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P-gp/ABCB1 exerts differential impacts on brain and fetal exposure to norbuprenorphine

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ABSTRACT

Norbuprenorphine is the major active metabolite of buprenorphine which is commonly used to treat opiate addiction during pregnancy. Norbuprenorphine produces marked respiratory depression and was 10 times more potent than buprenorphine. Therefore, it is important to understand the mechanism that controls fetal exposure to norbuprenorphine, as exposure to this compound may pose a significant risk to the developing fetus. P-gp/ABCB1 and BCRP/ABCG2 are two major efflux transporters regulating tissue distribution of drugs. Previous studies have shown that norbuprenorphine, but not buprenorphine, is a P-gp substrate. In this study, we systematically examined and compared the roles of P-gp and BCRP in determining maternal brain and fetal distribution of norbuprenorphine using transporter knockout mouse models. We administered 1 mg/kg norbuprenorphine by retro-orbital injection to pregnant FVB wild-type, *Abcb1a*$^{-/-}$/*1b*$^{-/-}$, and *Abcb1a*$^{-/-}$/*1b*$^{-/-}$/*Abcg2*$^{-/-}$ mice on gestation day 15. The fetal AUC of norbuprenorphine was ~64% of the maternal plasma AUC in wild-type mice, suggesting substantial fetal exposure to norbuprenorphine. The maternal plasma AUCs of norbuprenorphine in *Abcb1a*$^{-/-}$/*1b*$^{-/-}$ and *Abcb1a*$^{-/-}$/*1b*$^{-/-}$/*Abcg2*$^{-/-}$ mice were ~2 times greater than that in wild-type mice. Fetal AUCs in *Abcb1a*$^{-/-}$/*1b*$^{-/-}$ and *Abcb1a*$^{-/-}$/*1b*$^{-/-}$/*Abcg2*$^{-/-}$ mice were also increased compared to wild-type mice; however, the fetal-to-maternal plasma AUC ratio remained relatively unchanged by the knockout of *Abcb1a/1b* or *Abcb1a/1b/Abcg2*. In contrast, the maternal brain-to-maternal plasma AUC ratio in *Abcb1a*$^{-/-}$/*1b*$^{-/-}$ or *Abcb1a*$^{-/-}$/*1b*$^{-/-}$/*Abcg2*$^{-/-}$ mice was increased ~30-fold compared to wild-type mice. Protein quantification by LC–MS/MS proteomics revealed significantly higher amounts of P-gp protein in the wild-type mice brain than that in the placenta. These results indicate that fetal exposure to norbuprenorphine is substantial and that P-gp has a minor impact on fetal exposure to norbuprenorphine, but plays a significant role in restricting its brain distribution. The differential impacts of P-gp on norbuprenorphine distribution into the brain and fetus are likely, at least in part, due to the differences in amounts of P-gp protein expressed in the blood-brain and blood-placental barriers. BCRP is not as important as P-gp in determining both the systemic and tissue exposure to norbuprenorphine. Finally, fetal AUCs of the metabolite norbuprenorphine-β-D-glucuronide were 3–7 times greater than maternal plasma AUCs, while the maternal brain AUCs were <50% of maternal plasma AUCs, suggesting that a reversible pool of conjugated metabolite in the fetus may contribute to the high fetal exposure to norbuprenorphine.

Abbreviations: ABC, ATP-binding cassette; AUC, area under the concentration-time curve; BBB, the blood-brain barrier; BPB, the blood-placental barrier; P-gp, P-glycoprotein; BCRP, breast cancer resistance protein; ABCB1, the first member of subfamily B of the ABC transporter superfamily; ABCG2, the second member of subfamily G of the ABC transporter superfamily; *Abcb1a*, the murine isoform a of the human *ABCB1* gene; *Abcb1b*, the murine isoform b of the human *ABCB1* gene; PBS, phosphate-buffered saline; LC–MS, liquid chromatography–mass spectrometry; HPLC, high-performance liquid chromatography; BUP, buprenorphine; NBUP, norbuprenorphine; NBUP-d3, norbuprenorphine-d3; NBUP-G, norbuprenorphine-3-β-D-glucuronide; gd, gestation day; DMSO, dimethyl sulfoxide; SIL, stable isotope-labeled; SPE, solid-phase extraction.

