1521-009X/51/3/269–275$35.00
DRUG METABOLISM AND DISPOSITION

dx.doi.org/10.1124/dmd.122.001110
Drug Metab Dispos 51:269–275, March 2023

Maternal and Fetal Exposure to (-)-Δ^9-tetrahydrocannabinol and Its Major Metabolites in Pregnant Mice Is Differentially Impacted by P-glycoprotein and Breast Cancer Resistance Protein[S]

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Received September 8, 2022; accepted November 16, 2022

ABSTRACT

(-)-Δ^9-tetrahydrocannabinol (THC) is the primary pharmacological active constituent of cannabis. 11-hydroxy-THC (11-OH-THC) and 11-*nor*-9-carboxy-THC (THC-COOH) are respectively the active and nonactive circulating metabolites of THC in humans. While previous animal studies reported that THC could be a substrate of mouse P-glycoprotein (P-gp) and breast cancer resistance protein (Bcrp), we have shown, *in vitro*, that only THC-COOH is a weak substrate of human BCRP, but not of P-gp. To confirm these findings and to investigate the role of P-gp and/or Bcrp in the maternal-fetal disposition of THC and its metabolites, we administrated 3 mg/kg of THC retro-orbitally to FVB wild-type (WT), *P-gp*$^{-/-}$, *Bcrp*$^{-/-}$, or *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ pregnant mice on gestation day 18 and estimated the area under the concentration-time curve (AUC) of the cannabinoids in the maternal plasma, maternal brain, placenta, and fetus, as well as the tissue/maternal plasma AUC geometric mean ratios (GMRs) using a pooled data bootstrap approach. We found that the dose-normalized maternal plasma AUCs of THC in *P-gp*$^{-/-}$ and *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ mice, and the placenta-to-maternal plasma AUC GMR of THC in *Bcrp*$^{-/-}$ mice were 279%, 271%, and 167% of those in WT mice, respectively. Surprisingly, the tissue-to-maternal plasma AUC GMRs of THC and its major metabolites in the maternal brain, placenta, or fetus in *P-gp*$^{-/-}$, *Bcrp*$^{-/-}$ or *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ mice were 28–78% of those in WT mice. This study revealed that P-gp and Bcrp do not play a role in limiting maternal brain and fetal exposure to THC and its major metabolites in pregnant mice.

SIGNIFICANCE STATEMENT

This study systematically investigated whether P-gp and/or Bcrp in pregnant mice can alter the disposition of THC, 11-OH-THC, and THC-COOH. Surprisingly, except for Bcrp, which limits placental (but not fetal) exposure to THC, we found that *P-gp*$^{-/-}$, *Bcrp*$^{-/-}$, and/or *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ significantly decreased exposure to THC and/or its metabolites in maternal brain, placenta, or fetus. The mechanistic basis for this decrease is unclear and needs further investigation. If replicated in humans, P-gp- or BCRP-based drug–cannabinoid interactions are not of concern.

Introduction

Cannabis usage is escalating in the United States (Carliner et al., 2017; Grotenhermen and Müller-Vahl, 2017). Based on the self-reported prevalence of cannabis use in pregnant women in the United States from 2002–2003 to 2016–2017, past-month use doubled and past-month daily/nearly daily use quadrupled across the entire gestational period (Volkow et al., 2019). (-)-Δ^9-tetrahydrocannabinol (THC) is the major psychoactive constituent in cannabis. In humans, THC is primarily metabolized by the cytochrome P450 (CYP) enzyme cytochrome P450 2C9 to the psychoactive metabolite 11-hydroxy-THC (11-OH-THC) and sequentially to the non-psychoactive metabolite 11-*nor*-9-carboxy-THC (THC-COOH) (Grotenhermen, 2003; Patilea-Vrana et al., 2019). Notwithstanding the increasing understanding of cannabinoid metabolism by cytochrome P450 enzymes, little is known about the roles of transporters in cannabinoid disposition, including maternal and fetal exposure during pregnancy.

P-glycoprotein (P-gp), encoded by *ABCB1* in humans (Ueda et al., 1987), is a member of ATP-binding cassette (ABC) efflux transporter superfamily. The rodent P-gp orthologs are encoded by two genes with high homology to human *ABCB1*, namely *Abcb1a* and *Abcb1b* (Borst and Schinkel, 2013). Breast Cancer Resistance Protein (BCRP), encoded by *ABCG2* in humans, is another pivotal member of ABC efflux transporters (Allikmets et al., 1998; Doyle et al., 1998; Miyake et al., 1999). In rodents, Bcrp is encoded by *Abcg2* with 80–90% homology to human *ABCG2* (Allen et al., 1999). Both P-gp and BCRP are highly expressed in the brain capillary endothelial cells and placental syncytiotrophoblasts, exerting important roles in tissue distribution with a broad spectrum of substrates (Saidijam et al., 2018; Han et al., 2018). Both humans and rodents express P-gp and BCRP on the luminal membrane of brain capillary endothelial cells, directly facing the systemic circulation (Virgintino et al., 2002). On the other hand, human placental P-gp and BCRP are localized on the apical membrane of the syncytiotrophoblast monolayer, directly facing the maternal systemic circulation (Sun et al., 2006; Yeboah et al., 2006); however, rodent placental P-gp and BCRP are expressed on the apical membrane of the second layer of the syncytiotrophoblasts without direct contact with the maternal systemic

This work was supported by the National Institutes of Health's National Institute on Drug Abuse [Grant P01DA032507].
The authors declare no conflict of interest.
dx.doi.org/10.1124/dmd.122.001110.
[S] This article has supplemental material available at dmd.aspetjournals.org.

ABBREVIATIONS: 11-OH-THC, 11-hydroxy-Δ^9-tetrahydrocannabinol; ABC, ATP-binding cassette; AUC, area under concentration-time curve; BCRP/Bcrp, breast cancer resistance protein; CI, confidence interval; DPBS, Dulbecco's phosphate-buffered saline; GM, geometric mean; GMR, geometric mean ratio; LC-MS/MS, liquid chromatography-tandem mass spectrometry; P-gp, P-glycoprotein; THC, (-)-Δ^9-tetrahydrocannabinol; THC-COOH, 11-*nor*-9-carboxy-Δ^9-tetrahydrocannabinol; WT, wild-type.

circulation (Uchida et al., 2011). In rodents, connexin26 serves as the intracellular channel to connect the basal membrane of the first layer and the apical membrane of the second layer of the syntiotrophoblasts (Gabriel et al., 1998).

