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Efficient automated one-step synthesis of 2-[^{18}F]fluoroethylcholine for clinical imaging: optimized reaction conditions and improved quality controls of different synthetic approaches

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Abstract

[^{18}F]-labelled choline analogues, such as 2-[^{18}F]fluoroethylcholine (^{18}FECH), have suggested to be a new class of choline derivatives highly useful for the imaging of prostate and brain tumours. In fact, tumour cells with enhanced proliferation rate usually exhibit an improved choline uptake due to the increased membrane phospholipids biosynthesis. The aim of this study was the development of a high yielding synthesis of ^{18}FECH. The possibility of shortening the synthesis time by reacting all the reagents in a convenient and rapid one-step reaction was specially considered.

Methods: ^{18}FECH was synthesized by reacting [^{18}F]fluoride with 1,2-bis(tosyloxy)ethane and *N*,*N*-dimethylaminoethanol. The synthesis was carried out using both a one- and a two-step reaction in order to compare the two procedures. The effects on the radiochemical yield and purity by using different [^{18}F]fluoride phase transfer catalysts, reagents amounts and purification methods were assessed. Quality controls on the final products were performed by means of radio-thin-layer chromatography, gas chromatography and high-performance liquid chromatography equipped with conductimetric, ultraviolet and radiometric detectors.

Results: In the optimized experimental conditions, ^{18}FECH was synthesized with a radiochemical yield of 43±3% and 48±1% (not corrected for decay) when the two-step or the one-step approach were used, respectively. The radiochemical purity was higher than 99% regardless of the different synthetic pathways or purification methods adopted. The main chemical impurity was due to *N*,*N*-dimethylmorpholinium. The identity of this impurity in ^{18}FECH preparations was not previously reported.

Conclusion: An improved two-step and an innovative one-step reaction for synthesizing ^{18}FECH in a high yield were reported. The adaptation of a multistep synthesis to a single step process, opens further possibilities for simpler and more reliable automations.

Keywords: 2-[^{18}F]fluoroethylcholine; ^{18}FECH; Prostate cancer; Brain cancer; One-step reaction

1. Introduction

Choline plays a central role in human physiology being employed as a pool of methyl groups in biosynthetic pathways and being involved in cell-signalling mechanism neurotransmission and in synthesis of membrane lipids. In fact, choline is employed as a precursor of phosphatidylcholine in the biosynthetic pathway to cell membrane generation and growth [1–3]. Tumour cells with enhanced proliferation rate frequently exhibit an increased phospholipid membrane synthesis associated with the overexpression of choline transporters and choline kinase. Accordingly, [^{11}C]-labeled choline was synthesized and utilized in the imaging of a variety of tumours by means of positron emission tomography (PET) [4,5]. Unfortunately, due to the short half-life of ^{11}C, [^{11}C]choline can be properly employed only in PET centers with an on-site cyclotron. Conversely, ^{18}F is a radionuclide with optimal decay characteristics both for PET applications and for a widespread utilization. For these reasons, [^{18}F]-labeled analogues such as [^{18}F]fluoromethyl-dimethyl-2-hydroxyethylammonium (^{18}FCH) and 2-[^{18}F]fluoroethylcholine

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(^{18}FECH) have been developed and tested as an alternative choline derivatives for PET imaging of prostate and brain tumours [6–8]. The sterical and structural features imparted by the introduction of a fluorine atom in the choline backbone have been thoroughly investigated both in vitro and in vivo [9–12]. In these studies, it was reported that two methyl groups on the quaternary amine are essential for maintaining the affinity of the molecule for the choline transport system and for the specificity of choline kinase. The remaining third methyl group can be replaced by a longer alkyl chain where an ^{18}F atom is linked. As a result, the syntheses of ^{18}FCH and ^{18}FECH were developed in two-step reactions. Both the procedures resulted rather complicate to be carried out in an hospital environment and required time consuming purifications by means of gas chromatography (GC) or high-performance liquid chromatography (HPLC) [11,12]. In recent years, the synthesis of ^{18}FECH has been the most investigated of the [^{18}F]-radiolabeled choline derivatives likely because its synthesis was supposed to allow for easier automation. In the original method [12], [^{18}F]fluoride was reacted with a 1,2-bis (tosyloxy)ethane (diOTsEt) solution to give [^{18}F]fluoroethyltosylate (^{18}FEtOTs). Subsequently, a large excess of pure *N*,*N*-dimethylaminoethanol (DMAE) was added directly to the mixture in order to obtain ^{18}FECH. The reaction mixture was purified by evaporation, HPLC and then solid-phase extraction (SPE) obtaining the product with a 27% radiochemical yield (RCY), not decay corrected. This approach was recently improved with a fully automated synthesis by Pascali et al., achieving a yield of 36% [13]. In other studies, different [^{18}F] fluoroethylating agents (^{18}FEtX) were obtained by nucleophilic substitution on various precursors and reacted with DMAE solutions [14,15]. Therein, at least one SPE purification after the nucleophilic substitution and an HPLC purification after the alkylation reaction were performed. With some minor changes in the described approach [12], Zuhayra et al. were able to synthesize ^{18}FECH with a 47% RCY (not decay corrected) [14]. However, the use of a noncommercial precursor and of toxic solvent such as 1,2-dichlorobenzene is a strong drawback for applying this method in a clinical setting. Recently, it was reported that a drastic increase in [^{18}F] fluoroethylating agents yield could be obtained by adding alkali iodide to the reaction mixture [15]. However, when ^{18}FECH synthesis was fully performed using that approach, only a 30% of RCY was obtained [16].

