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Research Article

Radiolabeling of ginkgolide B with ^{18}F

Makiko Suehiro[1,*], Norman R. Simpson[2] and Ronald van Heertum[2]
[1] *Department of Chemistry, Columbia University, 3000 Broadway, New York, NY 10027, USA*
[2] *Department of Radiology, Columbia University, 630W 168th Street, New York, NY 10032, USA*

Summary

Ginkgolide B, a terpene trilactone constituent of *Gingko biloba* extracts and an antagonist for the platelet activating factor (PAF) receptor, was radiolabeled with the positron emitter ^{18}F for visualizing its *in vivo* behavior using positron emission tomography (PET). The 7-[^{18}F]fluoro analog of ginkgolide B (7-^{18}FGB) was synthesized via nucleophilic displacement of the triflate group at C7 with [^{18}F]fluoride. The reaction was complete in 5–10 min, affording 7-^{18}FGB in an average radiochemical yield of approximately 16% (decay corrected). The average specific activity at E.O.S. was approximately 40 GBq/μmol, and the radiochemical and chemical purity was greater than 95%. Copyright © 2004 John Wiley & Sons, Ltd.

Key Words: ^{18}F-labeled ginkgolide B; *Ginkgo biloba*; terpene trilactone; PET

Introduction

Extracts from the Ginkgo tree (*Ginkgo biloba* L.) such as EGb 761, which contain 24–25% flavone glycoside and 6% terpene lactones, i.e. ginkgolides and bilobalide, have been utilized as complimentary medications with therapeutic values for cerebral insufficiency including Alzheimer's disease (AD).[1–3] Their memory enhancing and anti-stress effects have also been reported.[4–6] However, the mechanisms underlying the bioactivities of the *Ginkgo biloba* components are not well understood although cumulative evidence indicates that they act as anti-inflammatories, free radical scavengers, anti-oxidants, platelet activating factor (PAF) antagonists and glucocorticoid synthesis regulators. Moreover, since the vast majority of the reports in the

*Correspondence to: M. Suehiro, Department of Chemistry, Columbia University, 3000 Broadway, New York, NY 10027, USA E-mail: ms630@columbia.edu

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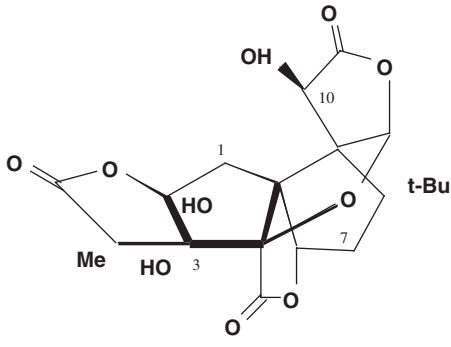

Figure 1. Structure of ginkgolide B

literature deal with extract mixtures, it is difficult to assess roles played by individual constituents of *Ginkgo biloba*.

Ginkgolides, active ingredients of *Ginkgo biloba*, are known as PAF receptor antagonists.[7] Considering the fact that PAF is one of the products generated in the cascades of inflammatory events, it is reasonable to assume that PAF receptor antagonism exerted by ginkgolides contributes to the neuroprotective effects of *Ginkgo biloba*. Recently, inhibitory effects of ginkgolides on glycine-gated ion channels have also been reported.[8,9] Non-steroidal anti-inflammatory drugs (NSAIDs) such as indomethacin and ibuprofen have also demonstrated to be effective to ameliorate neurodegenerative diseases including AD.[10,11] However, whether ginkgolides have other action sites than the PAF and glycine receptors, or how they initiate their actions on these receptors, or whether they share targets of action with NSAIDs is not known.

Mapping ginkgolides action sites by means of imaging techniques such as positron emission tomography (PET) with radiolabeled analogs of ginkgolides may provide unique and valuable information regarding the roles of ginkgolides in both physiological and pathological conditions. Based on this notion, we radiolabeled ginkgolide B (GB) with ^{18}F. We selected ginkgolide B (Figure 1) over other ginkgolides because of availability of a relatively large volume of literature focusing on CNS effects of ginkgolide B.