A previous study using a catharized pregnant macaque model demonstrated that in fetal plasma the area under concentration-time curve (AUC) of THC is ~30% of maternal plasma AUC of THC, while fetal exposure to THC-COOH is almost unquantifiable, indicating that the placental barrier where P-gp and BCRP are highly expressed might limit fetal exposure to THC and THC-COOH (Bailey et al., 1987). Afterward, animal studies revealed that the AUC of THC in plasma of *P-gp*-deficient CF1 mice was 2.17-fold higher than that in wild-type (WT) CF1 mice after oral administration of THC, suggesting the potential role of P-gp in limiting oral absorption of THC (Bonhomme-Faivre et al., 2008). Subsequently, Spiro et al. showed higher brain/blood THC concentration ratios in *P-gp*$^{-/-}$ or *Bcrp*$^{-/-}$ FVB mice compared with that in FVB WT mice at certain time points after intraperitoneal administration of THC (Spiro et al., 2012). These animal data suggest that THC is a P-gp and Bcrp substrate (Bonhomme-Faivre et al., 2008; Spiro et al., 2012). However, in the study by Spiro et al., the brain/blood THC concentration ratios in *P-gp*$^{-/-}$ or *Bcrp*$^{-/-}$ mice were not consistently higher than those in WT mice across all time points, making the conclusion that THC is a substrate of P-gp or Bcrp questionable. Our previous *in vitro* transport study using Madin-Darby canine kidney II cells and plasma membrane vesicles overexpressing human P-gp or human BCRP revealed that at pharmacologically relevant concentrations, only THC-COOH is a weak substrate and inhibitor of BCRP, but not of P-gp, while THC and 11-OH-THC are neither substrates nor inhibitors of P-gp or BCRP (Chen et al., 2021). Given these conflicting data, it is worthwhile to investigate whether P-gp and Bcrp play a role in determining the *in vivo* disposition of THC and its major metabolites, including exposure to the fetus. Due to logistical reasons, such studies in pregnant women are not possible, and thus studies in animal models are the only feasible alternatives to human *in vivo* studies. To date, no such *in vivo* animal studies have been reported. Therefore, in this study, we administered THC (3 mg/kg) retro-orbitally to FVB WT, *P-gp*$^{-/-}$, *Bcrp*$^{-/-}$, and *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ pregnant mice to determine if mouse P-gp and/or Bcrp limit brain, placental, and fetal exposure to THC, 11-OH-THC, and THC-COOH.

Materials and Methods

Materials. THC (10 mg/ml in ethanol, >95% pure) stock solution was provided by the National Institute on Drug Abuse (Bethesda, MD). THC, 11-OH-THC, THC-COOH and their deuterated internal standards (d^3-THC, d^3-11-OH-THC, d^3-THC-COOH) (100 μg/ml in methanol for each) were from Cerilliant (Round Rock, TX). Optima grade acetonitrile, water, and formic acid were from Thermo Fisher Scientific (Waltham, MA). Isoflurane was from Piramal Healthcare (Bethlehem, PA) through the University of Washington Medical Center Pharmacy (Seattle, WA). Hank's Balanced Salt Solution (1 ×) was purchased from Mediatech (Manassas, VA). Normal saline was from Quality Biologic (Gaithersburg, MD). Tween-80 and DMSO were from Sigma-Aldrich (St. Louis, MO). Dulbecco's phosphate-buffered saline was from Gibco Life Technologies (Carlsbad, CA). Ethanol was from Decon Laboratories (King of Prussia, PA). Homogenization tubes, beads, and bead ruptor homogenizer were obtained from Omni International (Kennesaw, GA). Heparinized microcentrifuge tubes, 1 ml syringes, and PrecisionGlide 26G × 1/2″ needles were from BD Bioscience (San Jose, CA). Low-binding tubes were from Genesee Scientific (San Diego, CA). Polycarbonate ultracentrifuge tubes were from Beckman Coulter (Brea, CA). Kimwipe was from Kimberly-Clark Professional (Irving, TX).

Animals. FVB wild-type (WT), *P-gp*$^{-/-}$ (FVB.129P2-ATP-binding cassette (*Abc*)*b1a*^{tm1Bor}*Abcb1b*^{tm1Bor} N12), *Bcrp*$^{-/-}$ (FVB.129P2-*Abcg2*^{tm1Ahs} N7), *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ (FVB.129P2-*Abcb1a*^{tm1Bor}*Abcb1b*^{tm1Bor}*Abcg2*^{tm1Ahs} N7) mice, aged 4–6 weeks upon delivery, were purchased from Taconic Biosciences (Germantown, NY) and cared for in accordance with the Guide for the Care and Use of Laboratory Animals published by the National Research Council. The animal protocol for this study was approved by the Institutional Animal Care and Use Committee at the University of Washington (Seattle, WA). Mice were maintained under 14/10 light/dark cycles while food and water were provided ad libitum. Female mice, 6–8 weeks of age, were mated with male mice of the same genotype overnight. The presence of a sperm plug after overnight housing was defined as gestation day 1. Progress of pregnancy was monitored by body weight increase and visual inspection.

Animal Studies. THC was dissolved in a solution containing 5.6% ethanol (v/v), 5.6% Tween-80 (v/v), and 88.8% normal saline (v/v) at a concentration of 1 mg/ml in a low-binding tube. Under anesthesia (2–5% isoflurane), pregnant mice on gestation day 18 were administered 3 mg/kg body weight of THC by retro-orbital injection. Depth of anesthesia was evaluated using the front-toe pinch method. The 3 mg/kg dose was selected according to Spiro et al. (Spiro et al., 2012). At various time points (2, 5, 10, 30, 60, 120, 180, and 240 minutes) after administration, mice (n = 3 per time point) were euthanized by cardiac puncture followed by organ collection under anesthesia. Maternal blood was collected in heparinized microcentrifuge tubes and centrifuged at 2,000 × g for 10 minutes at 4°C to harvest plasma. Maternal brain as well as individual placentas and fetuses were collected from pregnant mice immediately following maternal blood collection. Half of the fetuses collected were subject to separation of fetal brains from the remainder of the fetus (henceforth referred to as fetal remains) under a microscope. All tissues were immediately rinsed with Hank's Balanced Salt Solution. Maternal plasma and miscellaneous tissue samples were snap-frozen on dry ice and stored at -80°C until analysis.

