Effects of administration route on uptake kinetics of ^{18}F-sodium fluoride Positron Emission Tomography in mice

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Method Article

Keywords: 18F-NaF; calcification; intraperitoneal injection; CT; PET imaging

Posted Date: April 22nd, 2021

DOI: https://doi.org/10.21203/rs.3.pex-1377/v1

Abstract

^{18}F-sodium fluoride (^{18}F-NaF) is a positron emission tomography (PET) radiotracer widely used in skeletal imaging and has also been proposed as a biomarker of active calcification in atherosclerosis. Like most PET radiotracers, ^{18}F-NaF is typically administered intravenously. However in small animal research intravenous administrations can be challenging, because partial paravenous injection is common due to the small calibre of the superficial tail veins and repeat administrations via tail veins can lead to tissue injury therefore limiting the total number of longitudinal scanning points. This protocol allows to look at the feasibility of using intra-peritoneal route of injection of ^{8}F-NaF to study calcification in mice by looking at the kinetic and uptake profiles of normal soft tissues and bones versus intra-vascular injections.

Introduction

The short lived radioactive tracer ^{18}F-sodium fluoride (^{18}F-NaF) is a positron-emitting radiotracer widely used in skeletal imaging since the 1960s due to its high affinity for bone hydroxyapatite matrix (*1*). ^{18}F-NaF has minimal protein binding allowing for fast soft-tissue clearance where nearly half of the injected radiotracer is taken up by the bones immediately after injection (*2*,*3*). The remaining radiotracer is rapidly cleared from plasma and excreted by the kidneys (*1*). ^{18}F-NaF binds to areas of active bone formation, representing a marker of bone synthesis and blood flow (*4*,*5*). Bone deposition of this radiotracer occurs via chemisorption, in which the OH^- ions in hydroxyapatite crystals are exchanged for $^{18}F^-$ ions, converting hydroxyapatite to fluorapatite at sites of new bone formation (*1*,*6*) reflecting active mineralising bone not only at active skeletal lesions and bone metastasis but also importantly on sites with vascular microcalcification (*7*,*8*).

The use of ^{18}F-NaF for bone metastasis imaging was fuelled by the development and installation of the first clinical Positron Emission Tomography/Computed Tomography (PET/CT) scanner, allowing high-resolution functional imaging with significantly greater sensitivity, specificity, and accuracy than conventional planar bone scintigraphy with bisphosphonates (*9*,*10*). Recently, this old PET radiotracer has been re-purposed as a biomarker of active calcification in coronary atherosclerosis (*11*,*12*), an unfavourable event in the history of atherosclerosis that predicts cardiovascular morbidity and mortality (*13*). These vascular calcifications are a hallmark of atherosclerotic burden, and several biological mechanisms, including the release of calcifying extracellular vesicles, alterations in local microenvironment and increased mineralization (*14*,*15*). Studies have shown that the ^{18}F ions of ^{18}F-NaF bind to the surface of nanocrystalline hydroxyapatite and the intensity of ^{18}F-NaF uptake is higher for the smaller sized crystals (*16*), therefore supporting its use as a marker of active cardiovascular microcalcification. With expanding number of animal models of vascular calcification (*17*–*22*)and the advent of high-sensitivity/high-resolution preclinical PET systems (*23*,*24*), studies with ^{18}F-NaF in mice are likely to increase in coming years. Furthermore, since the mid-1980s, the effort to understand mammalian biology and the need to study disease models have caused the mouse to become the animal

of choice. Given that most human genes have a related mouse gene, this rodent can be used as a platform for simulating many human diseases (*25*,*26*). Therefore, it is unsurprising that many mouse models of human cancer are also available, many of which present with bone metastasis that could be imaged with ^{18}F-NaF PET technique(*27*–*30*).

Like most PET radiotracers, ^{18}F-NaF is typically administrated via intravenous injection. Although routine in humans, intravenous injections in small animals, namely mice, can be more challenging, since they require proper animal-handling techniques and partial paravenous injection is common because of the small calibre of the superficial tail veins, thus leading to technical challenges on small-animal PET research (*31*). Furthermore,reproducible intravenous injection is not always possible for longitudinal studies or for sequential or multiple-tracer injection studies due to residual activity from previous injections (*32*,*33*).