The aim of this study was to develop a reliable synthesis of ^{18}FECH by using a commercial synthesizer. Different synthetic approaches, reaction parameters, and purification methods were compared and optimized in order to achieve the highest radiochemical yield and purity. Aiming at a user-friendly and widely applicable procedure, the use of commercial reagents and one-pot reactions were preferred. With no regard for the synthetic procedure, the final ^{18}FECH solution was submitted to an in-depth set of quality controls for attesting the reliability of the synthesis and for evaluating a safe employment of the radiotracer for clinical trials.

2. Materials and methods

2.1. Reagents and instrumentation

diOTsEt, DMAE, ethanol and dry acetonitrile (ACN) were purchased from Sigma-Aldrich (Milan, Italy). Tetrabutylammonium bicarbonate (TBA) and Kryptofix 2.2.2/K_2CO_3 solutions were obtained by ABX (Radeberg, Germany). Fluoroethylcholine chloride (FECH) and N,N-dimethylmorpholinium (diMM) chloride (diMMCl), used as reference standards for the quality controls, were purchased from ABX and Labotest OHG (Dresden, Germany), respectively. All the reagents were used without further purification. Unless otherwise specified, the water used in any operation was distilled by using a MilliQ Simplicity 185 (Millipore, Milan, Italy). Sep-Pak light QMA (preconditioned CO_3^{2-} form), Sep-pak plus QMA, plus C-18 and CM cartridges were obtained by Waters (Milan, Italy). Cathivex-GS 0.22-μm filters were purchased from Millipore.

No-carrier-added [^{18}F]fluoride was produced by the ^{18}O(p,n)^{18}F nuclear reaction on a MINItrace cyclotron (GE Medical system, Uppsala Sweden) using a 9.6 MeV proton beam. Enriched (>98%) [^{18}O]water was purchased from SRICI (Shanghai, China). A $FX_{F\text{-}N}$ Tracer lab (GE Medical System, Uppsala, Sweden) was used for the synthesis of ^{18}FECH, without any modification from the original set up. Radioactivity measurements were performed in a calibrated ion chamber (Aktivimeter ISOMED 2000, MED Nuklear-Medizintechnik, Dresden, Germany). The radiochemical and chemical purity of the ^{18}FECH solutions were assessed by means of: (1) ion chromatography using an isocratic pump (Gilson, Milan, Italy) equipped with a Waters 432 conductimeter (Waters, Milan, Italy) in series with a β^+-flow count detector (Bioscan, Washington, DC) and with a 152 UV/VIS detector (Gilson, Milan, Italy); (2) thin-layer chromatography (TLC) using an AR 2000 Imaging Scanner (Bioscan, Washington, DC, USA) and silica gel plates 60 F254 (Merck, Milan, Italy). Residual solvents amounts and chemical purity were also measured by gas chromatography on a FOCUS GC system (Thermo Scientific, Waltham, MA, USA) equipped with a flame ionization detector (FID) and an AI/AS 3000 series autosampler. The absence of bacterial endotoxin in the final radiopharmaceutical preparations was checked with a chromogenic limulus amebocyte lisate test performed with an Endosafe-PTS instrument (Charles Rives Laboratories, Charleston, SC, USA).

2.2. Synthesis of ^{18}FECH

^{18}FECH was synthesized by two different approaches, as shown in Fig. 1. The first method is the well known two-step reaction [12]. The second method is an innovative approach which allows the two reactions to be carried out simultaneously.