bone uptake is attributed to defluorination. Initially high uptake in ileum/jejunum associated with increasing uptake in colon over time could be due to potential hepatobiliary clearance of [^{18}F]FE@SUPPY:2. This fact could not be observed for [^{18}F]FE@SUPPY. Calculating brain to blood ratios, we observed a dramatic increase of the ratio for [^{18}F]FE@SUPPY (2.73-18.33; see Fig. **4**), whereas [^{18}F]FE@SUPPY:2 remained constant over time. So far, we are left without an

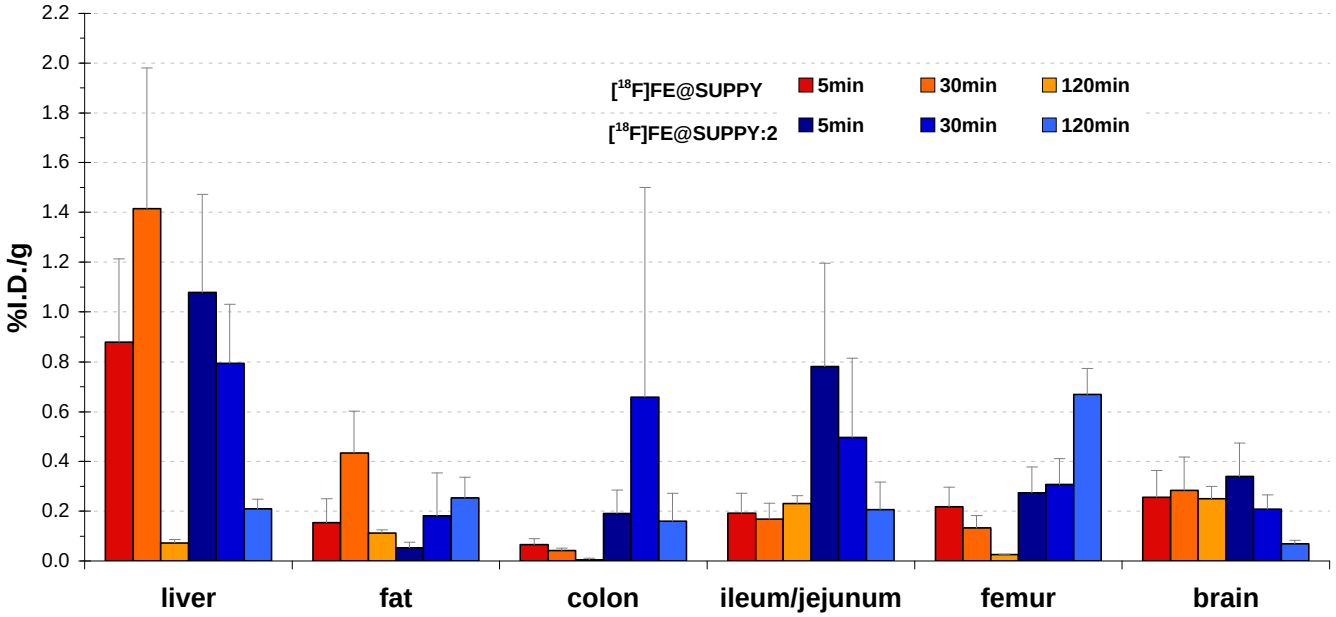


Fig. (3). A comparison of the uptake of [^{18}F]FE@SUPPY and [^{18}F]FE@SUPPY:2 in several organs. Values represent %I.D./g, n=4, error bars represent standard deviation.