Determination of Protein Binding in Maternal Plasma. Maternal plasma samples from pregnant mice of each genotype were diluted 10-fold by adding Dulbecco's phosphate-buffered saline in low-binding tubes. Maternal plasma from three randomly chosen pregnant mice per genotype was used. For each genotype, 0.8 ml of diluted plasma was spiked (1:100, v/v) with a cocktail of THC/11-OH-THC/THC-COOH (final concentration 333.3 ng/ml for each cannabinoid). After spiking, samples were mixed by placing them on a shaker (500 rpm) at 37°C for 20 minutes. For each sample, each 180 μl sample was aliquoted into four ultracentrifuge tubes. Half of the ultracentrifuge tubes were incubated at 37°C on a shaker (120 rpm) for 90 minutes, while the other half were centrifuged at 37°C at 435,000 ×g for 90 minutes. Then, 50 μl of the centrifuged (middle layer) or uncentrifuged sample was mixed with 100 μl of acetonitrile containing d^3-THC/d^3-11-OH-THC/d^3-THC-COOH (100 nM each) and centrifuged at 4°C at 25,314 ×g (maximal speed) for 20 minutes. For the ultracentrifuged samples, the outer surface of the pipette tip was gently wiped with Kimwipes to remove adhered lipids before mixing with the internal standards. Finally, the supernatant was transferred to disposable clean glass inserts and 10 μl of the sample were analyzed using liquid chromatography in tandem with mass spectrometry (LC-MS/MS) as described before (Patilea-Vrana et al., 2019). The unbound percentage in the diluted plasma ($f_{u,d}$, calculated by peak-area ratio of the ultracentrifuged sample by the peak-area ratio of the corresponding non-centrifuged sample) was extrapolated back to the unbound percentage in plasma ($f_{u,p}$) (Schuhmacher et al., 2000): $f_{u,p}$ = (dilution factor · $f_{u,d}$)/[1- $f_{u,d}$ · (1- dilution factor)].

Determination of Protein Binding in Mouse Maternal Brain Homogenate. Maternal brain homogenates from six female mice (time points of samples and animals were chosen randomly) of each genotype were diluted 10-fold by adding Dulbecco's phosphate-buffered saline in low-binding tubes. For each sample, 1 ml of diluted maternal brain homogenate was spiked (1:100, v/v) with a cocktail of THC/11-OH-THC/THC-COOH (final concentration: 333.3 ng/ml for each cannabinoid). After spiking, samples were placed on a shaker (500 rpm) at 37°C for 20 minutes. For each sample, each 180 μl was aliquoted into four ultracentrifuge tubes. Then, the unbound percentage was determined using the same procedure as described above for the plasma.

LC-MS/MS Quantification of THC, 11-OH-THC, THC-COOH in Maternal Plasma and Tissues. For maternal brains, individual whole fetuses, and fetal remains, 200 μl of normal saline was added to 100 mg of tissue and homogenized at 4°C using Omni Bead Ruptor Homogenizer at 5.8 m/s, 45 seconds/cycle, 2 cycles, 30-second dwell time. For placenta and fetal brain, 400 μl of normal saline was used due to the small quantity of these tissues. Then, all tissue homogenates were transferred to low-binding tubes. To every 50 or 100 μl of maternal plasma or tissue homogenate, 150 or 300 μl of acetonitrile

containing d^3-THC/d^3-11-OH-THC/d^3-THC-COOH (100 nM each) were added to precipitate proteins, respectively. All samples were vigorously vortexed for 30 seconds and centrifuged at 25,314 ×*g* for 20 minutes at 4°C. Supernatants were transferred to disposable clean glass inserts and, except for maternal brain samples, 10 μl per sample were injected for LC-MS/MS analysis as described previously (Patilea-Vrana et al., 2019). For analysis of the maternal brain concentration, the LC gradient was modified to separate interference peaks from the analytes, but the MS/MS method remained unchanged. Specifically, the gradient (0.3 ml/min) was: from 0 to 0.5 minutes, 90% A (water + 0.1% formic acid) and 10% B (acetonitrile + 0.1% formic acid); from 0.5 to 5 minutes, linearly decreased A from 90% to 5%, and maintained up to 6 minutes; from 6 to 6.1 minutes, linearly increased A from 5% to 90%, and then maintained up to 8 minutes. No obvious matrix effects were seen between mouse plasma or tissue of any genotype and human plasma (data not shown). Therefore, human plasma spiked with serially-diluted cannabinoid stocks (in DMSO) was used to prepare the calibrators (2.06 nM to 2000 nM) and the quality control samples. The lower quantification limits for THC/11-OH-THC/THC-COOH were 1 nM. The intraday and interday variation was below 15%. All plasma or tissue quantification concentrations were converted back to ng/ml for maternal plasma and ng/g tissue for tissue (assuming 1 g/cm^3 tissue density) based on the molecular weight and dilution of the tissue homogenate. Cannabinoid concentration in the placenta, fetal brain, fetal remains, and the whole fetus was determined and averaged for each dam.

Data Analyses. The placental concentrations of cannabinoids were corrected for blood in the placenta prior to further analysis using the equation proposed by Barker et al. (Barker et al., 1994; Eliesen et al., 2020). To fit the equation, cannabinoid concentrations in maternal blood were estimated from the maternal plasma concentrations and the previously published blood/plasma concentration ratios (Karschner et al., 2012), and cannabinoid concentrations in fetal blood were assumed to be the same as fetal concentrations. The maternal concentrations of cannabinoids were expressed in ng/ml/μg THC dose (3 mg/kg × body weight) when computing and comparing the dose-normalized AUCs among the genotypes. Using a bootstrapping method with 10,000 iterations, the AUCs over 0 to 240 minutes for each analyte in maternal plasma (normalized or not normalized) or tissues from each genotype were estimated and extrapolated back to time 0 using a noncompartmental approach using the linear trapezoidal rule (https://github.com/shirewoman2/LaurasHelpers). All AUCs were extrapolated to time infinity, and the percentage of extrapolation was calculated. A small number of not available (NA) values due to the algorithm in the bootstrapped AUCs were excluded for further processing. The geometric means of dose-normalized maternal plasma AUCs and tissue-to-maternal plasma AUC geometric mean ratios (GMRs), and 95% confidence intervals (95% CI) were calculated for each genotype-analyte-tissue group. The statistically significant differences in maternal plasma or tissue exposure of each cannabinoid between WT and each genotype mice were analyzed using two-way ANOVA followed by Dunnett's *post hoc* test assuming a significance level of 0.05. Differences with adjusted *p* values of <0.05 were considered statistically significant. The differences in unbound percentage of cannabinoids in plasma or maternal brain for all genotypes were analyzed using the ANOVA. All analyses were performed using R (version 4.0.5) or GraphPad Prism 9 (La Jolla, CA).

