

Synthesis and biological evaluation of [^{18}F]tetrafluoroborate: a PET imaging agent for thyroid disease and reporter gene imaging of the sodium/iodide symporter

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Abstract

Purpose The human sodium/iodide symporter (hNIS) is a well-established target in thyroid disease and reporter gene imaging using gamma emitters ^{123}I -iodide, ^{131}I -iodide and $^{99\text{m}}\text{Tc}$ -pertechnetate. However, no PET imaging agent is routinely available. The aim of this study was to prepare and evaluate ^{18}F -labelled tetrafluoroborate ([^{18}F]TFB) for PET imaging of hNIS.

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Methods [^{18}F]TFB was prepared by isotopic exchange of BF_4^- with [^{18}F]fluoride in hot hydrochloric acid and purified using an alumina column. Its identity, purity and stability in serum were determined by HPLC, thin-layer chromatography (TLC) and mass spectrometry. Its interaction with NIS was assessed in vitro using FRTL-5 rat thyroid cells, with and without stimulation by thyroid-stimulating hormone (TSH), in the presence and absence of perchlorate. Biodistribution and PET imaging studies were performed using BALB/c mice, with and without perchlorate inhibition.

Results [^{18}F]TFB was readily prepared with specific activity of 10 GBq/mg. It showed rapid accumulation in FRTL-5 cells that was stimulated by TSH and inhibited by perchlorate, and rapid specific accumulation in vivo in thyroid (SUV=72 after 1 h) and stomach that was inhibited 95% by perchlorate.

Conclusion [^{18}F]TFB is an easily prepared PET imaging agent for rodent NIS and should be evaluated for hNIS PET imaging in humans.

Keywords ^{18}F · PET · Tetrafluoroborate · Sodium/iodide symporter · Thyroid

Introduction

Historically, molecular imaging of the human sodium/iodide symporter (hNIS) [1, 2] with radioiodide has made a major contribution to the management of thyroid cancer and the development of nuclear medicine [3, 4]. The success of [^{131}I]iodide treatment for thyroid cancer and hyperthyroidism founded the field of therapeutic nuclear

medicine, and imaging with [^{131}I]iodide and [^{123}I]iodide has been fundamental to diagnosis of disease and monitoring treatment outcomes for over half a century. More recently, radioiodine imaging has found a role in dose planning in radionuclide therapy [5, 6]. In addition, recently hNIS has taken on a new research role as a reporter gene [7–9] in gene therapy and imaging of cell migration and differentiation. Reporter gene imaging with radioiodide SPECT is feasible because hNIS is normally expressed in only a few tissues (thyroid, gastric mucosa, salivary glands, kidneys and lactating breast) [10]. [$^{99\text{m}}\text{Tc}$]pertechnetate offers a more readily available and cheaper substitute for SPECT imaging where reduced radiation absorbed dose is important.

Despite the historical importance of hNIS imaging, current methods have limitations. SPECT has limited resolution and sensitivity for detecting low volume disease, especially if normal functioning thyroid remnants remain. With ^{131}I , and to a lesser extent with ^{123}I , “stunning” occurs—low activities administered for imaging may reduce the effectiveness of the therapy dose of ^{131}I [4]. The half-lives of both iodine radionuclides are longer than required for imaging, so absorbed radiation doses are unnecessarily high. Resolution and sensitivity of PET are significantly better than those of SPECT, resulting in better delineation of small regions of tracer uptake or lack of uptake. Better quantification of the absolute amount of tracer in small regions is also possible with PET. A PET alternative to SPECT as the primary hNIS imaging procedure could thus improve sensitivity of detection of metastases and cold nodules, tumour volume measurement and hNIS reporter gene imaging.

Current options for PET imaging of hNIS expression are very limited. The positron emitter [^{124}I]iodide [5, 6, 8, 11–16] has shown better sensitivity for detection of metastases than SPECT [13]. Volume determination, and hence accurate dosimetry, was feasible for lesions as small as 13 mm diameter [5, 16]. ^{124}I , however, suffers from several disadvantages that prevent routine diagnostic use. The low positron yield (23%) and long half-life mean that the radiation dose per unit positron yield from ^{124}I is approximately 18 times that from ^{18}F . This is compounded by high positron energy ($E_{\text{max}}=2.14$ MeV) and high energy gamma emission, both of which degrade image quality. Moreover, its availability is severely limited and its production relatively complex. [$^{94\text{m}}\text{Tc}$]pertechnetate [17] could be considered, but is not a plausible replacement because it is not widely available and its production is complex. The ideal radionuclide would be ^{18}F , as it is readily available, and has a half-life (110 min) compatible with typical imaging times and a low energy ($E_{\text{max}}=0.634$ MeV), high yield (97%) positron emission.

