Biological evaluation of [^{18}F]-nifedipine as a novel PET tracer for L-type calcium channel imaging

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Abstract. Due to interesting role of dihydropyridines in cardiovascular diseases and drug resistance studies and lack of a fluorine-18 labeled imaging agent for L-type calcium channel studies, this study was designed. [^{18}F]Dimethyl 2-(fluoromethyl)-6-methyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate **2** was prepared in no-carrier-added (n.c.a.) form from a starting brominated compound in one step at 80°C in Kryptofix2.2.2/[^{18}F]. Compound **2** was administered to normal rats via their tail veins for preliminary biodistribution studies and the ID/g% of the labeled compound was determined up to 3 h post injections. Coincidence images were obtained in rats 5 to 120 min. Radiofluorination on bromo precursor gave a fluorinated compound in 95% radiochemical purity and a 8% yield shown by RTLC and HPLC. Biodistribution studies showed that the tracer is accumulated in the heart in the first few minutes, followed by metabolism resulting in very soluble ^{18}F-containing metabolites eliminated through the urinary tract. In coincidence images, the target organ was shown to be the heart. Lung had high accumulation possibly due to the presence of Ca^{2+} channels and/or hydrolyzing enzymes showing a significant myocardial uptake at 120 min. The data demonstrates a significant agreement with the reported L-type calcium channels throughout the animal body. To our knowledge, this is the first example of ^{18}F-DHPs in the literature.

Key words: dihydropyridines • flourine-18 • PET • biodistribution

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Introduction

The 1,4-dihydropyridine nucleus serves as the scaffold for important cardiovascular drugs-calcium antagonists-including nifedipine, nitrendipine, amlodipine, and nisoldipine, which exert their antihypertensive and antianginal actions through actions at voltage-gated calcium channels of the CaV1 (L-type) class [12]. These compounds have been shown to be important in some pathological states, including seizures and central ischemic disorders [4, 7].

Imaging of calcium channels can be an interesting area of research in molecular imaging. Despite these interesting properties, providing great opportunities for design and radiosynthesis of radiolabeled DHP compounds, there are rare examples of PET radioisotope labeled DHPs in the literature, the few examples contain the preparation of a ^{11}C-labeled compound which was further evaluated in the animals [1, 13, 14].

Fluorine-18 is a suitable positron emitter for labeling various receptor ligands (positron energy = 0.64 MeV, tissue range = 2.4 mm, half-life = 109.7 min, steric similarity to hydrogen atom). This nuclide is the most widely used PET radionuclide in the nuclear medicine, and various lead compounds for molecular imaging are prepared in ^{18}F-labeled form for biological studies. In continuation of our previous works on the production

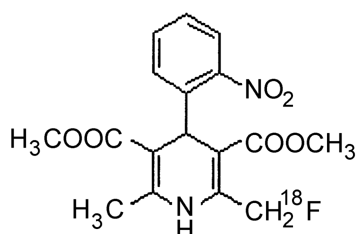

Fig. 1. Structural formula of ^{18}F-nifedipine analog.

and biological evaluation of fluorine-18 labeled compounds such as chelostryl esters [5] and benzodiazepine binding ligands [6], we decided to prepare a potent DHP ligand using a positron emitter (fluorine-18) to obtain a [^{18}F]-labeled nifedipine tracer. To our knowledge, there are no reports of ^{18}F-dihydropyridine in the literature. Recently, we have performed successful synthesis and quality control of [^{18}F]dimethyl 2-(fluoromethyl)-6-methyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate, **2** [8] (Fig. 1). Hereby, we report preliminary biological evaluation and imaging of compound **2** in the normal rats.