Reagents

Radiochemistry

- 18F-NaF in saline (Sodium chloride, 0.9% w/v, Hameln Pharmaceuticals)

Animals

- Nineteen 10- to 17-week-old Swiss mice (SD-1 males, 35.8 ± 3.6 g)

Surgical vessel cannulations

- Isoflurane (2–2.5%, oxygen 0.5 L/min, nitrous oxide 0.5 L/min, Mecan)

- Polyethylene catheters (PE-10 tubbing, ID 0.28 mm, OD 0.61 mm, 35 cm length, Fisher Scientific UK)

- Heparin sodium (20 IU/mL, B. Braun Medical Inc.)

- Sodium chloride (0.9% w/v, Hameln Pharmaceuticals)

- 6-0 silkthread (Interfocus)

- Surgical glue (Vetbond, 3M)

Equipment

Radiochemistry

- Cyclotron and a FASTlab synthesiser (GE Healthcare)

Surgical vessel cannulations

- Anaesthetic machine (Vet Equip)

- Stereomicroscope (Carl Zeiss)

- PET/CTscanner (nanoPET/CT, Mediso, Hungary)

PET/CT data acquisition and reconstruction

- Scanner (nanoPET/CT, Mediso, Hungary)

Processing and analysis of PET single organ time-activity curves

- PMOD 4.0 software (PMOD Technologies, Switzerland)

Data Analysis

- Prism, version 8.4.3 (GraphPad Software Inc.)

Procedure

Radiochemistry

1. Prepare ^{18}F-NaF in saline (0.9% w/v)

2. Use a cyclotron and a FASTlab synthesiser (GE Healthcare), using commercially available cassettes.

Animals

1. Use 10- to 17-wk- old Swiss mice (SD-1 males, 35.8 ± 3.6 g).

2. Keep all animals housed and maintained under standardised 12 h light:12 h dark conditions with food and water available *ad libitum.*

3. Split animals into experimental groups depending on the administration route of the radiotracer (i.e. femoral artery, femoral vein or intra-peritoneal injection).

Surgical vessel cannulations

For the femoral vein and femoral artery experimental groups:

1. Induce and maintain anaesthesia using isoflurane (2-2.5%, Oxygen 0.5 L/min, Nitrous Oxide 0.5 L/min) during the whole surgical procedure.

2. Using a stereomicroscope, insert polyethylene catheters (PE10) filled with heparinized (20 IU/mL) saline into the left femoral artery or vein.

3. Secure fastened with ligatures (6-0 silk thread).

4. Hold catheters in place with surgical glue.

5. Transfer the animals to the PET/CT scanner (nanoPET/CT, Mediso, Hungary) for all subsequent imaging procedures.

6. Use the right femur and the right tibia to obtain bone measurements.

PET/CT data acquisition and reconstruction

1. Administer ^{18}F-NaF via intra-vascular or intra-peritoneal injection .

2. Immediately post-radiotracer administration, obtain a 60 min whole-body emission scan using a 1:5 coincidence mode (where each detector module is in coincidence with five opposing ones, and is equivalent to maximum field of view of 102×102×94.7 mm).

3. Acquire a CT scan (semi-circular full trajectory, maximum field of view, 360 projections, 35 kVp, 170 ms and 1:4 binning) for attenuation correction.

4. Reconstruct PET data into 6×30 sec, 3×60 sec, 2×120 sec, 10×300 sec frames using Mediso's iterative Tera-Tomo 3D reconstruction algorithm.

5. Use the following settings: 4 iterations, 6 subsets, full detector model, low regularisation, spike filter on, voxel size 0.4 mm and 400-600 keV energy window.

6. Correct PET data for randoms, scatter and attenuation.

Processing and analysis of PET single organ time-activity curves

1.Import reconstructed scans into PMOD 4.0 software (PMOD Technologies, Switzerland).

2. Manually draw volumes of interest (VOIs) around organs and bones of interest using the CT data.

3. For kidneys and vena cava use PET data.

4. Estimate radioactivity in the blood using the blood pool in the vena cava.

5. Generate time-active curves (TACs) for each organ.

6. Calculate standardised uptake values (SUV, g/ml) using the following equation:

SUV(g/mL)= Radioactivity concentration in volume of interest (MBq/mL)/ [injected dose (MBq)/Animal weight (g)].

7. Perform kinetic modelling using Patlak analysis (t^*=16minutes) to estimate the influx constant (K_i).

8. Use the blood pool VOI in the vena cava as reference tissue VOI for Patlak modelling.

9. Take SUV and SUVr averages from the last 3 frames between 45-60 minutes.

Troubleshooting

Time Taken

Anticipated Results

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Acknowledgements