Many non-natural substrates for NIS are known, some of which are important medically as iodide uptake inhibitors (e.g. perchlorate) [2, 18–21]. [$^{99\text{m}}\text{Tc}$]pertechnetate is used for thyroid SPECT, and [^{188}Re]perrhenate [22, 23] and [^{211}At]astatide [24, 25] have been proposed for radionuclide therapy [22–24, 26, 27]. The key shared physico-chemical features are uninegative charge, similar ionic radius/volume and similar space-filling properties, i.e. roughly spherical (e.g. tetrahedral, non-dipolar) [2, 19]. Smaller anions such as fluoride, chloride and bromide do not compete as substrates [2]. Tetrafluoroborate (TFB) [19] is a fluorine-containing ion that interacts with NIS. It has not been used for PET imaging, but in the 1950s and early 1960s, before both the advent of clinical PET and the identification and characterisation of hNIS [2], Anbar et al. produced data suggesting to us that labelled TFB would have good potential for thyroid imaging [18, 19, 28]: TFB inhibits iodide uptake in thyroid [19, 29] *in vivo* and is specifically accumulated in thyroid [18], reaching a high thyroid to blood concentration ratio (at least 60:1 even at low specific activities) [19]. It is hydrolytically stable under physiological conditions [28] and is not significantly metabolised [19]. TFB has low toxicity (rat LD_{50} : 550 mg/kg when administered subcutaneously, according to data from NaBF_4 material safety data sheet). It is a constituent of $^{99\text{m}}\text{Tc}$ -sestamibi kits for myocardial imaging (78 $\mu\text{g}/\text{kit}$) and presents no hazard when intravenously administered. It has been radiolabelled with ^{18}F by isotopic exchange under acidic conditions, although published methods are not suitable for producing a radiotracer for clinical PET [28]. Despite this potential, [^{18}F]TFB has not been used for investigation of thyroid pathologies, or indeed for PET imaging in any context. Today’s nuclear medicine environment is better equipped, both to produce this tracer and to exploit its potential imaging applications. The relatively recent characterisation of cloned NIS [1, 20], and its use in gene therapy, have created new applications for NIS tracers. A simple and effective preparation of [^{18}F]TFB could permit quantitative, high-resolution imaging of NIS expression in this context without undue patient radiation burden.

Materials and methods

Radiochemistry

Initial investigations of isotopic exchange labelling of sodium tetrafluoroborate (Sigma-Aldrich, Gillingham, UK, 99%) were performed at various concentrations and temperatures with [^{18}F]fluoride, obtained directly from proton-irradiated [^{18}O]water (97 atom%, Isochem Ltd., Hook, UK; 11 MeV protons from a CTI RDS 112 cyclotron, beam

current 30 μA , irradiation time 10–20 min) without use of ion exchange, Kryptofix or azeotropic distillation. The pH was modified using hydrochloric acid (Fisher Scientific, Loughborough, UK).

Radioanalytical methods

Labelling was monitored by thin-layer chromatography (TLC) using alumina TLC strips with fluorescent indicator (length 100 mm, Sigma-Aldrich, Gillingham, UK) with methanol as mobile phase. Strips were scanned using a Mini-scan radio TLC scanner with Flow-Count detector (LabLogic, Sheffield, UK). The presence of TFB at R_f 0.80–0.85 was demonstrated by scraping the alumina from the TLC plate, extracting it with water and analysing by anion LC-electrospray mass spectrometry (MS) (Agilent 6520 Accurate-Mass Q-TOF LC/MS, Agilent Technologies UK Ltd.). A reverse UV HPLC method was developed [Agilent 1200 series system with a quadruple pump, UV detector (254 nm) and radioactivity detector (LabLogic, Sheffield, UK)] utilising a SGE Exsil ODS (reverse phase) column, 5 μm , 250 \times 4.0 mm (SGE Europe Ltd., Bucks, UK) with a mobile phase of 1 mM tetrabutylammonium hydroxide and 1.3 mM potassium hydrogen phthalate in water (pH 7.5) flowing at 1.0 ml/min, sample volume 20 μl . The method was validated by its ability to separate a range of uninegative ions of similar size and lipophilicity (in order of elution: fluoride, chloride, bromide, nitrate, sulphate, iodide and tetrafluoroborate). The system was calibrated to measure TFB concentration using standard aqueous sodium tetrafluoroborate solutions. The elution time of TFB and the identity of the eluted anion were further confirmed by LC-MS analysis of fractions as above.