Experimental

Materials

Thin-layer chromatography (TLC) of non-radioactive products was run on silica gel polymer-backed (F 1500/LS 254, 20 × 20 cm, TLC Ready Foils Schleicher & Schuell®) or glass plates (25 × 35 cm, E-Merck). Acetonitrile used for labeling experiments was of 'Sure-Seal™' grade (Aldrich). Radio-chromatography was performed by polymer-backed silica gel paper thin layer sheets using a thin-layer chromatography scanner, Bioscan AR2000, Paris, France. Analytical HPLC to determine the specific activity was performed by a Shimadzu™ LC-10AT, armed with two detector systems, a flow scintillation analyzer (Packard™-150 TR) and a UV-visible (Shimadzu) using C18 Partisphere 100, 5 µm 250 × 4.6 mm (Whatman), Inchrom™. Calculations were based on the 511 keV peak for ^{18}F. All values were expressed as mean ± standard deviation (Mean ± SD) and the data were compared using the Student's t-test. Statistical significance was defined as $P < 0.05$. Animal studies were performed in accordance with the United Kingdom Biological Council's Guidelines on the Use of Living Animals in Scientific Investigations, 2nd ed. For each time interval 3 rats were used. Images were taken in coincidence mode of a Dual-Head SPECT system (SMV, France, Sopha DST-XL).

Methods

Production of reactive ^{18}F-fluoride

^{18}F-Fluoride was produced via the ^{18}O(p,n)^{18}F nuclear reaction by bombardment of an isotopically enriched ^{18}O-H_2O target with an 18 MeV proton beam at the IBA Cyclone 30 cyclotron in a cylindrical gold plated silver target body. At EOB, aqueous solution of [^{18}F]-fluoride in ^{18}O-enriched water was captured on a cromafix PS-HCO_3 (Macherey-Nagel, Germany) Sep-Pak cartridge and then washed with deionized water (3 ml). [^{18}F]Fluoride on the cartridge was eluted using 500 µl of K_2CO_3 (0.166 M) solution. The eluted mixture was transferred to a conical vial containing 1 ml dry acetonitrile as well as Kryptofix (25 mg, 66.4 µmol) and then dried under a gentle stream of nitrogen gas at 85°C. The azeotropic drying step was repeated at least two times with 800 µl portions of acetonitrile.

Preparation of [^{18}F]-dimethyl 2-(fluoromethyl)-6-methyl-4-(2-nitrophenyl)-1,4-dihydropyridine-3,5-dicarboxylate (**2**)

Compound **2** was prepared from a brominated analog, according to the reported procedure [8]. The prepared mixture was evaporated and a solution of bromo precursor (6 mg, 0.01 mmol) in anhydrous acetonitrile (0.25 mL) was added to the dried residue and heated to 80°C for 10 min. The mixture was cooled in an ice bath and rapidly drained into a syringe containing water (5 mL) and passed through a C_{18} Sep-Pak column. The column was washed with acetonitrile (1 mL) and the elute solution was passed through a short silica column. The active solution was checked for radiochemical purity by developing one drop of the latter solution over a polymer-backed silica gel layer in chloroform as the mobile phase. HPLC and TLC showed a purity higher than 95% in the form of [^{18}F]-compound.

Stability of **2** in the final product

Stability tests were based on previous studies performed for radiolabeled compounds. A sample of **2** (185 MBq) was kept at room temperature for 2 h while being checked by RTLC every half an hour. A sample (5 µl) was taken from the shaken mixture and the ratio of free radiofluoride to the labeled compound was checked by RTLC using ethyl acetate:petroleum ether (40:60) as eluent.

Serum stability studies

To 36.1 MBq of **2** 500 µl of freshly prepared human serum was added and the resulting mixture was incubated at 37°C for 2 h, aliquots (5 µl) were analyzed by radio-TLC after 0, 0.25, 0.5, 1 and 2 h of incubation to determine the stability of the labeled compound.

Biodistribution of **2** in normal rats

To determine its biodistribution, compound **2** was administered to the normal rats. A volume (25–50 µl) of final **2** solution containing ~ 0.74 MBq radioactivity was injected intravenously to rats through their tail vein. The animals were sacrificed at exact time intervals (30, 60 and 120 min), and the specific activity of different organs was calculated as percentage of injected dose per gram using an HPGe detector.