Results and discussion

The radiosynthesis of 7-[^{18}F]fluoro-ginkgolide B (7-^{18}FGB) was performed via nucleophilic displacement of the triflate group at C7 with [^{18}F]fluoride (Figure 2).

The radiolabeling procedure described in Experimental was studied first using non-radioactive KF or Bu_4NF, and the identification of the final product isolated by semi-preparative HPLC was confirmed as 7-fluoro-GB by ^{1}H NMR spectroscopy and mass spectrometry. As expected, Walden inversion

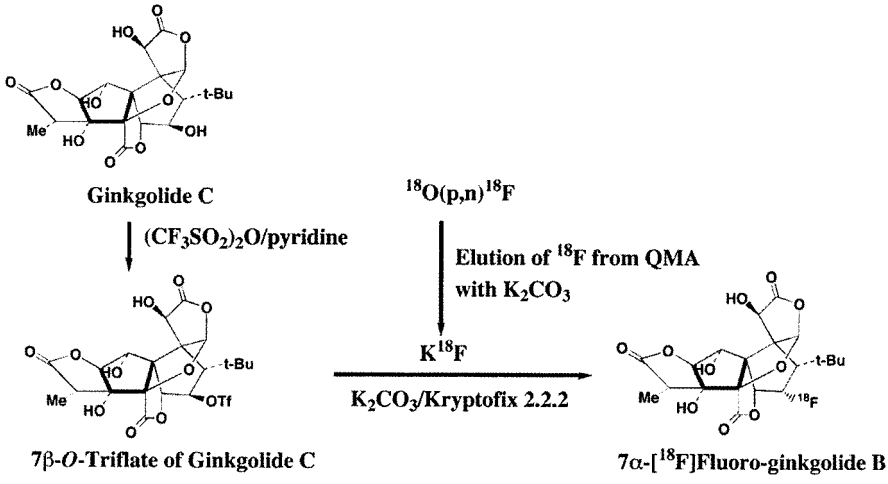

Figure 2. Radiosynthesis of 7α-[^{18}F]fluoro-ginkgolide B

accompanied the SN_2 displacement reaction resulting in formation of 7α-fluoro-ginkgolide B from 7β-*O*-triflate of GC, which was confirmed by ^{1}H NMR (400 MHz, DMSO-d_6) showing ^{1}H-^{19}F coupling constants at 8-H, 6-H and 7-H, 47.1, 10.8 and 49.0 (geminal) Hz, respectively. This indicates that the radiosynthesis product is 7α-[^{18}F]fluoro-ginkgolide B (Figure 2).

According to the HPLC analyses performed during the radiosynthesis, the reaction carried out in DMF at 120°C proceeded efficiently and was complete in 5–10 min with 18.4 ± 5.8% ($n = 5$) of the radioactivity incorporated into the GB structure (decay corrected). In contrast, the displacement reaction carried out in acetonitrile at 80°C for 30 min, which affords non-radioactive 7-fluoro-GB in good yields,[12] gave no significant amount of 7-^{18}FGB.

The purification of 7-^{18}FGB was performed on a C18 semi-preparative HPLC column (Figure 3). 7-^{18}FGB, which eluted at approximately 17 min (Peak c), was well separated from unreacted ^{18}F and radioactive by-products, which eluted near the void volume (Peak a and b). The small peak at the retention time of 2.3 min (Peak b) might be derived from 7-^{18}FGB with its lactone ring(s) open. The lactone ring opening in a basic medium is well documented[13,14] and can play a significant role in production of radiolabeled ginkgolides.[15] The decay-corrected radiochemical yield of 7-^{18}FGB was 15.7 ± 6.3% ($n = 5$) with an overall synthesis time, including HPLC purification and formulation, of 2.3 h. The specific activity at EOS was 40.7 ± 18.5 GBq/μmol ($n = 5$). The radiochemical and chemical purities were 95 ± 2% and 98 ± 2%, respectively. Raising the acetonitrile composition of the mobile phase from 20 to 30% resulted in lowering the radiochemical purity of the final product to approximately 70%. HPLC analyses suggested that this was caused by unreacted ^{18}F tailing into the 7-^{18}FGB elution, which peaked at