Synthesis of [^{18}F]TFB

From the above experiments the following automated (but not fully optimised) labelling protocol was developed using an Eckert and Ziegler Modular-Lab module (Imaging Equipment Ltd., Bristol, UK). [^{18}F]fluoride (12–18 GBq) was trapped by passage of the irradiated water (4 ml) through a QMA (Waters UK, Elstree, UK) cartridge conditioned with 1.0 M sodium hydrogen carbonate. Hydrochloric acid (1.5 M, 1.2 ml), prepared by dilution of 5 M hydrochloric acid with water for injections (BP, Hameln Pharmaceuticals, Gloucester, UK), was then passed through, eluting the [^{18}F]fluoride into the reactor which contained sodium tetrafluoroborate (1 mg in 0.1 ml 1.5 M HCl). The reaction mixture (1.3 ml) was heated to 120 $^\circ\text{C}$ for 10 min, cooled to 25 $^\circ\text{C}$ and passed through a silver ion-loaded cation exchange cartridge (OnGuard II AG, Dionex, Leeds, UK, conditioned with 10 ml water) to remove chloride and raise the pH, and through two alumina

columns (Waters SepPak Light Alumina N, conditioned with 10 ml water and 5 ml air, to remove unreacted [^{18}F]fluoride) and a sterile Millex-GS 0.22 μm filter unit (Millipore UK, Watford, UK), and washed through with a further 2 ml water for injections, into a nitrogen-filled sterile vial. The process, including irradiation, synthesis and purification, takes less than 1 h. Analysis and quality control were performed as described above before the product was used in biological studies.

Stability/shelf life

The product was subjected to HPLC and TLC analysis as described above after standing at room temperature for 5 h.

Serum stability

A total of 100 μl of the product was added to 1 ml human serum (male, type AB, Sigma-Aldrich, Gillingham, UK) and the mixture was incubated at 37 $^\circ\text{C}$. Aliquots (100 μl) were withdrawn hourly up to 4 h into microcentrifuge tubes and mixed with an equal volume of ethanol to precipitate serum proteins. After centrifugation the supernatant was analysed by HPLC and TLC as described above.

Cell culture

Media and reagents were obtained from PAA Laboratories (Yeovil, UK) and hormones from Sigma-Aldrich (Poole, UK). The Fisher rat thyroid cell line FRTL-5 was kindly donated by Prof. Jan Smit and Dr. Guido Hovens (Leiden University, The Netherlands) and grown in a humidified incubator at 37 $^\circ\text{C}$ with 5% CO_2 in Hams:F12 medium supplemented with 10% fetal bovine serum, penicillin/streptomycin, minimal essential medium amino acids and a hormone mixture containing insulin (10 $\mu\text{g}/\text{ml}$), hydrocortisone (3.6 ng/ml), glycyl-histidyl-lysine acetate (10 ng/ml), transferrin (5 $\mu\text{g}/\text{ml}$), somatostatin (10 ng/ml) and bovine thyroid-stimulating hormone (TSH) (1 mU/ml). For experiments without TSH stimulation, cells were maintained in the same medium with all hormones except TSH for 7 days prior to the experiment.

Tracer accumulation in FRTL-5 cells

Cells were seeded (150,000 per well, $n=3$ for each treatment in each of two independent experiments) into 24-well plates (one plate for each time point, three wells for each treatment) and incubated for 18–24 h. Prior to the assay, cells were washed twice in 1 ml phosphate-buffered saline (PBS), and Hanks' balanced salt solution (HBSS, 500 μl) was added to each well with or without 10 μM sodium perchlorate. The plate was incubated for 10 min

prior to the addition of 0.1 MBq [^{18}F]TFB (specific activity 10 GBq/mg) in a volume of 10 μl per well. At intervals from 1 to 120 min the radioactive medium was aspirated and cells were washed twice with 1 ml HBSS. Cell-bound radioactivity was extracted by addition of 500 μl 1 M NaOH for 10 min. Cell-associated activity was counted in a gamma counter (Wallac 1282-001 Compugamma CS). Accumulation was expressed as counts per minute (cpm) per μg cellular protein, which was measured using a Novagen bicinchoninic acid protein assay kit (Merck Chemicals Ltd., Nottingham, UK).

Tracer efflux from FRTL-5 cells

Cells were seeded (150,000 per well) and incubated in 24-well plates with triplicate wells for each time point as described above. After addition of 0.1 MBq [^{18}F]TFB to each well and incubation for 1 h, the radioactive medium was removed and cells were washed twice in 1 ml HBSS and 1 ml HBSS was added to each well. At intervals from 5 to 120 min the medium was collected and the activity remaining in the cells was extracted with 1 M NaOH (500 μl) for 10 min. Radioactivity was counted in HBSS using a gamma counter. Radionuclide efflux was expressed as the percentage of the total activity in cells immediately after washing in HBSS.

Biodistribution

Animal studies were carried out in accordance with UK Research Councils' and Medical Research Charities' guidelines on Responsibility in the Use of Animals in Bioscience Research, under a UK Home Office licence. Female BALB/c mice ($n=3$ per group, aged 9–12 weeks, 22.4 ± 1.76 g) were purchased from Harlan Laboratories, UK. One group received i.p. injections of 50 μl PBS and another received 50 μl sodium perchlorate (Sigma-Aldrich, Gillingham, UK, 100 mg/ml) in PBS. After 30 min, both groups received i.v. (tail vein) injections of approximately 3 MBq (50 μl) [^{18}F]TFB (specific activity 10 GBq/mg). Animals were killed at 60 min post-injection (p.i.) and tissues explanted, blotted dry, weighed (except thyroid, vide infra) and counted on a gamma counter (the carcass activity was measured with an ionisation chamber cross-calibrated with the gamma counter). Because of the small size and intimate attachment to trachea, normal thyroid glands were explanted along with a small piece of trachea and a standard thyroid tissue mass of 3.6 mg [30] used in all SUV calculations. A separate piece of thyroid-free trachea was taken and weighed to confirm that activity in trachea tissue did not significantly affect the thyroid SUV calculation. A third group ($n=4$, range: 20.6 ± 1.53 g), treated as above without sodium perchlorate, were killed at 30 min p.i. Uptake in each tissue was expressed as