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1. Introduction

Recent statistics indicate that the prevalence of opioid use among pregnant women ranges from 1 to 2% to as high as 21% in the United States [1], posing a major risk for maternal morbidities and neonatal complications. Methadone and buprenorphine are prescribed to pregnant women to treat opiate addiction [2,3]. Although the fetus is not the target of treatments, it is exposed to these drugs *de facto* when pregnant women use the medications. Randomized clinical trials comparing methadone and buprenorphine indicate that both medications are effective in preventing relapse to illicit opioids in opioid-dependent patients. Buprenorphine, however, results in less severe neonatal abstinence syndrome than methadone [4].

Buprenorphine (BUP) is a semisynthetic thebaine derivative that acts as a mixed partial agonist-antagonist opioid receptor modulator [5]. BUP undergoes *N*-dealkylation in the liver, forming the major metabolite norbuprenorphine (NBUP). NBUP is pharmacologically active with ~25% of buprenorphine's intrinsic analgesic effect [6]. Furthermore, NBUP is a μ-opioid, δ-opioid, and nociceptin receptor full agonist, and a κ-opioid receptor partial agonist. NBUP produces respiratory depression and was 10 times more potent than buprenorphine in rodents [7,8] and may be fatal to infants [9]. NBUP can be further glucuronidated at the third carbon to norbuprenorphine-3-β-D-glucuronide (NBUP-G), which is also biologically active [6]. Concheiro et al. [10] showed that both NBUP and NBUP-G could be detected in umbilical cord blood samples 5 h after administration of buprenorphine but before delivery with concentrations greater than those of the parent drug, suggesting that the fetus may have been exposed to substantial levels of pharmacologically active metabolites. To better predict fetal exposure and hence fetal safety of NBUP, it is critical that we first understand the mechanisms that control fetal exposure to NBUP and NBUP-G, which are currently still not clear.

P-gp/ABCB1 and BCRP/ABCG2 are the two most important ABC efflux transporters for drug disposition and have a broad spectrum of substrates, including many drugs routinely used by pregnant women [11–13]. P-gp and BCRP are highly expressed on the apical membrane of the liver hepatocyte, the brain endothelium and the placental syncytiotrophoblasts [14–16]. Hence, both transporters restrict penetration across tissue barriers and facilitate biliary elimination of drugs and xenobiotics. For example, it has been shown that P-gp and BCRP can protect the brain and fetus from potential chemical assaults by actively expelling drugs, xenobiotics and harmful substances from the brain or the fetus to the systemic or maternal circulation [17–21].

Previous studies have shown that P-gp can effectively transport NBUP but not BUP [6,22] and that P-gp is a major determinant of brain NBUP exposure [6]. BCRP is known to transport glucuronide conjugates of drugs, xenobiotics, and endogenous substances [23]. Given the fact NBUP is a P-gp substrate and NBUP-G is possibly a BCRP substrate, it is important to know whether P-gp or BCRP restricts fetal exposure to NBUP and/or its glucuronide metabolite. Murine knockout models have been developed to interrogate the effects of P-gp and BCRP on drug disposition [24–27]. Numerous studies using chemical inhibition and genetic knockout of P-gp and BCRP have demonstrated the importance of the two transporters in determining brain or fetal distribution of several prototypic substrates, including methadone, loperamide, fentanyl, glyburide, and nitrofurantoin [18,19,28–33]. Therefore, the aim of this study was to investigate whether P-gp and BCRP affect maternal pharmacokinetics of NBUP and its metabolite NBUP-G and whether P-gp and BCRP play important roles in restricting fetal penetration of NBUP and NBUP-G, using wild-type, *Abcb1a*$^{-/-}$/*1b*$^{-/-}$, and *Abcb1a*$^{-/-}$/*1b*$^{-/-}$/*Abcg2*$^{-/-}$ pregnant mice. It has previously been shown that P-gp in the blood-brain barrier (BBB) and the blood-retinal barrier (BRB) of mouse models has dissimilar impacts on tissue exposure to a substrate drug verapamil, with a much greater effect on limiting brain exposure than retinal exposure [34]. Hence, to investigate whether P-gp and BCRP in the BBB and the blood-placental barrier (BPB) display differential roles in restricting brain and fetal drug exposure, we also examined and compared brain and fetal exposure to NBUP and NBUP-G in the same wild-type and transporter knockout dams.