Imaging of compound **2** in normal rats

0.1 ml volumes of the final [18F]-compound solution containing 1.85 MBq activity were injected into the dorsal tail vein of healthy rats. The total amount of radioactive material injected into each rat was measured by counting the 1-ml syringe before and after injection in an activity meter with fixed geometry. The animals were relaxed by halothane and fixed in a relaxed suitable probe.

The animals were placed between the camera heads and images were taken 5–10, 50–55, 120–125 and 180–185 min after administration of the radioligand in coincidence mode. In order to keep the animals steady during the acquisition, small amounts of anesthetic ether was applied using diethyl ether cotton pieces.

To reach a suitable total count for image processing regarding the injected dose, 5–8 min were needed to complete the acquisition. Images were obtained using a coincidence software offered by SMV machine in tomography static mode. The final single mid-mouse coronal slice scans were chosen for presentation in this work. The useful field of view (UFOV) was 540 × 400 mm. The spatial resolution in the coincidence mode was 10 mm FWHM at the CFOV, and the sensitivity was 20 Kcps/37 kBq/cc. Sixty four projections were acquired for 30 s per view with a 64 × 64 matrix. Each rat was studied for 3 h.

Results

Chemistry

The precursor for most of 18F-labeled compounds is sulfonate ester which is mostly preferred in SN_2 reactions, however in our recent experiments we were not able to obtain the fluorinated compound by sulfonate esters and by enhancing the reaction conditions the precursor went through degradation. Thus brominated precursor was used and the reaction conditions were optimized based on this compound. The best condition was 80°C in the presence of K_{222}/F cryptate compound.

Biodistribution studies

The biodistribution of the tracer through animal body is demonstrated in Fig. 2. The compound is rapidly trapped in the liver in the first 30 min and then it is metabolized and observed in blood at 1 h. These metabolites are more water soluble (possibly fluoride anion) which are mostly excreted from the urinary tract after 2 h.

Due to GI excretion of the liver metabolites, the activity of stomach after 2 h is enhanced. The nature of these metabolites is not investigated, but it is believed that fluoride anion is an important metabolite since the sternum and bone uptake is enhanced after 2 h. Thus, defluorination is an important route of drug biodegradation in the liver.

At the first time course (0.5 h) which most of the labeled compound is still intact, heart uptake is observed, this can be in agreement with the general

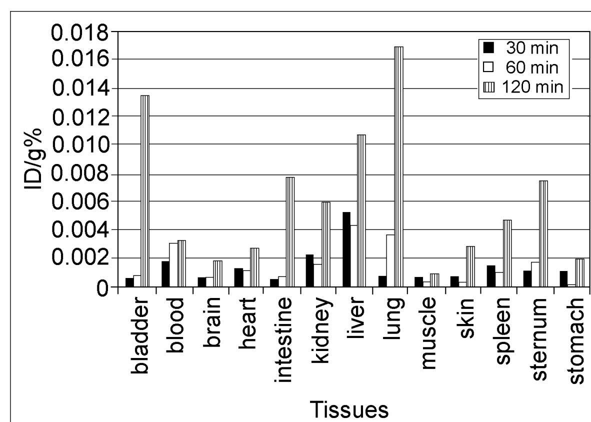

Fig. 2. ID/g% of 18F-nifedipine in normal rats 0.5, 1 and 2 h post injection.

distribution of calcium channels among mammalian tissues. Myocardial uptake, however is decreased in the next time intervals showing wash out of the tracer and/or biodegradation.

An interesting uptake is observed in lungs and this can be due to the presence of channels which are shown to be strongly expressed in human lung tissue [2, 3]. However, the accumulation in the lungs is much more higher to be considered just as a result of the Ca^{2+} channel distribution, the other possibility can be the presence of high amounts of hydrolase and esterase enzymes [9] in this tissue resulting in high production of more polar/ionic carboxylates produced from methyl ester hydrolysis in the tracer structure.