SUV, calculated as activity (cpm) per gram of tissue divided by total recovered activity (cpm) per gram of whole body mass. Excreted radioactivity, radioactivity in the tail and mass of the tail were excluded from the total recovered activity.

Imaging

A BALB/c mouse (age and weight as above) and a $TR\beta^{PV/PV}$ transgenic mouse [31] derived from a colony bred at King's College London (male, 11 months old, 27.8 g) received i.v. (tail vein) injections of 5 MBq of [^{18}F]TFB (50 μl). With the mice under isoflurane anaesthesia in a Minerve imaging chamber, PET/CT scans were acquired 30 min p.i. using a NanoPET/CT scanner (Bioscan, Paris, France) with PET acquisition time 2,400 s, coincidence relation: 1-3; image reconstruction: ordered subset expectation maximization (OSEM) with single-slice rebinning (SSRB) 2-D line of response (LOR), energy window: 400–600 keV, filter: Ram-Lak cut-off 1, number of iterations/subsets: 8/6. The biodistribution of the tracer in the $TR\beta^{PV/PV}$ mouse was then determined ex vivo by dissection and organ counting after sacrificing at 3.5 h p.i.

Results

Synthesis, purification and characterisation of [^{18}F]TFB

Initial experiments on exchange labelling of sodium tetrafluoroborate with [^{18}F]fluoride showed that the reaction rate and efficiency increased with increasing concentration of TFB (Online Resource 1), increasing temperature and increasing acid concentration, reaching a labelling efficiency of 45% after 10 min at 75°C with a NaBF_4 concentration of 10 mg/ml and HCl concentration of 1 M. These initial experiments also yielded a TLC method able to quantitatively distinguish [^{18}F]TFB (Rf 0.80–0.85) from [^{18}F]fluoride (Rf 0) (Fig. 1). It was difficult to prove that the radioactive spot at Rf 0.80–0.85 represents [^{18}F]TFB using TLC alone since cold TFB has no chromophore. However, electrospray MS confirmed the presence of TFB in the Rf 0.80–0.85 fraction {87 and 86, 100% (M^-); and 197, 25% ($[\text{Na}(\text{BF}_4)_2]^-$)}. To support the identification of labelled TFB, an HPLC method was developed and validated by demonstrating reproducible separation of a range of inorganic monoanions. In this method, [^{18}F]TFB eluted between 6 and 7 min while [^{18}F]fluoride eluted at 3 min (Fig. 2). These times agreed with those determined by spiking the radiotracer with the cold standards. [^{18}F]fluoride was easily removed by passage through two disposable alumina cartridges, eluting with water or saline, as demonstrated using the TLC (Fig. 1) and HPLC (Fig. 2)

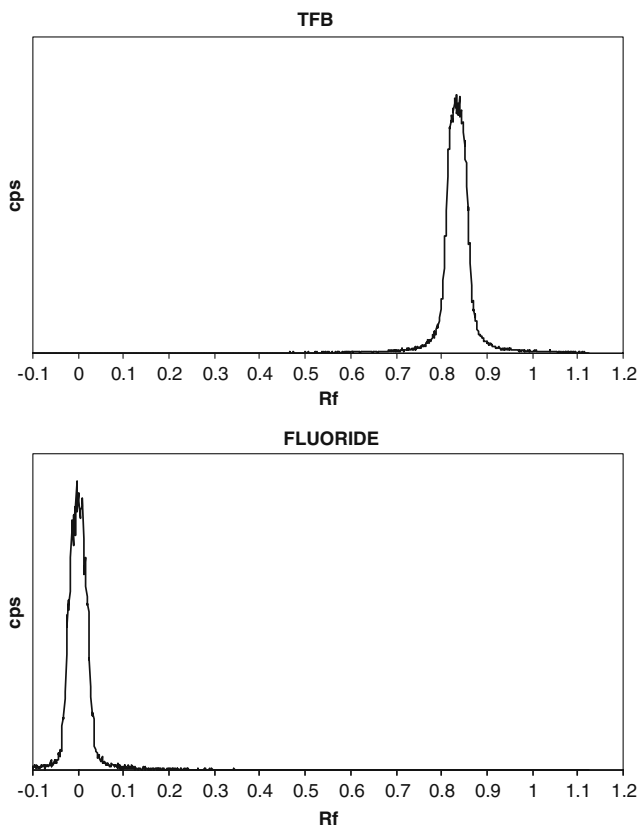


Fig. 1 TLC of purified [^{18}F]TFB (*upper*) and [^{18}F]fluoride (*lower*) using alumina stationary phase and methanol mobile phase, showing their clear separation and the purity of the final product

