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Enzymatic synthesis and biodistribution in mice of β-O-D-galactopyranosyl-(1,4′)-2′-[^{18}F]fluoro-2′-deoxy-D-glucopyranose (2′-[^{18}F]fluorodeoxylactose)

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Summary

We have synthesized β-O-D-galactopyranosyl-(1,4′)-2′-[^{18}F]fluoro-2′-deoxy-D-glucopyranose (2′-[^{18}F]fluorodeoxylactose, ^{18}FDL) using an enzymatic method starting from ^{18}FDG in order to evaluate this compound with regard to its usefulness for *in vivo* visualization of the expression of the LacZ gene. Incubation of ^{18}FDL with β-galactosidase results in the formation of ^{18}FDG. Biodistribution studies in normal mice showed that ^{18}FDL is cleared by urinary excretion and is not retained in any tissue. Biodistribution in Rosa-26 mice is identical to the biodistribution in normal mice, suggesting that ^{18}FDL is not able to cross the cell membrane.Copyright © 2001 John Wiley & Sons, Ltd.

Key Words: positron emission tomography; fluorine-18; β-Gal; LacZ

Introduction

Gene therapy for the treatment of human diseases has received a lot of attention during the last decade and several clinical trials are currently being performed.

Reporter gene expression has been evaluated *in vitro* by several methods assaying the presence of specific enzymes such as luciferase,[1]

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green fluorescent protein (GFP),[2] chloramphenicol acetyltransferase (CAT),[3] β-glucuronidase (GUS)[4] and β-galactosidase (β-Gal).[5]

In vivo monitoring of gene expression with radiolabeled or NMR probes that are substrates for either specific enzymes, receptors or transporters occurring as a result of the expression of the corresponding marker genes has been pursued by several groups and these applications have recently been reviewed by Bogdanov[6] and Gambhir.[7]

We are investigating substrates that could enable *in vivo* imaging of the expression of β-Gal resulting from the expression of the LacZ gene which is used frequently for *in vitro* evaluation of gene expression using substrates allowing colorimetric, fluorescence, immunoassay or luminescence detection.[8] Ideally, the substrate should be able to cross the cell membrane and be hydrolyzed by β-Gal resulting in a radiolabeled hydrolysis product which is retained intracellularly. The substrate should further be cleared from tissues not expressing the LacZ gene.

Hydrolysis of 2′-[^{18}F]fluoro substituted lactose results in the formation of both galactose and ^{18}FDG, the latter can be trapped intracellularly due to phosphorylation by hexokinase (Figure 1). It has been suggested that galactosylated compounds can cross the cell membrane by lectine-mediated binding followed by endocytosis and that hepatocytes express specific plasma membrane galactose transporters.[6]

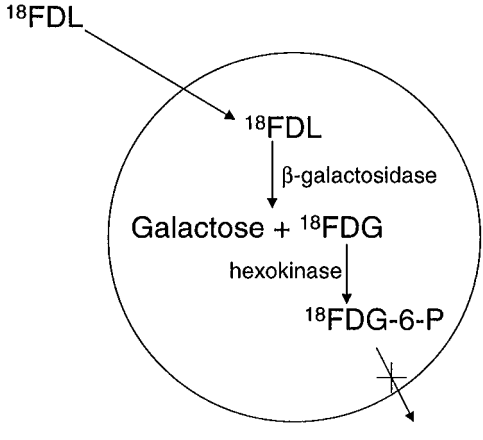

Figure 1. If ^{18}FDL crosses the cell membrane, it is hydrolyzed intracellularly by β-Gal, resulting in the formation of ^{18}FDG which is converted by hexokinase to FDG-6-phosphate. The latter is trapped in the cell

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We have therefore synthesized 2′-[^{18}F]fluorodeoxylactose using an enzymatic method and evaluated its biodistribution in normal mice and Rosa-26 mice that express bacterial LacZ in most of their tissues.[9]

Results and discussion

^{18}FDL was synthesized starting from ^{18}FDG following an enzymatic procedure published by Thompson and Chassy[10] (Figure 2).

The reaction was followed by HPLC-purification on a Carbopac anion exchanger column that allows the separation of the starting compound ^{18}FDG from ^{18}FDL (Figure 3(A)) and the identity of ^{18}FDL was confirmed by mass spectrometry on a sample obtained using the same synthesis procedure in the presence of carrier FDG. The yield of ^{18}FDL was comparable to the reported yield (50% conversion of

