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In vitro metabolism studies of ^{18}F-labeled 1-phenylpiperazine using mouse liver S9 fraction

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Abstract

The in vitro metabolism of 1-(4-[^{18}F]fluoromethylbenzyl)-4-phenylpiperazine ([^{18}F]**1**) and 1-(4-[^{18}F]fluorobenzyl)-4-phenylpiperazine ([^{18}F]**2**) was investigated using mouse liver S9 fraction. Results were compared to those of in vivo metabolism using mouse blood and bone and to in vitro metabolism using mouse liver microsomes. Defluorination was the main metabolic pathway for [^{18}F]**1** in vitro and in vivo. Based on TLC, HPLC and LC-MS data, [^{18}F]fluoride ion and less polar radioactive metabolites derived from aromatic ring oxidation were detected in vitro, and the latter metabolites were rapidly converted into the former with time, whereas only the [^{18}F]fluoride ion was detected in vivo. Similarly, the in vitro metabolism of [^{18}F]**2** using either S9 fraction or microsomes showed the same pattern as the in vivo method using blood; however, the radioactive metabolites derived from aromatic ring oxidation were not detected in vivo. These results demonstrate that liver S9 fraction can be widely used to investigate the intermediate radioactive metabolites and to predict the in vivo metabolism of radiotracers.
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Keywords: Metabolism; In vitro method; Liver S9 fraction; Radiotracers; ^{18}F

1. Introduction

When a novel radiotracer is prepared for biological evaluation, the characterization of its metabolism is a prerequisite. Although drug metabolism can occur in lungs, skin, or kidneys, it is mainly performed in the liver. In the same vein, radiopharmaceuticals are metabolized mainly in the liver [1–3]. Most radiotracers are metabolized for detoxification purposes, but some radiotracers undergo metabolic activation, and thus act as prodrugs. Metabolism studies of radiotracers have been performed in vivo using animals, whereby radiotracers are injected into animals, and samples of blood and tissue are collected and analyzed for radioactive metabolites [4–6]. This requires relatively high doses of radiotracers and involves the costs of managing animal experiment systems. Our previous in vitro metabolism studies using mouse liver microsomes demonstrated that this method can be used to predict the in vivo metabolism of radiotracers [7]. Liver S9 fraction has been used to assess mutagenicity in the Ames test, in which cytochrome P-450 in the S9 fraction catalyzes the metabolic activation of nonmutagenic substances [8]. As a result, S9 fraction was used to investigate the metabolic conversion of various compounds [9–11], because liver S9 fraction is not only easily obtained during the early stage of liver microsomal preparation [10–12], but also contains both microsomal and cytosolic fractions, which can provide more metabolic information than microsomes alone due to the presence of cytosolic enzymes. Therefore, metabolism by S9 fraction may be preferable to that by microsomes if the former shows a similar metabolism pattern to the latter.

In this study, we investigated a simple and efficient in vitro method for performing metabolism studies using model radiotracers, [^{18}F]**1** and [^{18}F]**2**, and mouse liver S9 fraction. Results obtained were compared to in vivo metabolism findings using mouse bone and blood and to in vitro metabolism findings using mouse liver microsomes.

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2. Materials and methods

Chemicals were purchased from Aldrich (Milwaukee, WI, USA), and NADPH and calcium phosphate tribasic were from Sigma (St. Louis, MO, USA). ^{1}H NMR spectra were performed on a Varian 500NB spectrometer. Chemical shifts (δ) were reported in parts per million downfield from tetramethylsilane as an internal reference. Electron impact (EI) and fast atom bombardment (FAB) mass spectra were obtained on a JMS-700 Mstation (JEOL Ltd) instrument. In vitro incubations were performed at 37°C using a digital block heater (Digi-Block Laboratory Devices, Holliston, MA, USA). LC-MS was performed on an 1100LC/MSD trap (Agilent Technologies, Palo Alto, CA, USA) using an HPLC column (Phenomenex Gemini C18, 5 μ, 4.6×250 mm). For purification and analysis of radioligand, HPLC was carried out on a Thermo Separation Products System (Fremont, CA, USA) equipped with a semipreparative column (Alltech Econosil silica gel, 10 μ, 10×250 mm) or an analytical column (YMC-Pack C18, 5 μ, 4.6×250 mm). The eluant was simultaneously monitored by a UV detector (254 nm) and a NaI(T1) radioactivity detector. Thin-layer chromatography (TLC) was performed on Merck F_{254} silica plates and analyzed using a Bioscan scanner (Washington, DC, USA). The radioactivity was measured in a dose calibrator (Biodex Medical Systems, Shirley, NY, USA) and tissue radioactivity in a Wallac automatic gamma counter (Perkin Elmer, Wellesly, MA, USA). All animal experiments were performed in compliance with the rules of the Samsung Medical Center Laboratory Animal Care based on the NIH guidelines.