Results

Maternal Plasma Exposure to THC, 11-OH-THC, and THC-COOH in WT, *P-gp*$^{-/-}$, *Bcrp*$^{-/-}$, or *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ Pregnant Mice. The bootstrapped dose-normalized maternal plasma geometric mean $AUC_{0\text{-}240}$ of THC in *P-gp*$^{-/-}$ or *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ pregnant mice was significantly increased 2.79-fold or 2.71-fold, respectively, as compared with that in WT pregnant mice (Table 1). However, such significant differences in $AUC_{0\text{-}\infty}$ of THC disappeared after extrapolation to infinity (Table 1). We did not calculate $AUC_{0\text{-}\infty}$ of 11-OH-THC in *P-gp*$^{-/-}$ or *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ pregnant mice due to lack of discernible terminal phase (Fig. 1), even though the terminal phase could be observed in unnormalized maternal plasma concentration-time profiles for 11-OH-THC (Supplementary Fig. 1). No significant differences were observed in dose-normalized geometric mean AUC ($AUC_{0\text{-}240}$ or $AUC_{0\text{-}\infty}$) of THC between WT and *Bcrp*$^{-/-}$ pregnant mice or in dose-normalized geometric mean AUCs of 11-OH-THC and THC-COOH between WT and any of the transporter knockout genotype (Table 1). While the percentages of extrapolation of AUCs for THC were below 6%, the percentages of extrapolation of AUCs for THC-COOH exceeded 20% in *P-gp*$^{-/-}$ or *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ pregnant mice (Table 1).

TABLE 1

Dose-normalized maternal plasma geometric mean AUCs of THC, 11-OH-THC, and THC-COOH in WT and knockout mice

Dose-normalized geometric mean $AUC_{0\text{-}240}$ (95% CI) (μg/mL/μg dose · min)	WT	*P-gp*$^{-/-}$	Adjusted *P* value	*Bcrp*$^{-/-}$	Adjusted *P* value	*P-gp*$^{-/-}$/*Bcrp*$^{-/-}$	Adjusted *P* value
THC	0.56 (0.39, 0.80)	1.56 (0.94, 2.57)	0.0030	0.88 (0.56, 1.38)	0.26	1.52 (0.93, 2.48)	0.0037
11-OH-THC	0.07 (0.06, 0.08)	0.12 (0.08, 0.19)	0.23	0.14 (0.08, 0.24)	0.068	0.11 (0.07, 0.17)	0.28
THC-COOH	0.21 (0.19, 0.23)	0.13 (0.10, 0.17)	0.23	0.18 (0.14, 0.23)	0.91	0.14 (0.11, 0.18)	0.36
Dose-normalized geometric mean $AUC_{0\text{-}\infty}$ (95% CI) (μg/ml/μg dose · min)	WT	*P-gp*$^{-/-}$	Adjusted *P* value	*Bcrp*$^{-/-}$	Adjusted *P* value	*P-gp*$^{-/-}$/*Bcrp*$^{-/-}$	Adjusted *P* value
THC	0.59 (0.36, 0.99)	1.56 (0.65, 3.74)	0.50	0.89 (0.30, 2.69)	0.92	1.56 (0.63, 3.86)	0.49
11-OH-THC	0.08 (0.07, 0.09)	NA[a]	NA[a]	0.15 (0.05, 0.45)	0.37[b]	NA[a]	NA[a]
THC-COOH	0.24 (0.22, 0.26)	0.21 (0.05, 0.89)	1.0	0.21 (0.04, 1.05)	1.0	0.21 (0.05, 0.79)	1.0

Extrapolation%	WT	*P-gp*$^{-/-}$	*Bcrp*$^{-/-}$	*P-gp*$^{-/-}$/*Bcrp*$^{-/-}$
THC	5.54	0.03	1.55	2.40
11-OH-THC	11.24	NA[a]	4.39	NA[a]
THC-COOH	13.34	37.85	16.03	32.41

[a] NA stands for not available. [b] Statistical analysis performed using unpaired *t* test. As shown in Fig. 1, the dose-normalized plasma concentrations of 11-OH-THC in *P-gp*$^{-/-}$ and *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ pregnant mice the terminal phase could not be discerned and therefore could not be extrapolated to infinity. Data shown are the bootstrapped geometric means (95% confidence interval) (n = 3) of dose-normalized maternal plasma $AUC_{0\text{-}240}$ and $AUC_{0\text{-}\infty}$ of THC, 11-OH-THC, and THC-COOH after retro-orbital injection of THC (3 mg/kg) to pregnant mice on gestation day 18. Percentages of extrapolation were also calculated for each cannabinoid in each genotype. Differences between WT and *P-gp*$^{-/-}$, *Bcrp*$^{-/-}$, or *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ pregnant mice were analyzed by two-way ANOVA followed by the Dunnett's *post hoc* test. Differences indicated in underline were statistically significant with *p* values of <0.05 that were adjusted for multiple comparison.