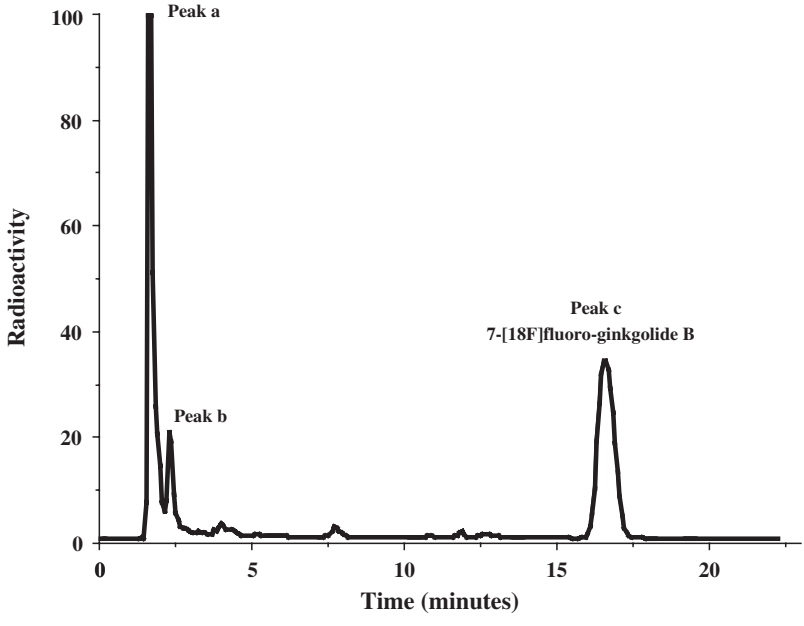

Figure 3. Separation of 7α-[^{18}F]fluoro-ginkgolide B by semi-preparative HPLC

7.4 min ($k' = 2.5$). The chemical purity was also reduced to 82% due to residual Kryptofix 2.2.2 from its broad peak at 5.5 min.

UV characteristics of ginkgolides such as the lack of absorption maxima above 200 nm and low molar absorptivities make quantitative HPLC analyses of these compounds with UV detection difficult: at λ max 219 (ethanol), their molar absorptivities range from 200 to 500 l/mol cm.[16] Usable solvents are also limited. Van Beek reported that the detection limit of ginkgolides at 219 nm was 30 ng when a reversed phase HPLC system with a 4.6 mm i.d. × 23 cm C18 column and a mobile phase of methanol/water (33/67) was used.[17] A similar system was evaluated in our laboratory. However, inconsistencies in retention times were observed especially when a reaction mixture containing KF-Kryptofix2.2.2 and 7-fluoro-GB was analyzed. Under the analytical HPLC conditions described here, the detection limit of 7-fluoro-GB was approximately 80 ng or 0.2 nmole. Other HPLC solvent systems such as water/acetonitrile (20/80), water/methanol/THF (75/5/15) and water/isopropanol (9/1) have also been used in the past for the separation of ginkgolides mixtures.[17,18]

As we previously reported,[12] fluorination at the 7α position of GB did not diminish its potency as an antagonist for the PAF receptor, suggesting that the 7α-[^{18}F]fluoro analog behaves similarly to GB. Thus, it is reasonable to assume that the dynamic biodistribution of 7-^{18}FGB visualized by PET represents *in vivo* behavior of GB itself, and, therefore, PET studies with this analog radiotracer of GB may provide insight into the mechanisms by which

ginkgolides exert their protective effects against various neurotoxic factors causing neurodegenerative diseases, aging, dementia, apoptosis and psychiatric disorders.