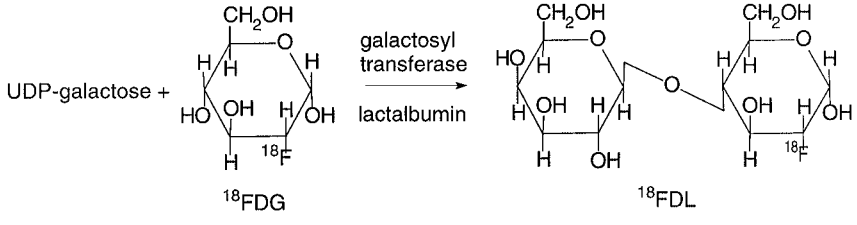

Figure 2. Synthesis of ^{18}FDL

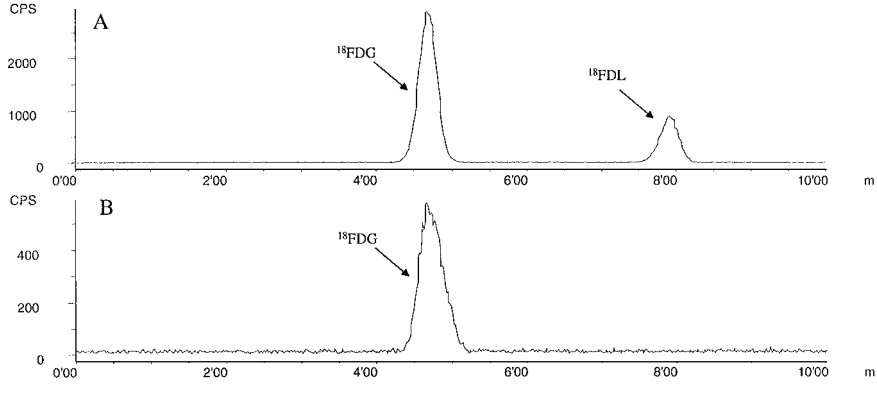

Figure 3. HPLC chromatograms of (A) reaction mixture during synthesis of ^{18}FDL starting from ^{18}FDG after 5 hours of incubation, (B) reaction mixture 30 min after incubation of ^{18}FDL with β-galactosidase

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^{18}FDG to ^{18}FDL after about 20 h of incubation) and was not different if HPLC-purified ^{18}FDG (free from carrier glucose) or non-purified ^{18}FDG was used.

The decay of ^{18}F taken into consideration, a maximum yield of 3.4% (from starting ^{18}FDG activity) was obtained after about 3 h of incubation in the conditions used.

This enzymatic method is not suited to obtain clinically relevant amounts of ^{18}FDL but provides enough to perform preliminary evaluations.

As could be expected, incubation of ^{18}FDL with β-Gal from *E. coli* results in complete hydrolysis whereas in a control experiment in the absence of β-Gal no hydrolysis was observed. The radiometric trace of the HPLC analysis shows the presence of only ^{18}FDG after 30 min of incubation (Figure 3(B)).

Biodistribution studies of HPLC-purified ^{18}FDL in normal mice showed that the compound is cleared from plasma by urinary excretion and is not retained in any particular organ (Table 1).

In order to evaluate whether ^{18}FDL is retained in tissue expressing the LacZ gene, we have also investigated its biodistribution in Rosa-26 mice.

This biodistribution is almost identical to that of ^{18}FDL in normal mice (Table 1) indicating that ^{18}FDL either does not penetrate the cell membrane or is hydrolyzed to ^{18}FDG that back-diffuses out of the cell. In view of the absence of brain uptake, it is unlikely that hydrolysis yielding ^{18}FDG occurred in any tissue. The lack of tissue retention of

Table 1. Biodistribution of ^{18}FDL in normal mice ($n = 3$) at 10 and 30 min p.i. and in Rosa-26 mice ($n = 3$) at 30 min p.i. Results are expressed as percentage of injected dose $\pm$ standard deviation

	Normal mice		Rosa-26 mice
	10′ p.i.	30′ p.i.	30′ p.i.
Urine	44.5 $\pm$ 6.7	59.3 $\pm$ 7.4	54.2 $\pm$ 9.9
Kidneys	5.3 $\pm$ 1.3	2.2 $\pm$ 0.2	4.1 $\pm$ 1.8
Liver	4.1 $\pm$ 0.3	3.1 $\pm$ 0.8	3.8 $\pm$ 1.6
Spleen	0.2 $\pm$ 0.1	0.3 $\pm$ 0.0	0.3 $\pm$ 0.2
Lungs	0.8 $\pm$ 0.3	0.7 $\pm$ 0.0	0.7 $\pm$ 0.3
Heart	0.2 $\pm$ 0.0	0.2 $\pm$ 0.1	0.3 $\pm$ 0.1
Intestines	2.5 $\pm$ 0.0	1.6 $\pm$ 0.2	2.3 $\pm$ 0.4
Stomach	0.4 $\pm$ 0.1	0.3 $\pm$ 0.1	0.3 $\pm$ 0.1
Blood	10.1 $\pm$ 1.5	5.6 $\pm$ 0.5	8.4 $\pm$ 2.1
Carcass	30.7 $\pm$ 4.9	27.1 $\pm$ 2.6	25.5 $\pm$ 6.2

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^{18}FDL in Rosa mice is therefore probably due to the inability of ^{18}FDL to penetrate the cell membrane. Cellular retention of ^{18}FDL therefore seems to require the presence of a specific transporter protein such as lactose permease, the gene of which has been sequenced by Büchel *et al.*[11]

Experimental

α-Lactalbumin (type 1), galactosyltransferase, UDP-galactose disodium salt and β-galactosidase (*E. coli*) were obtained from Sigma (Bornem, Belgium).

