Nuclear Medicine and Biology 61 (2018) 56–62

Contents lists available at ScienceDirect

Nuclear Medicine and Biology

journal homepage: www.elsevier.com/locate/nucmedbio

N-(4-[^{18}F]fluorobenzyl)cholylglycine, a novel tracer for PET of enterohepatic circulation of bile acids: Radiosynthesis and proof-of-concept studies in rats☆

Kim Frisch [a,*], Damion H.R. Stimson [b], Taracad Venkatachalam [b], Gregory K. Pierens [b], Susanne Keiding [a,c], David Reutens [b], Rajiv Bhalla [b]

[a] Department of Nuclear Medicine & PET Centre, Aarhus University Hospital, Aarhus, Denmark
[b] Centre for Advanced Imaging, University of Queensland, St. Lucia, Brisbane, Australia
[c] Department of Hepatology & Gastroenterology, Aarhus University Hospital, Aarhus, Denmark

ARTICLE INFO

Article history:
Received 9 March 2018
Received in revised form 20 April 2018
Accepted 27 April 2018
Available online xxxx

Keywords:
Bile acids
Bile Salt Export Pump
Enterohepatic circulation
Fluorine-18 labelling
Metabolism
Positron emission tomography

ABSTRACT

Introduction: Enterohepatic circulation (EHC) of conjugated bile acids is an important physiological process crucial for regulation of intracellular concentrations of bile acids and their function as detergents and signal carriers. Only few bile acid-derived imaging agents have been synthesized and hitherto none have been evaluated for studies of EHC. We hypothesized that *N*-(4-[^{18}F]fluorobenzyl)cholylglycine ([^{18}F]FBCGly), a novel fluorine-18 labeled derivative of endogenous cholylglycine, would be a suitable tracer for PET of the EHC of conjugated bile acids, and we report here a radiosynthesis of [^{18}F]FBCGly and a proof-of-concept study by PET/MR in rats.
Methods: A radiosynthesis of [^{18}F]FBCGly was developed based on reductive alkylation of glycine with 4-[^{18}F]fluorobenzaldehyde followed by coupling to cholic acid. [^{18}F]FBCGly was investigated *in vivo* by dynamic PET/MR in anesthetized rats; untreated or treated with cholyltaurine or rifampicin. Possible *in vivo* metabolites of [^{18}F]FBCGly were investigated by analysis of blood and bile samples, and the stability of [^{18}F]FBCGly towards enzymatic de-conjugation by Cholylglycine Hydrolase was tested *in vitro*.
Results: [^{18}F]FBCGly was produced with a radiochemical purity of 96% ± 1% and a non-decay corrected radiochemical yield of 1.0% ± 0.3% (mean ± SD; $n = 12$). PET/MR studies showed that i.v.-administrated [^{18}F]FBCGly underwent EHC within 40–60 min with a rapid transhepatic transport from blood to bile. In untreated rats, the radioactivity concentration of [^{18}F]FBCGly was approximately 15 times higher in bile than in liver tissue. Cholyltaurine and rifampicin inhibited the biliary secretion of [^{18}F]FBCGly. No fluorine-18 metabolites of [^{18}F]FBCGly were observed.
Conclusion: We have developed a radiosynthesis of a novel fluorine-18 labeled bile acid derivative, [^{18}F]FBCGly, and shown by PET/MR that [^{18}F]FBCGly undergoes continuous EHC in rats without metabolizing. This novel tracer may prove useful in PET studies on the effect of drugs or diseases on the EHC of conjugated bile acids.

1. Introduction

The formation of bile acids in the liver and their secretion into bile canaliculi and onwards into the small intestine play an essential role in digestion and detoxification, and in metabolic signaling [1–3]. Before secretion from hepatocytes, bile acids are conjugated (*N*-acyl amidated) with glycine or taurine. <10% of the secreted bile acids stem from *de novo* synthesis; the rest have undergone enterohepatic circulation (EHC), *i.e.* the continuous circulation of bile acids between liver and small intestine [1,2,4]. The EHC, which occurs 6–10 times per day in humans [5], results from active vectorial transport of bile acids across hepatocyte and enterocyte membranes, and involves several transporter proteins [6]. These proteins include the Bile Salt Export Pump (BSEP), which accounts for the rate-limiting step of the overall transport across the hepatocyte [7], and the Apical Sodium-dependent Bile acid Transporter (ASBT), which is located mainly in ileal enterocytes and accounts for >90% of the absorption of bile acids from the intestinal lumen [8,9]. The EHC ensures optimal concentrations of bile acids at the sites of their physiologic actions and hence allows bile acids to form micelles and dissolve digested lipids in the small intestine and to function as signal molecules, while keeping the intracellular concentration of bile acids low to avoid cellular damages [1,10]. Several factors may perturb the EHC and lead to liver and gastrointestinal disorders [2,11–13].