2.1. Synthesis of nonradioactive standards

*2.1.1. Synthesis of 1-(4-fluoromethylbenzyl)-4-phenylpiperazine (**1**)*

4-Fluoromethylbenzyl methanesulfonate ester (40 mg, 0.18 mmol) and Et_3N (28 μl, 0.20 mmol) were added to 1-phenylpiperazine (35 μl, 0.23 mmol) in CH_3CN (4 ml). The reaction mixture was refluxed for 1 h. Flash column chromatography (3:1 hexane–ethyl acetate) afforded **1** as a colorless oil (32 mg, 62.5%). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 2.61 (t, J=5 Hz, 4H), 3.20 (t, J=5 Hz, 4H), 3.58 (d, J=1.5 Hz, 2H), 5.42 (t, J=47.5 Hz, 2H), 6.84 (m, 1H), 6.92 (dd, J=8.75, 0.5, 2H), 7.25 (m, 2H), 7.36 (m, 4H); MS m/z (FAB): 285 (M^++H); HRMS calcd for $C_{18}H_{22}FN_2$, 285.1767; found, 285.1754.

*2.1.2. Synthesis of 1-(4-fluorobenzyl)-4-phenylpiperazine (**2**)*

To 4-fluorobenzaldehyde (180 μl, 1.68 mmol) in methanol (5 ml) was added 1-phenylpiperazine (254 μl, 1.66 mmol). The reaction mixture was adjusted with acetic acid to pH 5 and then reacted with $NaBH_3CN$ (285 mg, 4.54 mmol) at 80°C overnight. After removal of the solvent in vacuo and basification with 10% NaOH, the resulting solution was extracted with ethyl acetate, washed with water and dried over Na_2SO_4. Flash column chromatography (4:1 hexane–ethyl acetate) gave **2** as a white solid (312 mg, 69%). ^{1}H NMR (500 MHz, $CDCl_3$) ^{1}H NMR (500 MHz, $CDCl_3$, δ): 2.59 (t, J=3.75 Hz, 4H), 3.19 (t, J=5 Hz, 4H), 3.53 (s, 2H), 6.84 (td, J=7.25, 0.5, 1H), 6.91 (dd, J=10.5, 0.5, 2H), 7.01 (m, 2H), 7.27 (m, 4H); MS m/z (FAB): 271 (M^++H); HRMS calcd for $C_{17}H_{20}FN_2$ 271.1611; found, 271.1612.

*2.1.3. Synthesis of 1-(4-fluoromethylbenzyl)-4-(4-hydroxyphenyl)piperazine (**3**)*

4-Fluoromethylbenzyl methanesulfonate ester (20 mg, 0.09 mmol) and Et_3N (15.3 μl, 0.11 mmol) were added to 1-(4-hydroxyphenyl)piperazine (32.7 mg, 0.18 mmol) in CH_3CN (3 ml). The reaction mixture was refluxed for 1 h. Flash column chromatography (3:1 hexane–ethyl acetate) gave **1** as a colorless oil (23 mg, 85.1%). ^{1}H NMR (500 MHz, $CDCl_3$, δ): 2.65 (t, J=4.5 Hz, 4H), 3.1 (t, J=5 Hz, 4H), 3.58 (m, 2H), 5.4 (d, J=47.5 Hz, 2H), 6.76 (m, 2H), 6.84 (dd, J=9.75, 2, 2H), 7.37 (m, 4H); MS m/z (EI): 300 (M^+); HRMS calcd for $C_{18}H_{21}FN_2O$ 300.1638; found, 300.1636.

*2.1.4. Synthesis of 1-(4-fluorobenzyl)-4-(4-hydroxyphenyl)piperazine (**4**)*

1-(4-Hydroxyphenyl)piperazine (398.7 mg, 2.24 mmol) was added to 4-fluorobenzaldehyde (200 μl, 1.86 mmol) in methanol (5 ml). The reaction mixture was adjusted with acetic acid to pH 5 and then reacted with $NaBH_3CN$ (288 mg, 4.58 mmol) at 80°C overnight. Flash column chromatography (3:1 hexane–ethyl acetate) afforded **2** as a white solid (398 mg, 74.6%). ^{1}H NMR (500 MHz, $CDCl_3$, δ) 2.62 (t, J=7 Hz, 4H), 3.1 (t, J=5 Hz, 4H), 3.55 (s, 2H), 6.76 (dd, J=7.5, 3, 2H), 6.85 (dd, J=9.3, 2, 2H), 7.03 (m, 2H), 7.33 (m, 2H); MS m/z (EI): 286 (M^+); HRMS calcd for $C_{17}H_{19}FN_2O$ 286.1481; found, 286.1471.

2.2. Preparation of radiotracers

*2.2.1. Preparation of 1-(4-[^{18}F]fluoromethylbenzyl)-4-phenylpiperazine ([^{18}F]**1**)*

4-[^{18}F]Fluoromethylbenzyl methanesulfonate ester was synthesized from 1,4-benzenedimethanol bismethanesulfonate ester (1 mg, 3.4 μmol) and nBu$_4$N[^{18}F]F in CH_3CN (200 μl) at 90°C for 10 min [7]. To the resulting mixture were added 1-phenylpiperazine (2.5 μl, 16.4 μmol), Et_3N (9 μl, 64.6 μmol) and CH_3CN (200 μl). The reaction mixture was then heated at 130°C for 15 min and then passed through a short plug filled with 1-cm silica gel and 1-cm Na_2SO_4 using a 9:1 mixture of CH_2Cl_2 and methanol. The eluate was concentrated under a stream of N_2 and purified by HPLC using a semipreparative column eluted with an 85:15 mixture of hexane and 95:5:0.1 CH_2Cl_2–2-propanol–NH_4OH at a flow rate of 4.0 ml/min. The desired product was eluted at 17–20 min. Effective specific activity was determined by comparing the UV peak area of the desired radioactive peak and those of different concentrations of

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