Other reagents were obtained from Acros (Geel, Belgium) and were of reagent quality.

^{18}FDG was produced in an IBA synthesis module (Ion Beam Applications, Louvainla-Neuve, Belgium) starting from [^{18}F]fluoride obtained by proton irradiation of $H_2^{18}O$ (97% enrichment) in an IBA cyclone 10/5 cyclotron.

HPLC analyses and purifications were performed using a Dionex Carbopac PA-10 column (25 cm × 0.4 cm) eluted with freshly prepared NaOH 0.1 M (1 ml/min). Radioactivity in the column eluate was monitored with a 3-in NaI(Tl) crystal and the signal was processed by RaChel data acquisition equipment (LabLogic, Sheffield, UK).

β-O-D-Galactopyranosyl-(1,4′)-2′-[^{18}F]fluoro-2′-deoxy-D-glucopyranose (2′-[^{18}F]fluorodeoxylactose, ^{18}FDL)

One millilitre of a solution of ^{18}FDG (74-740 MBq either HPLC-purified or used as obtained from the FDG synthesis module) was added to 0.5 ml of a solution containing HEPES buffer pH 7.5 (50 mM), $MnSO_4$ (60 mM), α-lactalbumin (0.2 mg), galactosyltransferase (0.3 mg, 1 U) and UDP-galactose (10 mg, 16.6 μ mol). The mixture was incubated at 37°C during 30 min to 6 h. The reaction mixture was purified by passage through a Maxi-Clean IC-H 0.5 ml cartridge (Alltech, Laarne, Belgium) and a SepPak QMA cartridge (Waters, Brussels, Belgium) and analysed using HPLC to monitor the conversion of ^{18}FDG to ^{18}FDL.

For hydrolysis and biodistribution experiments ^{18}FDL was isolated from HPLC and neutralized by passage through a Maxi-Clean IC-H 0.5 ml cartridge (Alltech).

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For characterization of FDL with mass spectrometry the same synthesis procedure as described above was used but 5 mg of carrier 2-FDG (Sigma, Bornem, Belgium) was added to the reaction mixture which was incubated for 12 h. FDL was isolated by HPLC and the solution was neutralized by passage over a Maxi-Clean IC-H 0.5 ml cartridge (Alltech). The mass spectrum was recorded on a Micromass Q-TOF-2 system (ES + mode) and showed a molecular ion mass of 345 (FDL-H +), 367 (FDL + Na^+) and 383 (FDL + K^+).

Hydrolysis of ^{18}FDL with β-galactosidase

18.5 MBq of HPLC purified ^{18}FDL in 0.8 ml water was added to 0.2 ml of a solution containing 25 mg (500 U) β-Gal (E. Coli), HEPES buffer pH 7.0 (120 mM), $MgS0_4$ (4 mM) and NaCl (180 mM). The solution was incubated for 30 min at 37°C, purified over a Maxi-Clean IC-H 0.5 ml cartridge and a SepPak QMA cartridge and analysed by HPLC. A control experiment was carried out using the same procedure but in the absence of β-Gal.

Biodistribution experiments

HPLC-purified ^{18}FDL solutions were diluted with normal saline to a radioactive concentration of 3.7 MBq/ml.

The biodistribution was determined in normal NMRI mice (body mass 20–25 g) and in ROSA-26 mice (body mass 20–25 g, gift from the Laboratory of Molecular and Vascular Biology, University of Leuven). Homozygous expression of β-Gal in ROSA-26 mice was verified by X-Gal staining experiments on ear and tail biopsies of newborn pups obtained by crossing ROSA-26 mice with wild type mice. The animal studies were performed according to the Belgian code of practice for the care and use of animals. The mice were sedated by intraperitoneal injection of 1 mg of pentobarbital (Nembutal®, Sanofi, Brussels, Belgium) 1 h prior to tracer injection. A volume of 0.1 ml of the tracer solution was injected via a tail vein. The mice were sacrificed by decapitation at 10 or 30 min post injection. Blood was collected in a tared tube and weighed. All organs and other body parts were dissected and their activity was counted in a 3-in NaI(Tl) well crystal, coupled to a multichannel analyzer (Wallac, Turku, Finland). Corrections were made for background radiation and physical decay during counting. Results were expressed as percentage of injected dose (%ID) equal to

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the sum of the net counts in all body parts. The percentage of the tracer present in the urine was calculated from the activity measured in the urinary bladder. For calculation of total blood activity, blood mass was assumed to be 7% of the body mass.

Conclusion

^{18}FDL was synthesized from ^{18}FDG in a relatively low yield using an enzymatic method. Conversely, incubation of ^{18}FDL with β-Gal results in the formation of ^{18}FDG.

In normal mice, ^{18}FDL is not retained in any particular organ and is cleared from plasma by urinary excretion. Biodistribution of ^{18}FDL in Rosa mice is almost identical to that in normal mice. The lack of tissue retention in Rosa mice is probably due to the absence or slow cell membrane penetration.

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