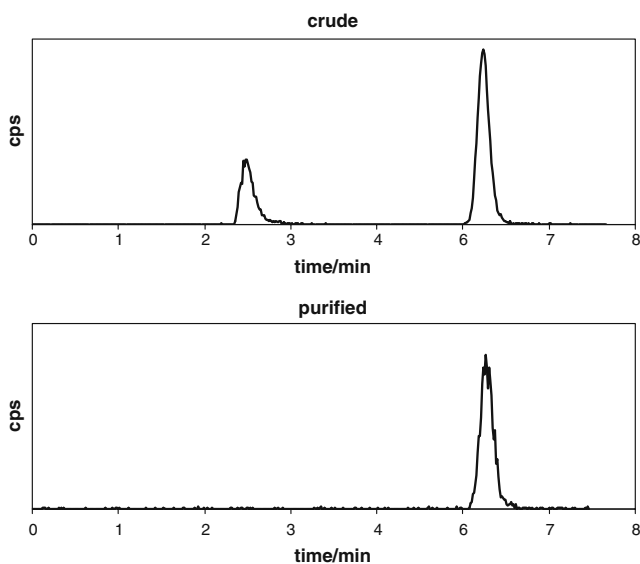


Fig. 2 Radio-HPLC of product of exchange ^{18}F labelling of tetrafluoroborate, before (*upper*) and after (*lower*) purification using alumina column. For HPLC conditions see “[Materials and methods](#)” section

methods described above. To produce samples suitable for biological evaluation, it was necessary to reduce the acidity and chloride concentration without substantial dilution, for which filtration through a Dionex AG (a cation exchanger loaded with silver ions) cartridge proved efficacious, raising the pH to between 6 and 7 without trapping [^{18}F]TFB.

Further development was conducted with use of an automated synthesis apparatus. Labelling efficiency was increased further by increasing the reaction temperature (120°C) and time (20 min) and HCl concentration (1.5 M). The specific activity of the product varied depending on the starting activity of ^{18}F . By starting with 12–18 GBq ^{18}F and 1 mg sodium tetrafluoroborate, a specific activity of 10 GBq/mg (1 GBq/ μmol) was routinely achieved, with an isolated radiochemical yield of approximately 10% uncorrected for decay in a volume of 2 ml. Radiochemical purity consistently exceeded 96%. The pH was between 6 and 7. The production was complete within 30 min from end of bombardment (EOB).

Shelf life and stability in serum

The speciation of radioactivity in the [^{18}F]TFB preparation, as determined by TLC and HPLC, was unchanged after standing at room temperature for 5 h. Similarly, incubation in human serum at 37°C for 4 h gave no change in the TLC or HPLC patterns.

In vitro accumulation in rat thyroid cells

[^{18}F]TFB showed rapid accumulation in NIS-expressing FRTL-5 cells, reaching a plateau between 5 and 20 min. The plateau count rate shown in Fig. 3 corresponds to an intracellular to extracellular concentration ratio of approximately 22:1. High accumulation was dependent on pretreatment with TSH [32]. If TSH was excluded from the medium for several days prior to the experiment, accumulation was reduced by >90% under otherwise identical conditions (Fig. 4). Accumulation by both TSH-stimulated and untreated cells was dramatically inhibited (approximately 90%) by sodium perchlorate (10 μM), a well-established specific inhibitor of iodide uptake by hNIS [33, 34] (Fig. 4). After pre-incubating cells in medium containing [^{18}F]TFB, replacement of the medium with fresh medium containing no [^{18}F]TFB led to rapid efflux of tracer (more than 90% within 20 min).

In vivo biodistribution and imaging

The biodistribution of [^{18}F]TFB (Fig. 5) at 60 min p.i. shows marked uptake in thyroid (mean SUV=71.9). Stomach, which also is well known to express NIS [10], also showed significant uptake (SUV=4.4) compared to