2. Methods and methods

2.1. Materials

Norbuprenorphine (NBUP) and norbuprenorphine-3-β-D-glucuronide (NBUP-G) used in animal studies were provided by the National Institute on Drug Abuse (Bethesda, MD). NBUP, norbuprenorphine-d3 (NBUP-d3) and NBUP-G used for analytical calibrations in LC–MS/MS analysis were from Cerilliant (Round Rock, TX). Optima grade or high-performance liquid chromatography grade methanol, acetonitrile, polyethylene glycol 400, ethanol, formic acid, DMSO, dimethyl sulfoxide and water were from Thermo Fisher Scientific (Waltham, MA) or Acros Organics (Pittsburgh, PA). Isoflurane was purchased from Piramal Healthcare (Mumbai, India) through the University of Washington Medical Center Pharmacy. Synthetic signature peptides for absolute protein quantification by LC–MS/MS were obtained from New England Peptides (Boston, MA). The corresponding stable-isotope-labeled (SIL) peptides were purchased from Thermo Fisher Scientific (Rockford, IL). The protein extraction kit for ProteoExtract native membrane was from Calbiochem (Temecula, CA). Ammonium bicarbonate and sodium deoxycholate were obtained from Thermo Fisher Scientific and MP Biomedicals (Santa Ana, CA), respectively. BCA protein assay and in-solution trypsin digestion kits, iodoacetamide and dithiothreitol were obtained from Pierce Biotechnology (Rockford, IL).

2.2. Animals

Wild-type FVB, *Abcb1a*$^{-/-}$/*1b*$^{-/-}$, and *Abcb1a*$^{-/-}$/*1b*$^{-/-}$/*Abcg2*$^{-/-}$ mice, 7–10 weeks of age, were purchased from Taconic Farms (Germantown, NY) and cared for in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Research Council. Mice were maintained under 12-h light/dark cycles, and food was provided ad libitum. Female mice, 7–10 weeks of age, were mated with male mice of the same genotype and the same age overnight using a female to male ratio of 2:1. Gestation day (gd) 1 was defined as the presence of a sperm plug following overnight housing. Progress of pregnancy was monitored by visual inspection and body weight increase. Body weight was recorded on the day of dosing. Pregnant mice used in this study were on gd 15, which approximately corresponds to the late second trimester in humans. On gd 15, high levels of both P-gp and BCRP are expressed in mouse placenta, and hence gd 15 was chosen in this study [35–37]. This animal protocol was approved by the Institutional Animal Care and Use Committee of the University of Washington.

2.3. In vivo animal studies

NBUP was dissolved in a solution that contained 0.5% (v/v) dimethyl sulfoxide, 10% (v/v) ethanol, 39.5% (v/v) saline, and 50% (v/v) polyethylene glycol 400 at a concentration of 0.5 mg/ml. Under 2–5% isoflurane anesthesia, pregnant mice on gd 15 were administered 1 mg/kg NBUP by retro-orbital injection (60–70 μl each mouse). The 1 mg/kg dose was selected based on literature data about elicitation of a significant decrease in respiratory rate in mice and achievement of maternal plasma exposure of NBUP in