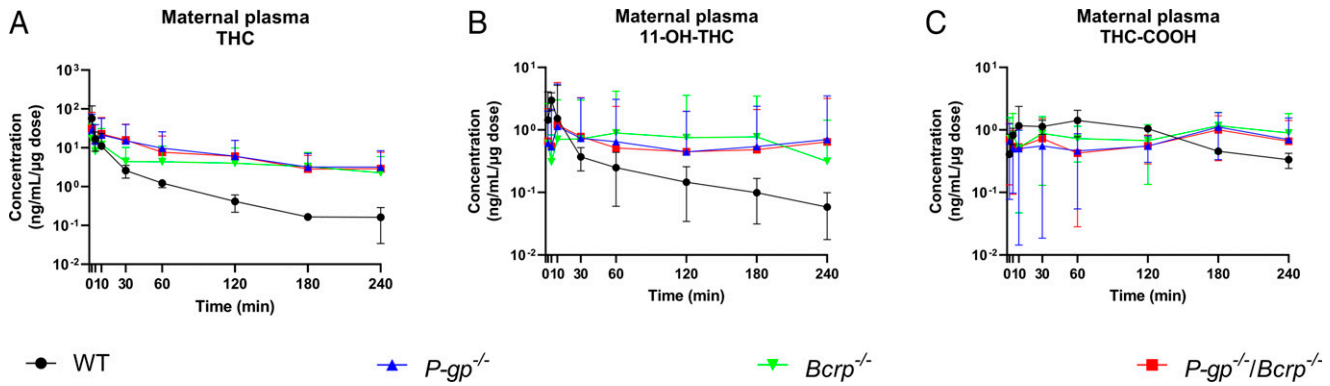

Fig. 1. Dose-normalized maternal plasma concentration-time profiles of THC, 11-OH-THC, and THC-COOH. Compared with the WT mice (•), the mean dose-normalized maternal plasma concentration-time profiles of THC (**A**), were significantly higher in *P-gp*$^{-/-}$ (▲) and *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ (□), but not in *Bcrp*$^{-/-}$ (▼) mice. However, the mean dose-normalized maternal plasma concentration-time profiles of 11-OH-THC (**B**), and THC-COOH (**C**) were not different across the genotypes. Mice were administered 3 mg/kg of THC by retro-orbital injection on gestation day 18. Data are mean ± S.D. (n = 3 at each time point).

Maternal Brain Exposure to THC, 11-OH-THC, and THC-COOH in WT, *P-gp*$^{-/-}$, *Bcrp*$^{-/-}$, or *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ Pregnant Mice. Next, we examined maternal brain exposure to THC and its major metabolites in WT, *P-gp*$^{-/-}$, *Bcrp*$^{-/-}$, and *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ pregnant mice. Since the absolute tissue exposure is driven by the absolute maternal plasma exposure, we determined the maternal brain-to-maternal plasma AUC geometric mean ratios (GMRs). We found that the maternal brain/maternal plasma $AUC_{0\text{-}240}$ GMR of THC in *P-gp*$^{-/-}$ pregnant mice was significantly decreased by 72% compared with that in WT pregnant mice (Table 2). While the maternal brain/maternal plasma $AUC_{0\text{-}240}$ GMRs of 11-OH-THC in *P-gp*$^{-/-}$ and *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ pregnant mice were significantly, but modestly, decreased by 35% and 39%, respectively, versus WT pregnant mice, the difference between *Bcrp*$^{-/-}$ and WT pregnant mice was not statistically significant (Table 2). The maternal brain/maternal plasma $AUC_{0\text{-}240}$ GMR of THC-COOH in *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ pregnant mice was significantly, but modestly, lower by 24% compared with that in WT pregnant mice, whereas no significant differences were observed between WT and *P-gp*$^{-/-}$ *or* *Bcrp*$^{-/-}$ pregnant mice (Table 2). Compared with WT pregnant mice, we found similar observations for the maternal brain/maternal plasma

TABLE 2

The maternal brain, placental, and fetal to maternal plasma $AUC_{0\text{-}240}$ GMRs of THC, 11-OH-THC, and THC-COOH

	$AUC_{0\text{-}240}$ GMR (95% CI)	$AUC_{0\text{-}240}$ GMR (95% CI)	Adjusted *P* value	$AUC_{0\text{-}240}$ GMR (95% CI)	Adjusted *P* value	$AUC_{0\text{-}240}$ GMR (95% CI)	Adjusted *P* value
THC	WT	*P-gp*$^{-/-}$		*Bcrp*$^{-/-}$		*P-gp*$^{-/-}$/*Bcrp*$^{-/-}$	
Maternal brain	0.55 (0.37, 0.80)	0.15 (0.09, 0.26)	<0.0001	0.64 (0.55, 0.74)	0.77	0.35 (0.24, 0.51)	0.065
Placenta	0.36 (0.24, 0.55)	0.28 (0.23, 0.34)	0.40	0.61 (0.52, 0.71)	0.029	0.53 (0.40, 0.69)	0.14
Fetal remains	0.20 (0.14, 0.30)	0.14 (0.12, 0.16)	0.12	0.27 (0.23, 0.32)	0.31	0.20 (0.15, 0.26)	1.0
Fetus	0.17 (0.11, 0.25)	0.16 (0.14, 0.19)	1.0	0.22 (0.18, 0.27)	0.39	0.16 (0.12, 0.21)	0.97
11-OH-THC	WT	*P-gp*$^{-/-}$		*Bcrp*$^{-/-}$		*P-gp*$^{-/-}$/*Bcrp*$^{-/-}$	
Maternal brain	1.26 (1.07, 1.49)	0.82 (0.72, 0.93)	0.0011	1.45 (1.26, 1.67)	0.48	0.77 (0.61, 0.96)	0.0002
Placenta	1.38 (1.18, 1.62)	1.02 (0.88, 1.18)	0.026	1.51 (1.31, 1.75)	0.77	1.25 (1.08, 1.45)	0.71
Fetal remains	1.17 (1.00, 1.36)	0.76 (0.67, 0.86)	0.0013	1.01 (0.88, 1.17)	0.47	0.78 (0.70, 0.88)	0.0026
Fetus	1.15 (0.98, 1.35)	0.57 (0.50, 0.64)	<0.0001	1.02 (0.87, 1.21)	0.60	0.59 (0.51, 0.68)	<0.0001
THC-COOH	WT	*P-gp*$^{-/-}$		*Bcrp*$^{-/-}$		*P-gp*$^{-/-}$/*Bcrp*$^{-/-}$	
Maternal brain	0.25 (0.22, 0.28)	0.23 (0.19, 0.26)	0.48	0.22 (0.20, 0.26)	0.43	0.19 (0.15, 0.24)	0.0064
Placenta	0.87 (0.76, 0.98)	0.75 (0.68, 0.83)	0.25	1.02 (0.92, 1.13)	0.14	0.67 (0.61, 0.74)	0.012
Fetal remains	0.54 (0.47, 0.61)	0.40 (0.36, 0.44)	0.0020	0.46 (0.40, 0.52)	0.16	0.33 (0.29, 0.36)	<0.0001
Fetus	0.50 (0.43, 0.58)	0.27 (0.23, 0.31)	<0.0001	0.39 (0.33, 0.46)	0.013	0.29 (0.26, 0.31)	<0.0001