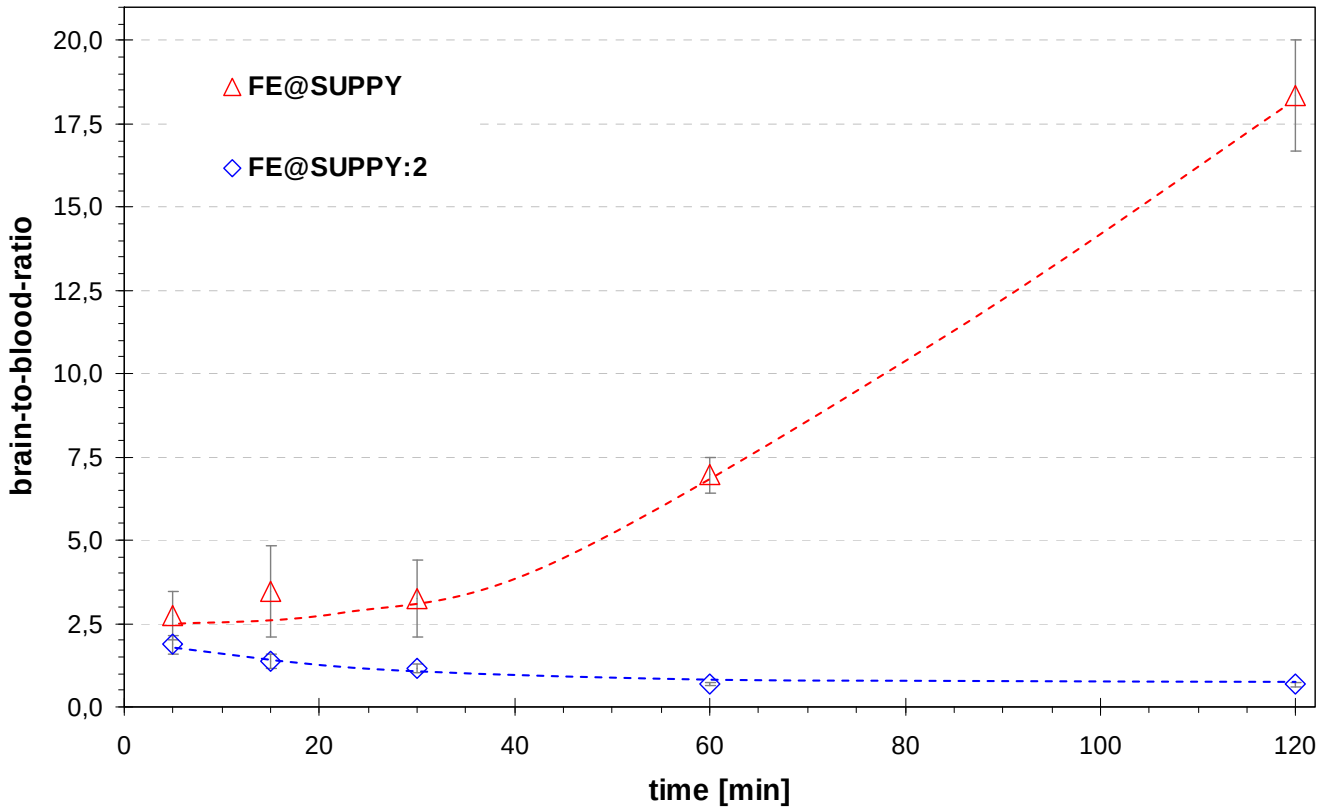


Fig. (4). Brain to blood ratios of [^{18}F]FE@SUPPY and [^{18}F]FE@SUPPY:2 after various time points. Values represent arithmetic means of individually calculated ratios, n=4, error bars represent standard deviation.

explanation for this phenomenon.

Lipophilicity

As a prerequisite for its successful application as a radiopharmaceutical, a molecule has to exhibit several properties: it should be widely available, it should accumulate in the target tissue in stable condition and within a reasonable time flow and it should bind to the desired structures (receptor sites, enzymes, transporters, proteins…) with high affinity, selectivity and specificity. Since logP is known to influence many of these pharmacokinetic and pharmacodynamic parameters, especially unspecific binding of radiopharmaceuticals and blood brain barrier permeability [32], we tried to pay specific deference to this measure although we are well aware of the fact, that logP is of limited value as a predictor for lipophilicity. Nevertheless, it is the most commonly used and prominent measure.