☆ Conflicts of interest: The authors declare that they have no conflicts of interest.

* Corresponding author at: Department of Nuclear Medicine & PET Centre, Aarhus University Hospital, DK-8000 Aarhus C, Denmark.
E-mail address: kimfrisc@rm.dk. (K. Frisch).

https://doi.org/10.1016/j.nucmedbio.2018.04.004
0969-8051/

Cholestatic liver diseases and drug-induced inhibition of BSEP result in accumulation of bile acids in hepatocytes, which leads to liver injury, treatable only by liver transplantation in severe cases [7,14]. Impaired intestinal uptake caused by lack of the ileum or by drug-induced inhibition of ASBT results in bile acid malabsorption and excessive excretion of bile acids to the colon, which leads to severe diarrhea, alteration of the gut microbiome and, potentially, development of colorectal cancer [13].

A method for non-invasive quantitative imaging of the dynamic EHC of conjugated bile acids *in vivo* would be a valuable tool for investigations of the underlying pathological mechanisms of diseases that affect the EHC and of drugs inhibiting hepatic and intestinal transport of conjugated bile acid.

The development of such method is likely to be successful only if the imaging agent is derived from an endogenous bile acid conjugate, since these are the only molecules that so far have been shown to go through all features of the EHC [10]. The current standard of practice for quantification of the EHC do involve an analog, 23-[^{75}Se]seleno-25-homotaurocholic acid, closely related to endogenous cholyltaurine, but the method provides only a measure of the retention of the labeled analog in the body; not quantifiable images of its circulation between liver and ileum [15]. Other labeled bile acid analogs have been investigated for single photon emission computed tomography [16], magnetic resonance [17–19], and PET imaging [20–26], but so far no imaging agent has been reported for imaging of the EHC. In particular, [^{11}C]cholylsarcosine, a carbon-11 labeled analog of endogenous cholylglycine [20], has proven useful for quantification of hepatic transport of conjugated bile acids in healthy persons and in patients with cholestatic liver disease by PET/CT [27,28]. However, although cholylsarcosine is known to undergo EHC, the use [^{11}C]cholylsarcosine as PET tracer for EHC is limited by the relatively short half-life of the carbon-11 radioisotope (^{11}C; $T_{½}$ = 20.3 min). We therefore set out to develop a novel bile acid tracer with properties comparable to [^{11}C]cholylsarcosine, but labeled with a positron emitting isotope with a radioactive half-life suitable for studies of EHC. Here we report a radiosynthesis of *N*-(4-[^{18}F]fluorobenzyl)cholylglycine ([^{18}F]FBCGly), a novel fluorine-18 (^{18}F; half-life = 109.8 min) labeled derivative of endogenous cholylglycine, and show in a proof-of-concept study in rats that [^{18}F]FBCGly is a PET tracer for EHC.

2. Materials and methods

2.1. General information

Chemicals and solvents were obtained from Sigma-Aldrich or VWR International Ltd. and used as received. Water was sterile or MilliQ water. Cholylglycine Hydrolase from *Clostridium perfringens* (*C. welchii*) (lyophilized powder, ≥100 units/mg) was obtained from Sigma-Aldrich. Cholylglycine-2,2,4,4-d_4 was obtained from CDN Isotopes. (4-Formylphenyl)trimethylammonium triflate [29] and unlabeled cholylsarcosine [20] were prepared as described in the literature. 4-Fluorobenzylalcohol (used for identification purposes only) was prepared by reduction of 4-fluorobenzaldehyde with sodium borohydride in methanol. Unlabeled *N*-(4-fluorobenzyl)glycine methyl ester (FBGly-ME), *N*-(4-fluorobenzyl)cholylglycine methyl ester (FBCGly-ME), and *N*-(4-fluorobenzyl)cholylglycine (FBCGly) were prepared and characterized (^{1}H, ^{13}C, ^{19}F, COSY, HSQC, HMBC and ROESY NMR) as described in Supplementary material. For comparison, NMR spectra of cholic acid are also reported. Sep-Pak® Accell Plus QMA Carbonate Plus Light Cartridge (130 mg sorbent/cartridge, 37–55 μm particle size) and Sep-Pak® Alumina N Plus Light Cartridges (280 mg sorbent/cartridge, 50–300 μm particle size) were obtained from Waters® and used as received. Sep-Pak® C_8 Plus Short Cartridges (400 mg sorbent/cartridge, 37–55 μm particle size), also obtained from Waters®, were conditioned before use with 10 mL ethanol, then 10 mL water, and finally 10 mL air.

