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Influence of 24-*Nor*-Ursodeoxycholic Acid on Hepatic Disposition of [^{18}F]Ciprofloxacin, a Positron Emission Tomography Study in Mice

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ABSTRACT

24-*nor*-ursodeoxycholic acid (*nor*UDCA) is a novel therapeutic approach to cholestatic liver diseases. In mouse models of cholestasis, *nor*UDCA induces basolateral multidrug resistance–associated proteins 4 (Mrp4) and 3 in hepatocytes, which provide alternative escape routes for bile acids accumulating during cholestasis but could also result in altered hepatic disposition of concomitantly administered substrate drugs. We used positron emission tomography imaging to study the influence of *nor*UDCA on hepatic disposition of the model Mrp4 substrate [^{18}F]ciprofloxacin in wild-type and *Mdr2*$^{(-/-)}$ mice, a model of cholestasis. Animals underwent [^{18}F]ciprofloxacin positron emission tomography at baseline and after *nor*UDCA treatment. After *nor*UDCA treatment, liver-to-blood area under the curve ratio of [^{18}F]ciprofloxacin was significantly decreased compared to baseline, both in wild-type (−34.0 ± 2.1%) and *Mdr2*$^{(-/-)}$ mice (−20.5 ± 6.0%). [^{18}F]Ciprofloxacin uptake clearance from blood into liver was reduced by −17.1 ± 9.0% in wild-type and by −20.1 ± 7.3% in *Mdr2*$^{(-/-)}$ mice. Real-time PCR analysis showed significant increases in hepatic Mrp4 and multidrug resistance–associated protein 3 mRNA after *nor*UDCA. Transport experiments in organic anion transporting polypeptide (OATP)1B1-, OATP1B3-, and OATP2B1-transfected cells revealed weak transport of [^{14}C]ciprofloxacin by OATP1B3 and OATP2B1 and no inhibition by *nor*UDCA. In conclusion, our data suggest that changes in hepatic [^{18}F]ciprofloxacin disposition in mice after *nor*UDCA treatment were caused by induction of basolateral Mrp4 in hepatocytes.

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Introduction

Generation of bile is a key function of the liver. Its impairment leads to accumulation of cytotoxic bile acids and other potentially toxic cholephils in liver and systemic circulation, a syndrome commonly known as cholestasis.[1] Primary sclerosing cholangitis (PSC) is a chronic cholestatic liver disease with bile duct inflammation and fibrosis leading to end-stage liver disease and/or hepatobiliary cancer requiring liver transplantation.[2]

24-*nor*-ursodeoxycholic acid (*nor*UDCA) is a side chain–modified derivative of ursodeoxycholic acid, which has been shown to reverse PSC features in animal models [3,4] and which is currently undergoing clinical trials in patients.[5] The therapeutic mechanisms of *nor*UDCA involve the stimulation of bile flow and biliary bicarbonate secretion as well as the induction of detoxification and elimination routes for bile acids.[3,4] Mice lacking the adenosine triphosphate-binding cassette (ABC) transporter multidrug resistance protein 2 (Mdr2), which is located in the canalicular membrane of hepatocytes, where it transports phospholipids into bile, represent a well-characterized animal model with morphologic features of PSC.[6] These mice develop sclerosing cholangitis due to "hypertoxic" bile enriched with nonmicellar-bound bile acids. In this mouse model, *nor*UDCA was shown to induce the hepatic bile acid detoxifying enzymes Cyp2b10, Cyp3a11, and Sult2a1 as well as multidrug resistance–associated proteins 4 and 3 (Mrp4, Mrp3),

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which are located in the basolateral membrane of hepatocytes and which serve as a backup system for elimination of bile acids across the hepatocellular membrane into the blood.[3]

Patients with PSC often are on several different drugs including antibiotics for treatment of bacterial infections of the bile duct, such as penicillins, cephalosporins, fluoroquinolones, and carbapenems. Changes in hepatic ABC transporter expression induced by *nor*-UDCA may lead to altered hepatic disposition of drugs and drug metabolites which are recognized by these transporters, which could in turn lead to side effects and/or loss of efficacy (transporter-mediated drug-drug interaction, DDI).[7] The fluoroquinolone ciprofloxacin, which is frequently used for treatment of bacterial cholangitis, is actively secreted into bile by the canalicular ABC transporter breast cancer resistance protein (Bcrp1).[8] In addition, several studies have shown that ciprofloxacin is also a substrate of human MRP4 and murine Mrp4.[9-11]

Positron emission tomography (PET) imaging enables the noninvasive measurement of the tissue distribution of carbon-11-(^{11}C) or fluorine-18-(^{18}F) labeled drugs. PET has been proposed as a powerful tool to assess the impact of drug transporters on drug pharmacokinetics on a tissue level.[12,13] Ciprofloxacin contains fluorine in its structure and can therefore be labeled with ^{18}F without structural modification.[14] [^{18}F]Ciprofloxacin PET has been used before to study *in vivo* ciprofloxacin disposition in humans.[15]

Aim of the present study was to use small-animal PET and [^{18}F]ciprofloxacin to study the influence of *nor*UDCA treatment on hepatic disposition of ciprofloxacin as a model Mrp4 substrate in wild-type and *Mdr2*$^{(-/-)}$ mice. We hypothesized that induction of hepatic Mrp4 caused by *nor*UDCA would lead to alterations in liver distribution of [^{18}F]ciprofloxacin.