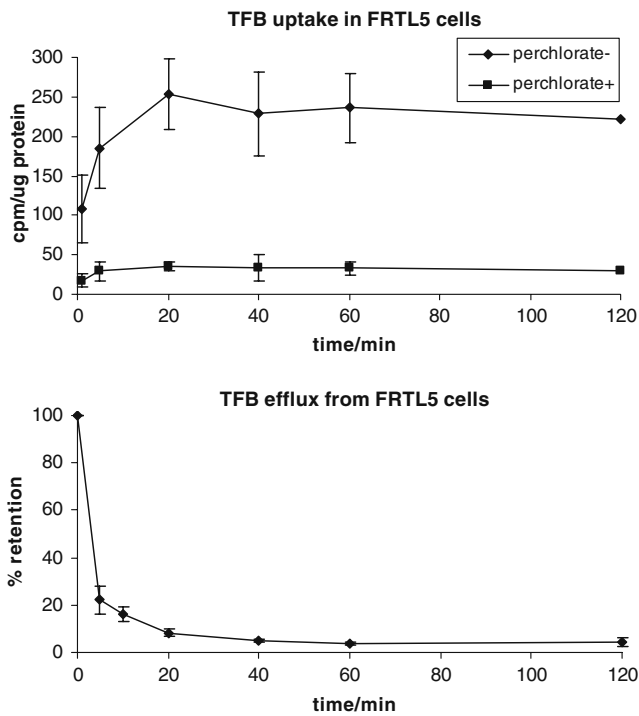


Fig. 3 Upper panel Accumulation of [¹⁸F]TFB in FRTL-5 rat thyroid cells, with and without blocking with perchlorate. Lower panel Efflux of radioactivity from FRTL-5 cells after treatment with [¹⁸F]TFB without perchlorate blocking, followed by replacement of media with [¹⁸F]TFB-free media. Error bars represent 1 SD

other tissues. At 30 min p.i. thyroid uptake was slightly higher (SUV=86; data not shown) than at 60 min. There was no significant uptake in visceral organs or bones and elimination was exclusively renal. To demonstrate that [¹⁸F]TFB uptake was mediated through NIS, a group of mice receiving sodium perchlorate 30 min prior to [¹⁸F]TFB administration showed >95% suppression of [¹⁸F]TFB accumulation in thyroid (SUV=3.2) and stomach (SUV=0.6) at 60 min. In the tumour-bearing *TRβ^{PV/PV}* mouse, with greatly and symmetrically enlarged lobes of the thyroid

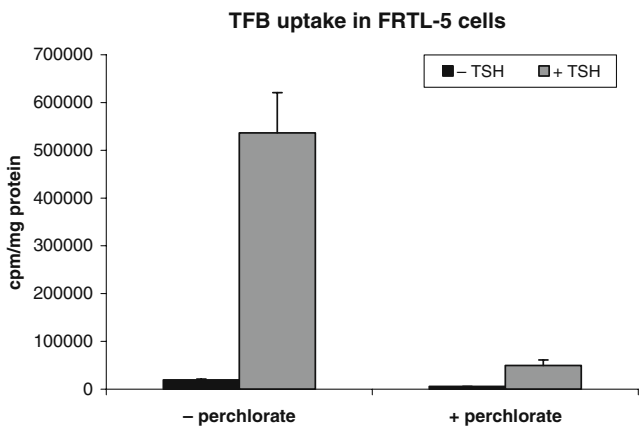


Fig. 4 Effect of perchlorate and TSH on plateau accumulation of [¹⁸F]TFB in FRTL-5 rat thyroid cells (n=3, error bars represent 1 SD)

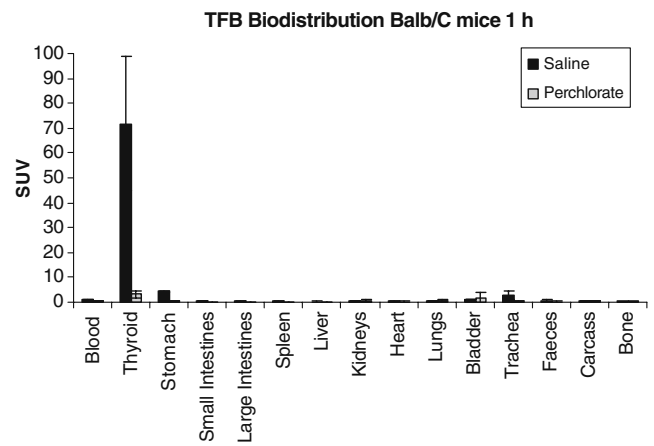


Fig. 5 Biodistribution of [¹⁸F]TFB in normal mice 1 h p.i.; with and without perchlorate blocking, determined by ex vivo counting of tissues; n=3 except bone (femur) where n=1. Relative uptake in organs is represented as SUV. Error bars represent 1 SD