Brain uptake is also observed in the first time intervals and almost remains intact due to an unknown trapping mechanism which is in agreement with the distribution of the L-type channel in rat cerebellum [10]. There are however many reports regarding the high activity of dehydrogenase enzymes in the brain tissue that yield in pyridines from dihydropyridines. Pyridine ring can be then protonised to pyridinium cation which leads to the accumulation of the tracer in the brain tissue [11].

Imaging of [18F]-nifedipine in normal rats

Figure 3 demonstrates the coincidence images of the tracer among animal model body. Five minutes post injection, most of the tracer is accumulated in the myocardial tissue, as well as traces of the activity in the liver and GI tract. After 1 h post injection, there is still considerable heart uptake, but most of the activity is accumulated in the liver and GI tract. At hour 2, however the activity appears mostly in the kidneys and slightly in the vertebrae as well as skull, this can be due to the release of fluoride-18 anion which can be absorbed in bones as well as showing up in blood after possible liver metabolism.

Finally, 3 h after injection most of the activity is accumulated in the vertebrae and skull and the remainder is excreted in urine. The best imaging time for myocardial tissue would be few minutes after injection (1–5 min). This is a very interesting result since short scanning time after administration of the tracer is an important clinical characteristic of a PET tracer not only reducing

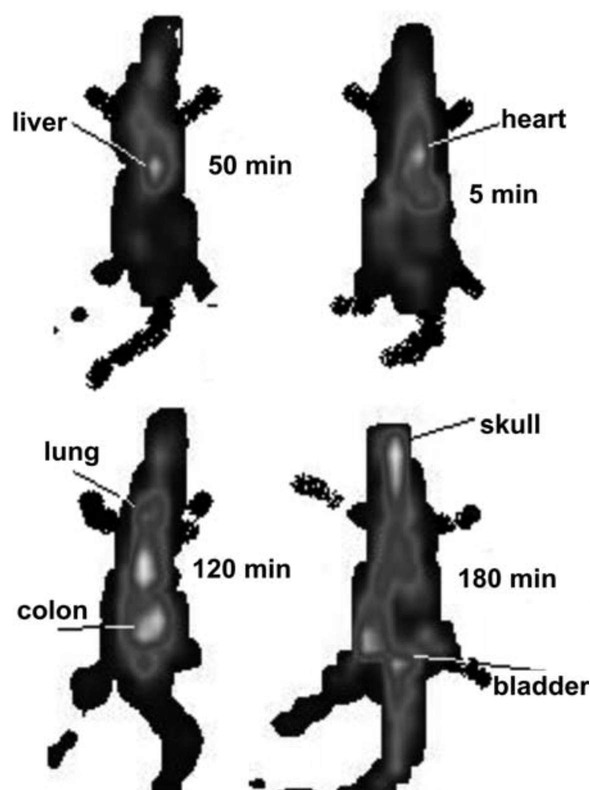

Fig. 3. Coincidence scans of [^{18}F]-nifedipine (20 μCi), 5–180 min post injection.

the injected dose, but also fast release of patient for possible future PET scan centers.

Discussion

In this report, we used the approved conditions for the recently prepared nifedipine radioligand. The radiolabeled complex was stable in mice serum for at least 2 h and no significant amount of free ^{18}F was observed. Target organ was shown to be the myocardial tissues. The best time interval *in vivo* was shown to be up to 5 min post injection. After 3 h, the radioactivity is cleared from blood circle and most of the tracer accumulates in bones. This is in agreement with reported ^{18}F-labeled tracer going through liver metabolism. [^{18}F]-nifedipine is a suitable probe for biodistribution study of L-type calcium channels with importance in many diseases. Since the tracer can rapidly accumulate in myocardium, further studies in higher mammals can be interesting.

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