Materials and Methods

Chemicals and Drugs

If not stated otherwise chemicals were purchased from Sigma-Aldrich Chemie GmbH (Schnelldorf, Germany) or Merck (Darmstadt, Germany) and were of at least analytical grade. [6,7-^{3}H(N)]estrone-3-sulfate ([^{3}H]E3S, specific activity: 1.7 GBq/μmol) was purchased from PerkinElmer (Boston, MA). [2-^{14}C] Ciprofloxacin (specific activity: 2.2 MBq/μmol) was purchased from Hartmann Analytic GmbH (Braunschweig, Germany). *Nor*UDCA and tauro-*nor*UDCA were synthesized as described elsewhere.[4]

Cell Lines

Wild-type Chinese hamster ovary (CHO) cells were maintained in Dulbecco's Modified Eagle Medium supplemented with 10% fetal bovine serum (Gibco, Paisley, UK), 0.05 mg/mL L-proline, 100 units/mL penicillin, and 100 g/mL streptomycin at 37°C in a humidified atmosphere with 5% CO_2. Organic anion transporting polypeptide (OATP)1B1-, OATP1B3- and OATP2B1-stably transfected CHO cells[16,17] were grown under selective pressure with Geneticin G-418 (500 μg/mL) (Gibco).

Animals

Female wild-type and *Mdr2*$^{(-/-)}$ mice (FVB/N background) were obtained from the Division of Laboratory Animal Science and Genetics (Medical University of Vienna). At the time of experiment, animals had an age of 20-25 weeks and weighted 27 ± 2 g. All animals were housed in individually ventilated cages (3 or 4 mice per cage) under controlled environmental conditions (24 ± 2°C, 40%-70% humidity, 12 h light/dark cycle) and permitted *ad libitum* access to water and standard mouse diet (Sniff, Soest, Germany). All animal experiments were approved by the national authorities (Amt der Niederösterreichischen Landesregierung) and all study procedures were performed in accordance with the European Communities Council Directive of September 22, 2010 (2010/63/EU).

Transport Studies in Intact Cells

Initial uptake of radioactive compounds was measured using a protocol designed for rapid uptake determination in cells.[18] Cells were seeded in 35-mm dishes at a density of 2.5 × 10^5 cells per dish. Twenty-four h before the uptake measurement, cells were treated with 5 mM sodium butyrate to induce gene expression.[19] Medium was aspirated and cells rinsed twice with prewarmed transport buffer (116.4 mM NaCl, 5.3 mM KCl, 1 mM NaH_2PO_4, 0.8 mM $MgSO_4$, 5.5 mM D-glucose, and 20 mM Hepes/Tris, pH 7.4). Cells were equilibrated in transport buffer at 37°C; then the buffer was aspirated, and transport buffer containing the radiolabeled reagents ([^{14}C]ciprofloxacin or [^{3}H]E3S, each 10 μM) in presence or absence of either *nor*UDCA or tauro-*nor*UDCA (each 100 μM) was added. Uptake was stopped after 5 min by quick aspiration of the radiolabeled cocktail followed by extensive washing with ice-cold transport buffer. Cells were digested with 1 mL of 1% Triton X-100. Five hundred microliters of the lysate was mixed with 10 mL of scintillation liquid (Ultima Gold, PerkinElmer, Switzerland) and assessed for intracellular radioactivity by liquid scintillation counting. Protein content was determined by the bicinchoninic acid protein assay on a 25-μL aliquot. Each experiment was performed in triplicate, and uptake is expressed as picomoles of substrate per milligram of protein.

Synthesis of [^{18}F]Ciprofloxacin

[^{18}F]Ciprofloxacin was synthesized as described previously[20] with a radiochemical purity >98% and a specific activity of 1.5 ± 0.7 GBq/μmol (n = 11). [^{18}F]Ciprofloxacin was formulated in phosphate-buffered saline solution for intravenous (i.v.) injection into animals.

Experimental Design

Groups of wild-type (n = 4) and *Mdr2*$^{(-/-)}$ mice (n = 4) underwent magnetic resonance imaging (MRI) followed by a dynamic 90-min PET scan after i.v. injection of [^{18}F]ciprofloxacin both at baseline and after 5 days of oral *nor*UDCA treatment. For *nor*UDCA treatment, mice received a diet supplemented with *nor*UDCA (0.5%, wt/wt) as reported previously.[3]

PET/MR Imaging

Animals were preanesthetized in an induction chamber using isoflurane (2.5%-3.5% in oxygen), placed on a heated animal bed (38°C), and the lateral tail vein was cannulated. Anesthesia and warming were maintained for the whole imaging period. Whole-body anatomical MRI was performed on a 1 Tesla benchtop MR system (ICON, Bruker BioSpin GmbH, Ettlingen Germany) using a modified 3D T1-weighted gradient echo sequence (T1-fast low angle shot). After MRI, the animal bed was transferred into the gantry of a microPET scanner (Focus 220, Siemens Medical Solutions, Knoxville, TN) and a 10-min transmission scan using a ^{57}Co point source was recorded. Subsequently, [^{18}F]ciprofloxacin (7.9 ± 2.4 MBq, 21.2 ± 20.5 nmol, 0.10 mL, n = 16) was administered as an i.v. bolus over 1 min, and a 90-min dynamic PET scan (energy window: 250-750 keV; timing window: 6 ns) was initiated at the start of radiotracer injection.

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