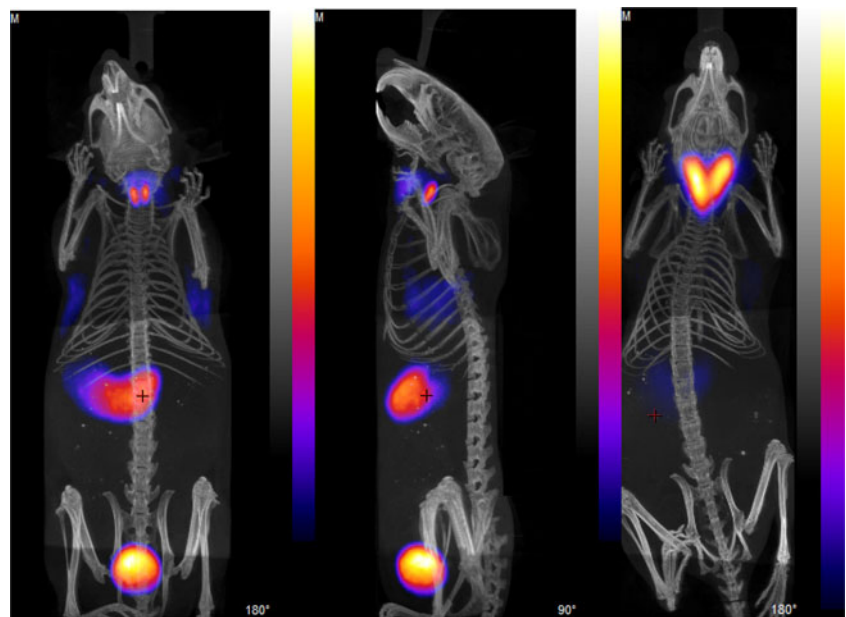
gland, the thyroid SUV (52 at 3.5 h p.i.) was reduced compared to normal thyroid tissue but, nevertheless, thyroid was by far the most radioactive organ.

As a preliminary assessment of the potential for PET imaging, a BALB/c mouse was administered i.v. 5 MBq of [¹⁸F]TFB and imaged 30 min later. This gave clear images of thyroid with high target to background ratio and both lobes clearly resolved (Fig. 6). In addition to thyroid and stomach, salivary glands were clearly visualised overlying the thyroid glands. There was also accumulation in lateral parts of the thorax which we attribute to mammary tissue. High radioactivity in the bladder confirmed renal excretion of the tracer. The images also showed that tissues not specifically counted in the biodistribution experiments did not have significant uptake, except salivary glands. A *TRβ^{PV/PV}* transgenic mouse with a greatly enlarged spontaneous follicular thyroid tumour was also imaged and despite lower thyroid SUV (52 at 3.5 h p.i.), the abnormal morphology and increased volume were readily apparent (Fig. 6). Accumulation in salivary glands, stomach, mammary tissue and bladder was less evident in this animal. This is consistent with clinical experience that tissues with low hNIS expression such as thyroid cancer metastases are often less evident when normally functioning thyroid or thyroid remnants are present.

Discussion

Early production of [¹⁸F]TFB was limited by contemporary methodology for producing ¹⁸F, which utilised the ¹⁶O(³H, n)¹⁸F or ¹⁸O(p, n)¹⁸F reactions on natural abundance Li₂CO₃ or ¹⁸O-enriched Li₂CO₃, using a Van der Graaf generator. An exchange reaction of ¹⁸F-fluoride with potassium tetrafluoroborate in acid (pH 0) at room

Fig. 6 PET/CT images. *Left and centre* Anterior (*left*) and lateral (*centre*, to distinguish thyroid from salivary gland uptake) view maximum intensity projections of normal mouse 30 min after injection of [^{18}F]TFB. *Right* PET/CT image of $TR\beta^{PV/PV}$ transgenic mouse with thyroid tumour, 30 min after injection with [^{18}F]TFB



temperature or 100°C gave [^{18}F]KBF₄, which was purified by recrystallisation [18, 28], a method which inherently leads to low specific activity. The route described here is similar in principle, but with modern methods for ^{18}F production and a new purification method, efficient production is possible to give higher specific activity [^{18}F]TFB adequate for imaging in humans while avoiding saturation of NIS *in vivo*. This required a compromise between the best % radiochemical yield and the best specific activity. A satisfactory compromise is described here although some features of the method remain open to significant further optimisation. The neutralisation step involves removal of HCl by displacing Ag⁺ from a cation exchange resin, with precipitation of AgCl on the column, a method previously applied to $^{99\text{m}}\text{Tc}$ generator eluates [35]. This raises the pH from 0 to between 6 and 7. The purification method exploits the affinity of fluoride ions for an alumina Sep-Pak cartridge. The method is easily adapted to GMP production. The processing was simple and complete within 30 min from EOB to readiness for quality control and administration. Quality control was performed using HPLC and TLC. The TLC method described is ideal for routine determination of radiochemical purity of the GMP product. The chromatographic behaviour of the radioactive species identified it as tetrafluoroborate. The final pH, volume and composition are compatible with clinical use. The radiopharmaceutical has a shelf life and serum stability at least commensurate with the physical half-life of ^{18}F , placing no limitations on clinical use.

The rapid uptake and efflux of [^{18}F]TFB in the rat thyroid cell line parallels the behaviour of [$^{99\text{m}}\text{Tc}$]pertechnetate (Online Resource 1), which is known to be taken up in cells expressing NIS [23, 36]. The plateau per cent

accumulation represents an intracellular to extracellular concentration ratio of 22:1. The rapid efflux on removal of TFB from the supernatant indicates that radioactivity is not irreversibly trapped. It also implies that the washing steps employed before measurement of uptake would have washed out a significant fraction of the cellular radioactivity, and hence the measured plateau intracellular to extracellular [^{18}F]TFB concentration ratio is likely to significantly underestimate the true ratio. It is clear from the TSH stimulation of uptake, and blocking by perchlorate, that uptake of [^{18}F]TFB is specific and mediated by NIS. TSH is known to upregulate NIS activity in thyroid cells [32, 37], and perchlorate is a known specific inhibitor of NIS [20, 23, 33]. At a specific activity of 10 GBq/mg the TFB concentration in the wells was about 0.2 μM . This is within the range of the measured IC₅₀ for inhibition of NIS-mediated iodide uptake [29], and hence uptake may increase at higher specific activity. Nevertheless, the data provide justification for *in vivo* biological evaluation of [^{18}F]TFB.