2.2. Radiochemistry

The developed radiosynthesis of [^{18}F]FBCGly is illustrated in Fig. 1. No-carrier-added [^{18}F]fluoride was produced by the ^{18}O(p,n)^{18}F nuclear reaction from irradiation of isotopically enriched [^{18}O]H_2O (Rotem Industries, Deer Sheva, Israel) using a cyclotron (GE PETtrace 16.4-MeV proton beam or IBA Cyclone 18/18). The [^{18}F]fluoride (40–60 GBq) was trapped on a QMA cartridge and eluted into a 3 mL reaction vessel with a solution of K222 (Kryptofix™; 5 mg; 13 μmol) and K_2CO_3 (0.7 mg; 5 μmol) in a mixture of water (0.30 mL) and acetonitrile (0.30 mL). The [^{18}F]fluoride was dried in the reactor by azeotropic distillation from anhydrous acetonitrile (3 × 0.6 mL) under a stream of helium at 120 °C. The temperature was lowered to 80 °C, a solution of (4-formylphenyl)trimethylammonium triflate (2 mg; 6 μmol) in anhydrous dimethylsulfoxide (0.3 mL) was added, and the mixture was heated at 105 °C for 10 min. The crude [^{18}F]FBA (dark orange to brown in color) was purified by passing it through an alumina N cartridge, which was subsequently washed with anhydrous dimethylsulfoxide (0.2 mL), into a second 3 mL reaction vessel. A solution of glycine methyl ester hydrochloride (2 mg; 16 μmol) and triethylamine (3 μL; 22 μmol) in anhydrous dimethylsulfoxide (0.1 mL) was added to the purified [^{18}F]FBA and the mixture was heated at 60 °C for 10 min. The reaction vessel was cooled to 25 °C using a stream of cold air, a solution of $NaBH_4$ (2 mg; 53 μmol) in anhydrous dimethylsulfoxide (0.1 mL) was added, and the mixture was allow to stand for 5 min. A solution of cholic acid (16 mg; 39 μmol) and triethylamine (9 μL; 65 μmol) in anhydrous dimethylsulfoxide (0.1 mL) was added, followed by a solution of diethyl phosphoryl cyanide (12 μL; 79 μmol) in anhydrous dimethylsulfoxide (0.1 mL). The temperature was raised to 60 °C and the mixture was heated for 10 min. All added solutions were freshly prepared. After cooling to 25 °C, the reaction mixture was diluted with water (9 mL) and [^{18}F]FBCGly-ME was trapped on a conditioned C_8 cartridge. The cartridge was washed with water (10 mL) and 50% aqueous ethanol (10 mL), and eluted with 100% ethanol (1 mL) into a product vial. Aqueous NaOH (2 mL; 0.25 N) was added to the product vial and the mixture was allowed to stand at room temperature for 10 min with occasional shaking. The basic mixture was finally neutralized with aqueous NaH_2PO_4 (7 mL; 70 mM). Total synthesis time was 90 min from delivery of [^{18}F]fluoride.

The product formulation of [^{18}F]FBCGly was analyzed by reverse-phase HPLC with a PDA-detector (Dionex Ultimate® 3000 system) connected in series with a gamma-detector (Gabistar; Nuclear Interface) and an ESI mass spectrometer (Bruker Daltonics HCT Plus ion trap mass spectrometer) running in negative ionization mode. The column was a Phenomenex® Luna® 5 μ C18(2) 100A (5 μm, 150 × 4.6 mm) with an isocratic eluent of 50% acetonitrile in aqueous 1 mM NH_4OAc (pH 3.0; adjusted with glacial acetic acid) and a flow of 1 mL/min. The product formulation of [^{18}F]FBCGly was also tested for free [^{18}F]fluoride by radio-iTLC (Supplementary material). The identity of [^{18}F]FBCGly (retention time: 8.2 min) was confirmed by co-injection of unlabeled reference material by monitoring its UV (254 nm) and MS (572.3 m/z) signals. For determination of molar radioactivity, the amount of FBCGly was determined by MS using a series of standard solutions (2, 5, 10, and 20 μg/mL) and with cholylglycine-2,2,4,4-d_4 as internal standard. To investigate the individual steps of the radiosynthesis, the formation of reaction intermediates was analyzed by radio-HPLC (Supplementary material).

2.3. In vivo PET/MR and metabolite studies

All animal experiments were carried out at the Dept. of Nuclear Medicine & PET Centre, Aarhus University Hospital, Aarhus, Denmark. The animal experiments were performed according to the Danish Animal Experimentation Act and the European convention for the protection of vertebrate animals used for experimental and other purposes