Data shown are the bootstrapped tissue/maternal plasma AUC GMRs of THC, 11-OH-THC, and THC-COOH after retro-orbital injection of THC (3 mg/kg) to pregnant mice on gestation day 18 over 240 min. Data are reported as geometric mean ratio (GMR) (95% confidence interval) (n = 3). Differences in AUC GMRs between WT and *P-gp*$^{-/-}$, *Bcrp*$^{-/-}$, or *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ pregnant mice were analyzed by two-way ANOVA followed by the Dunnett's *post hoc* test. Differences indicated in underline were statistically significant with p values of <0.05 that were adjusted for multiple comparisons.

$AUC_{0-\infty}$ GMR of THC in *P-gp*$^{-/-}$ pregnant mice and the maternal brain/maternal plasma $AUC_{0-\infty}$ GMR of 11-OH-THC in *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ pregnant mice, but not of any other cannabinoids in any other genotypes (Supplementary Table 1). The percentages of extrapolations of AUCs of cannabinoids were generally not greater than 20%, except for AUC of THC-COOH in WT pregnant mice and AUCs of 11-OH-THC and THC-COOH in *P-gp*$^{-/-}$ pregnant mice (Supplementary Table 2).

Placental Exposure to THC, 11-OH-THC, and THC-COOH in WT, *P-gp*$^{-/-}$, *Bcrp*$^{-/-}$, or *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ Pregnant Mice. The placenta/maternal plasma AUC_{0-240} GMRs of THC in *Bcrp*$^{-/-}$ pregnant mice was increased 1.67-fold as compared with that in WT pregnant mice (Table 2). The placenta/maternal plasma AUC_{0-240} GMR of 11-OH-THC in *P-gp*$^{-/-}$ pregnant mice was significantly, but modestly, decreased by 26% compared with that in WT pregnant mice (Table 2). The placenta/maternal plasma AUC_{0-240} GMR of THC-COOH in *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ pregnant mice was significantly, but modestly, decreased by 22% versus that in WT pregnant mice (Table 2). None of the other placenta/maternal plasma AUC_{0-240} GMRs in transporter knockout mice significantly differed from those in WT pregnant mice (Table 2). None of the $AUC_{0-\infty}$ GMRs of the cannabinoids in transporter knockout mice significantly differed from those in WT pregnant mice (Supplementary Table 1). About half of the percentages of extrapolations of the placental AUCs exceeded 20% (Supplementary Table 2).

Fetal Exposure to THC, 11-OH-THC, and THC-COOH in WT, *P-gp*$^{-/-}$, *Bcrp*$^{-/-}$, or *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ Pregnant Mice. We found no statistically significant differences in fetal (both fetus and fetal remains) AUC_{0-240} GMRs of THC among all four mouse groups (Table 2). The fetal (fetus and fetal remains) AUC_{0-240} GMRs of 11-OH-THC in *P-gp*$^{-/-}$ and *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ pregnant mice were significantly, but modestly, decreased by 33–51% compared with that in WT pregnant mice (Table 2). The fetal (fetus and fetal remains) AUC_{0-240} GMRs of THC-COOH in *P-gp*$^{-/-}$ and *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ pregnant mice were significantly, but modestly, decreased by 26–46% versus WT pregnant mice (Table 2). While the fetus AUC_{0-240} GMR of THC-COOH in *Bcrp*$^{-/-}$ pregnant mice was moderately decreased by 22% versus WT pregnant mice, its fetal remains AUC_{0-240} GMR in *Bcrp*$^{-/-}$ pregnant mice was not significantly different from that in WT mice despite with the same trend (Table 2). Compared with WT pregnant mice, we did not observe significant differences in $AUC_{0-\infty}$ GMRs of 11-OH-THC/THC-COOH in fetal remains of *P-gp*$^{-/-}$ pregnant mice, or in $AUC_{0-\infty}$ GMR of THC-COOH in fetus of *Bcrp*$^{-/-}$ pregnant mice (Supplementary Table 1). The majority of the percentages of extrapolation of the fetal AUCs exceeded 20% (Supplementary Table 2).

Fetal Brain Exposure to THC, 11-OH-THC, and THC-COOH. THC and its major metabolites in fetal brain tissues were not quantifiable by LC-MS/MS in any of the mouse groups investigated in this study. The peaks for THC, 11-OH-THC, and THC-COOH in their corresponding channel were either not greater than the lower limit of detection or the lower limit of quantification except for only a few individual samples (data not shown).

Protein Binding of Cannabinoids in Maternal Plasma. To investigate whether the differential tissue distributions of the cannabinoids described above were due to differential plasma protein binding of THC and its major metabolites among different genotypes, we determined the unbound percentages of THC, 11-OH-THC, and THC-COOH in maternal plasma samples from WT, *P-gp*$^{-/-}$, *Bcrp*$^{-/-}$, *and P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ mice using ultracentrifugation. The unbound percentages of THC, 11-OH-THC, and THC-COOH in maternal plasma were within the ranges of 2.84–5.87%, 2.38–5.45%, and 2.31–6.92%, respectively, and did not differ significantly among the four mouse genotypes (Table 3).

TABLE 3

Unbound percentages of THC, 11-OH-THC, and THC-COOH in maternal plasma

f_u (%)	THC	11-OH-THC	THC-COOH
WT	3.09 ± 1.54	5.87 ± 5.47	3.65 ± 0.34
P-gp$^{-/-}$	2.38 ± 2.04	5.45 ± 3.70	4.20 ± 0.94
Bcrp$^{-/-}$	2.31 ± 2.01	6.92 ± 4.76	4.56 ± 0.47
P-gp$^{-/-}$/*Bcrp*$^{-/-}$	3.09 ± 1.54	5.87 ± 5.47	3.65 ± 0.34

Data shown are the unbound percentages of THC, 11-OH-THC, and THC-COOH in maternal plasma of WT, *P-gp*$^{-/-}$, *Bcrp*$^{-/-}$, and *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ male mice after spiking 1:10 diluted blank male mouse plasma with 0.33 μg/ml (final concentration 333.3 ng/ml) of each cannabinoid. Data are reported as mean ± S.D. plasma samples from three pregnant mice per genotype with duplicate determinations for each plasma sample. Differences among the four mouse groups were analyzed by ANOVA and considered statistically significant with p values of <0.05. No statistically significant differences were found.