Experimental

The precursor for the radiosynthesis of 7-^{18}FGB, 7-trifluoromethanesulfonyloxy-ginkgolide C, 7β-*O*-triflate of GC, was synthesized from ginkgolide C, (7β)-7-hydroxy-ginkgolide B, according to Cazaux *et al.*[19] as we previously reported.[12] Non-radioactive 7-fluoro-GB was synthesized from the triflate precursor and fluoride anions derived from Bu_4NF as previously reported.[12] 1H-NMR spectra were recorded on a Bruker DMX using $(CH_3)_4Si$ as an internal standard. Mass spectra were obtained on a JEOL JMS-HX110/100A using a 3-nitrobenzyl alcohol matrix.

^{18}F was produced by the $^{18}O(p,n)$ reaction on 98% atom [^{18}O]H_2O using the CTI RDS 112 biomedical cyclotron installed at the Kreitchman PET Center of Columbia University. Anhydrous acetonitrile and anhydrous DMF used in the radiosynthesis were purchased from Pierce (silylation grade). HPLC grade acetonitrile, potassium carbonate (ReagentPlus grade) and Kryptofix 2.2.2 were obtained from Aldrich.

Analytical HPLC was performed with a Waters 515 pump, a Rheodyne 7125 injector, a Nova-Pak C18 column (4 µ, 15 cm × 4.6 mm i.d., Waters), a Dynamax UV-1 detector at 219 nm and a Bioscan Flow-Count equipped with a NaI gamma detector. HPLC purification of the radiolabeled product was carried out with a Waters 515 pump, a Rheodyne Lab Pro automated injector, a semi-preparative Nova-Pak C18 column (6 µ, 30 cm × 7.8 mm i.d.), a Spectroflow 757 UV detector at 219 nm and a Bioscan PIN diode gamma detector (HOTCELL system). Chromatography data acquisitions and peak integration calculations were carried out with the Millenium software (Waters).

A Capintec CRC 35R dose calibrator was used for radioactivity measurements.

Radiosynthesis of 7α-[^{18}F]fluoro-ginkgolide B

[^{18}F]Fluoride was separated from [^{18}O]H_2O using a QMA anion exchange cartridge (Waters) and eluted into a glass Reacti-Vial (Pierce) with an acetonitrile-water solution containing Kryptofix 2.2.2 (4 mg) and K_2CO_3 (4 mg). The solvent was evaporated azeotropically to dryness at 90°C with anhydrous acetonitrile. A solution of 7-*O*-triflate of GC (5–8 mg) in 0.2 ml of anhydrous DMF was added to the V-vial, and the reaction mixture was heated at 120°C for 5–10 min. The reaction was followed with time by applying an aliquot to an analytical C18 HPLC column eluted with a mobile phase of acetonitrile and water (20/80) containing 0.1 M ammonium formate at a flow

rate of 3 ml/min. Under these conditions, the elution time for 7-^{18}FGB was 5.5 min ($k' = 2.2$).

The product 7-^{18}FGB was separated from the reaction mixture by semi-preparative HPLC with a mobile phase of acetonitrile/water (20:80) containing 0.1 M ammonium formate at a flow rate of 5 ml/min. The radioactive fraction eluting at the retention time corresponding to that of non-radioactive 7-fluoro-GB (16.7 min, $k' = 9.2$) was collected, diluted with 20 ml of sterile water and passed through a Classic C18 Sep-Pak (Waters). The Sep-Pak was washed with 10 ml of water, and 7-^{18}FGB was eluted into a sterile vial with 1.0 ml of ethanol (200 proof, USP, Aaper). An aliquot of the ethanol solution was applied to the above-mentioned analytical C18 HPLC system to determine the specific activity and the radiochemical and chemical purities of the final product. The rest of the ethanol solution containing 7-^{18}FGB was diluted with 10 ml of sterile normal saline. The radiochemical yield was determined with a dose-calibrator and decay corrected to the end of bombardment (EOB).

Conclusion

Ginkgolide B, a terpene trilactone constituent of *Gingko biloba*, can be radiolabeled with ^{18}F at the 7 position in good yields, high specific activity and high radiochemical and chemical purity, suggesting that the radiotracer can be used for visualizing *in vivo* distribution of ginkgolide B by PET.

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