LogP (or more precisely $_{OW}$logP where OW stands for octanol/water) is defined as the decadal logarithm of the partition coefficient between equal volumes of 1-octanol and water. Normally, an aliquot of the solid compound is dissolved directly in a mixture of 1-octanol and water, the phases are separated and then the content is determined using e.g. chromatographic or spectroscopic methods. But in the case of radiopharmaceutical preparations, on the one hand, one has the advantage of using the radioactivity for very accurate measurement of the product concentration but, on the other hand, one rarely has access to solid compounds. Furthermore, the final preparation is always contaminated by small amounts of other radioactive species (e.g. by-products, educts…). Even these small amounts (1-3%) would significantly bias the outcome of the measurement of the $_{OW}$logP: if, for instance, the final product solution of a typical lipophilic compound displayed a radiochemical purity of 99% and the remaining 1% could be attributed to a contamination with hydrophilic [^{18}F]fluoride, the partition coefficient would never be higher than 99 to 1 and therefore the $_{OW}$logP measurement would always give a result lower than 2! Thus, optimized methods have been proposed to overcome this problem [33]. In our mind, the most elegant solution to get an accurate and simple measure of the lipophilicity is using the $_{HPLC}$logP instead of $_{OW}$logP. Since contaminations are separated within the HPLC run they do not interfere with the retention of the main compound. Additionally, the used HPLC method is very simple and inexpensive, it may be used both with radioactive and non-radioactive compounds and the pH of the aqueous phase may be adjusted easily to whatever value is desired.

As shown in Table **2**, logP values for both [^{18}F]FE@SUPPY-derivatives are comparatively high. It is known, that high lipophilicity significantly contributes to the amount of unspecific binding. However, $_{HPLC}$logP values measured for well established PET-tracers such as [^{11}C]DASB ($_{HPLC}$logP: 3.81), [^{11}C]carfentanil ($_{HPLC}$logP: 3.37) or [^{11}C]verapamil ($_{HPLC}$logP: 3.35) are also high and found their way into scientific routine. Hence, although be-

ing in the upper range, logP values should not be an obstacle for further expedient application of [^{18}F]FE@SUPPY-derivatives.

CONCLUSION

Aims of the present study were (1) the automatized preparation of both [^{18}F]FE@SUPPY-derivatives, (2) the biodistribution of [^{18}F]FE@SUPPY:2 in rats, (3) the characterisation of the lipophilicity and (4) the comparison of the findings of [^{18}F]FE@SUPPY and [^{18}F]FE@SUPPY:2. Results show that:

(1) [^{18}F]FE@SUPPY:2, an alternative to [^{18}F]FE@SUPPY, the first PET-ligand for the adenosine A_3 receptor, was prepared in a reliable and feasible manner. Automation yields for both molecules were sufficient for further preclinical and clinical applications.

(2) Biodistribution experiments evinced bowels and liver as organs with highest uptake, suggesting metabolic activity and hepatobiliary excretion. Intermediate uptake was found in kidney, lung and heart, all organs known to express A3AR.

(3) LogP values are in the upper range but should not pose any hindrance for successful application of [^{18}F]FE@SUPPY and [^{18}F]FE@SUPPY:2 in future.

(4) The uptake pattern of [^{18}F]FE@SUPPY:2 differed from [^{18}F]FE@SUPPY, especially brain to blood ratios are considerably higher for [^{18}F]FE@SUPPY.

Hence, from a radiopharmaceutical perspective, drastically better specific radioactivities would militate in favour of [^{18}F]FE@SUPPY:2; preclinical evaluations, so far, seem to point in favour of [^{18}F]FE@SUPPY. Taken together, our preliminary data do not yet permit the decision upon the selection of the optimum [^{18}F]FE@SUPPY-derivative. With [^{18}F]FE@SUPPY:2, we are able to provide a second potential tracer that could help to further characterize the still quite unexplored A3AR.

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