Protein Binding of Cannabinoids in Maternal Brain Homogenates. To investigate whether the differential tissue distributions of the cannabinoids described above were due to differential tissue binding of THC and its major metabolites, we determined the unbound percentages of THC, 11-OH-THC, and THC-COOH in maternal brain homogenates from WT, *P-gp*$^{-/-}$, *Bcrp*$^{-/-}$, *and P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ pregnant mice using ultracentrifugation. We chose maternal brain homogenates in these analyses because the greatest statistically significant difference we observed was in maternal brain exposure to THC between WT and *P-gp*$^{-/-}$ pregnant mice (Table 2). The unbound percentages of THC, 11-OH-THC, and THC-COOH fell within the ranges of 0.009–0.010%, 0.098–0.115%, and 1.08–1.23%, respectively, and did not significantly differ among the four mouse genotypes (Table 4).

Discussion

The primary goal of this study was to compare the maternal brain, placental, and fetal exposure to THC and its metabolites between WT and *P-gp*$^{-/-}$, *Bcrp*$^{-/-}$ or *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ pregnant mice. Tissue exposure is driven by maternal plasma exposure. Since maternal plasma exposure could be influenced by cofounding factors, such as variation in body weight of pregnant mice due to variation in litter size and potential impact of transporter knockout on systemic clearance of the cannabinoids, we focused our analyses on tissue-to-maternal plasma AUC ratios as the primary endpoint, which should not be affected by systemic clearance of the cannabinoids provided the maternal plasma and tissue AUCs are sufficiently captured over the duration of sampling. We used 240 minutes as the last time point of plasma sampling based on a previous study (Bonhomme-Faivre et al., 2008) which well captured maternal plasma AUC. However, the high percentages of extrapolation for calculating $AUC_{0-\infty}$ values of the cannabinoids, especially in fetal tissues, made the interpretation of $AUC_{0-\infty}$ GMR of concern. Therefore, we primarily interpreted AUC_{0-240} GMR in this study.

TABLE 4

Unbound percentages of THC, 11-OH-THC, and THC-COOH in maternal brain homogenates

f_u (%)	THC	11-OH-THC	THC-COOH
WT	0.009 ± 0.001	0.098 ± 0.019	1.08 ± 0.16
P-gp$^{-/-}$	0.009 ± 0.001	0.101 ± 0.013	1.12 ± 0.08
Bcrp$^{-/-}$	0.010 ± 0.002	0.110 ± 0.027	1.15 ± 0.07
P-gp$^{-/-}$/*Bcrp*$^{-/-}$	0.010 ± 0.003	0.115 ± 0.031	1.23 ± 0.12

Data shown are unbound percentages of THC, 11-OH-THC, and THC-COOH in maternal brain homogenates of WT, *P-gp*$^{-/-}$, *Bcrp*$^{-/-}$, and *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ pregnant mice after spiking 1:10 diluted pregnant mouse maternal brain homogenates with 0.33 μg/ml (final concentration 333.3 ng/ml) of each cannabinoid. Data are reported as mean ± S.D. (n = 6). Differences among the four mouse groups were analyzed by ANOVA, but none were found. Differences were considered statistically significant with p values of <0.05.

We found ~3-fold higher maternal plasma exposure to THC in *P-gp*$^{-/-}$ or *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ pregnant mice, suggesting that the systemic clearance of THC in *P-gp*$^{-/-}$ or *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ pregnant mice is decreased given that retro-orbital injection mimics intravenous administration. Since the metabolite to parent molar AUC ratios did not significantly differ among all the genotypes (Supplementary Table 3), changes in cannabinoid-metabolizing enzymes by P-gp knockout seem unlikely. Given that the blood clearance of THC in the WT pregnant mice is ~2.7 ml/min, it appears to be blood flow-limited (mouse hepatic blood flow is ~1.8 ml/min) (Davies and Morris, 1993). Therefore, if hepatic blood flow is affected by the P-gp knockout, this could partially explain our findings. Nevertheless, we cannot rule out the contribution of other transporters to the systemic disposition of THC. Such transporters remain to be determined in future studies.

Surprisingly, we found that the maternal brain/maternal plasma AUC GMR of THC in *P-gp*$^{-/-}$ mice was decreased by 72% (Table 2). We also observed a general trend of decrease in maternal brain/maternal plasma or fetal/maternal plasma AUC GMRs of 11-OH-THC or THC-COOH in *P-gp*$^{-/-}$ and *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ mice or no change (mostly in *Bcrp*$^{-/-}$ mice) compared with WT mice (Table 2). Likewise, the fetal/maternal plasma AUC GMR of THC were not affected by P-gp and/or Bcrp knockout (Table 2). These data strongly indicate that THC and its metabolites are not substrates of P-gp or Bcrp because, if they were, knocking out these efflux transporters at the blood-brain or blood-placental barrier should result in an increase (not a decrease) in their AUC GMR in the knockout mice. In addition, when drugs are substrates of these transporters, knocking them out results in a larger increase in maternal brain/maternal plasma AUC GMR versus the fetus/maternal plasma AUC GMR (Liao et al., 2017; Fujita et al., 2022). Here we observed a reverse trend in *P-gp*$^{-/-}$ mice for THC-COOH where the fetal/maternal plasma AUC GMR was significantly reduced while the maternal brain/maternal plasma AUC GMR was unchanged (Table 2). Moreover, the direction of transport by P-gp cannot be inverted unless artificial mutations are introduced in P-gp (Sajid et al., 2020), which is unlikely to occur *in vivo*. Collectively, these data are consistent with our *in vitro* findings that THC, 11-OH-THC, or THC-COOH are not substrates of human P-gp and only THC-COOH is a weak substrate of human BCRP (Chen et al., 2021).