The biodistribution of [^{18}F]TFB in normal mice and in a $TR\beta^{PV/PV}$ mouse (a transgenic mouse with the “knock-in” PV mutation that spontaneously develops follicular thyroid cancer [31]) was investigated to evaluate its potential use as a tool to image thyroid disease. The SUV in normal thyroid may be subject to systematic error because “standard” weights were used in the calculations; values for standard weights of mouse thyroids ranging from 1.6 to 5 mg have been used by previous authors [30, 38, 39]. The most apparently rigorous measurements were reported by Rugh [30] and we used the value of 3.6 mg accordingly. Nevertheless, we can be confident that the high and specific accumulation of [^{18}F]TFB in normal thyroid (SUV > 70 at

1 h, higher at 30 min), stomach and salivary glands [10, 23, 40], and rapid renal clearance, parallel the behaviour of [^{99m}Tc]pertechnetate. The lack of significant uptake in bone is consistent with the expectation from the observed stability in serum that fluoride ion is not released in vivo. These pharmacokinetic attributes are very similar to those of [^{99m}Tc]pertechnetate (Online Resource 1) and are ideal for PET imaging, providing a good match with the half-life of ^{18}F and offering favourable dosimetry and imaging convenience in humans. The PET/CT images confirm this and show no uptake in bones or joints; hence, there is no in vivo release of [^{18}F]fluoride. Accumulation in thyroid tumour tissue of the $TR\beta^{PV/PV}$ mouse was also marked and indicates the potential value of thyroid tumour imaging with this tracer. The SUV was somewhat reduced compared to that of the normal thyroid tissue; however, this may be because the animal was killed at a later time point (3.5 h) than the normal mice.

The preliminary PET/CT images shown here represent the first biological imaging data produced with this model of scanner and demonstrate the excellent resolution and sensitivity of the instrument. Full evaluation of the technical capability of the instrument will be published in due course. The two lobes (each <2 mg) of the normal mouse thyroid are clearly resolved and the altered morphology of the thyroid tumour is clearly evident. The imaging quality supports the precept that a ^{18}F -labelled tracer for NIS has potential to improve radionuclide imaging of thyroid disease.

The present data are not able to show whether TFB ions are transported into cells by NIS, or whether they merely occupy a binding site. Previously published data show that a transmembrane potential is generated during iodide transport by NIS, consistent with symport of two sodium ions per iodide. The transmembrane potential generated by TFB (and of other competitors such as pertechnetate and perhenate) is much smaller [2], indicating either that TFB transport occurs with little or no coupling to sodium transport, or that TFB occupies a binding site on NIS but is transported very inefficiently.

Without further optimisation, the present synthesis generates a product with a modest specific activity of 10 GBq/mg (1 GBq/ μmol) when starting with 12–18 GBq ^{18}F . A likely scenario for production for human use is as follows. Starting with 10 GBq ^{18}F , assuming 50% incorporation of ^{18}F into TFB and a production time of 30 min, the specific activity at the time of injection would be in the order of 5 GBq/mg TFB or 0.5 GBq/ μmol . An administered activity of 400 MBq would thus contain 0.8 μmol TFB. This does not present a significant toxicity hazard (a commercial kit for the preparation of [^{99m}Tc]sestamibi contains about this amount). However, upon dilution into the vascular and interstitial compartment the

initial concentration in vivo to which NIS is exposed would be in the order of 0.1 μM . The IC_{50} of TFB for inhibition of iodide uptake by NIS is between 0.1 and 1 μM [29]. Thus, although the images and biodistribution show that the specific activity is clearly adequate for thyroid imaging purposes, optimisation to improve the specific activity perhaps tenfold, preferably without starting with excessive activity of ^{18}F , might well provide a superior target to background ratio.

Conclusion

We report a simple synthesis of [^{18}F]TFB, with sufficient specific activity for imaging the sodium/iodide symporter in vivo. Preliminary biological assessment shows behaviour very similar to that of ^{99m}Tc -pertechnetate and justifies evaluation of [^{18}F]TFB in humans. It may find applications in pre- and post-surgical evaluation of primary thyroid malignancy and detection of metastases, and for imaging expression of hNIS as a reporter gene, offering the possibility of improved image quality and quantification compared to ^{99m}Tc -pertechnetate at the expense of a higher effective radiation dose per injected activity.

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