We attempted to interpret our surprising finding of reduced distribution of the cannabinoids into maternal brain or fetus in *P-gp*$^{-/-}$ or *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ mice. The cannabinoid concentrations we quantified in this study were total concentrations. Theoretically, only the unbound cannabinoids can cross the tissue membrane barriers by passive diffusion. Therefore, one possibility could be that the differences in protein binding of the cannabinoids in tissues between WT and *P-gp*$^{-/-}$ or *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ mice led to greater asymmetric distributions of the cannabinoids across the tissue barriers in transporter knockout mice. For instance, lower brain tissue binding in knockout mice versus WT, while maintaining equal degree of binding to maternal plasma, could result in lower total THC tissue/maternal plasma AUC ratio in knockout mice versus WT mice, as observed in *P-gp*$^{-/-}$ mice (72% decrease). Indeed, previous investigations using *in vitro* autoradiography by incubating ^{11}C-tariquidar (a P-gp and BCRP substrate and inhibitor) with isolated brain sections showed that the binding of ^{11}C-tariquidar (a highly lipophilic compound as are the cannabinoids) to isolated brain sections from *P-gp*$^{-/-}$ and/or *Bcrp*$^{-/-}$ mice is ~50% of that in WT mice (Bauer et al., 2010). Therefore, we determined the unbound percentages of THC and its major metabolites in mouse plasma and paired maternal brain homogenate samples of six randomly selected mice. However, unlike Bauer et al., we found no significant difference in unbound percentages of the cannabinoids in maternal brain (or plasma) between WT and transporter-knockout mice (Tables 3 and 4) (Bauer et al., 2010). A limitation of this aspect of this study is that our plasma protein binding study was done with male and not pregnant female plasma (these studies were conducted after all the pregnant females had been sacrificed and there was a lack of sufficient blank pregnant female plasma).

Another possible explanation is the potential induction of other transporters at the blood-brain and blood-placental barrier in the transporter-knockout mice. However, previous studies have shown that the expressions of 12 ABC transporters and ten solute carrier transporters in the brain capillary endothelial cells isolated from *P-gp*$^{-/-}$, *Bcrp*$^{-/-}$, and *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ FVB mice are not significantly altered compared with those isolated from WT mice (Agarwal et al., 2012). Whether other transporters are induced in the placenta of *P-gp*$^{-/-}$ or *Bcrp*$^{-/-}$ mice is currently unknown. Moreover, to the best of our knowledge, whether THC and its metabolites are substrates of any other transporters has not been systemically investigated.

In WT mice, fetal exposure to THC was only about 17–20% of its maternal plasma exposure (Table 2), suggesting either extensive placental/fetal metabolism and/or efflux by transporters other than P-gp or Bcrp. This finding is consistent with the previous macaque study which showed that fetal exposure to THC was ~30% of its maternal plasma exposure (Bailey et al., 1987). In humans, THC is not metabolized by placental microsomes, but it is rapidly metabolized by fetal liver microsomes (Kumar et al., 2022). Thus, the fetal/maternal plasma AUC GMR of <1 for THC suggests that these processes may act to reduce fetal exposure to this potentially toxic cannabinoid.

Since THC is thought to produce long-term deleterious effect on the developing human brain (Day et al., 1994), it is important to determine if THC and its metabolites distribute into the fetal brain. Both P-gp and Bcrp are known to be expressed in fetal brain (Han et al., 2018). However, THC and its metabolites were not detectable or quantifiable by LC-MS/MS in the fetal brain samples regardless of genotypes in our study.

In summary, in this study, we showed that *P-gp*$^{-/-}$, *Bcrp*$^{-/-}$, and/or *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ significantly decrease exposure to THC and/or its metabolites in maternal brain, placenta, or the fetus, except for Bcrp which limits placental (but not fetal) exposure to THC. The mechanistic basis for this decreased tissue exposure is puzzling, not clear and needs further investigation. Based on these findings, we conclude that mouse P-gp or Bcrp plays a minor or no role in limiting maternal brain and fetal exposure to the cannabinoids and, in fact, P-gp and Bcrp knockout appears to reduce such exposure. These data, together with our *in vitro* data that these cannabinoids are not substrates or weak substrates (THC-COOH) of human P-gp and BCRP (Chen et al., 2021), make it unlikely that P-gp or BCRP-mediated drug-cannabinoid interactions are of concern. In addition, it is unlikely that people with reduced function genotype variants of P-gp or BCRP will experience enhanced psychoactive effects or fetal toxicity to these cannabinoids. Interestingly, the fetal/maternal plasma AUC GMR of <1 for THC suggests that the placental/fetal metabolism or placental efflux by transporters other than P-gp or Bcrp may act to reduce fetal exposure to this potentially toxic cannabinoid. Studies with human tissues (*e.g.*, perfused placenta) are needed to confirm these findings.

Acknowledgements

The authors thank Dr. Sumit Bansal, Josefin Koehn, Neha Saxena, and Dale Whittington for technical assistance in using LC-MS/MS, we also acknowledge the Department of Comparative Medicine of University of Washington, Dr. Lyrialle W. Han, and Dr. Hideaki Tomita for technical assistance in animal operation.

Authorship Contributions

Participated in research design: Chen, Unadkat, Mao.
Conducted experiments: Chen.
*Performed data analysis and interpretation:*Chen, Unadkat, Mao.

Wrote or contributed to the writing of the manuscript: Chen, Unadkat, and Mao.

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DMD-AR-2022-001110R1

Supplementary Information

Drug Metabolism and Disposition

Maternal and Fetal Exposure to (-)-Δ^9-tetrahydrocannabinol and Its Major Metabolites in Pregnant Mice Is Differentially Impacted by P-glycoprotein (P-gp) and Breast Cancer Resistance Protein (BCRP)

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Supplementary Table 1. The maternal brain, placental, and fetal to maternal plasma $AUC_{0-\infty}$ GMRs of THC, 11-OH-THC, and THC-COOH

	$AUC_{0-\infty}$ GMR (95% CI)	$AUC_{0-\infty}$ GMR (95% CI)	Adjusted *P* value	$AUC_{0-\infty}$ GMR (95% CI)	Adjusted *P* value	$AUC_{0-\infty}$ GMR (95% CI)	Adjusted *P* value
THC	WT	*P-gp*$^{-/-}$		*Bcrp*$^{-/-}$		*P-gp*$^{-/-}$/*Bcrp*$^{-/-}$	
Maternal brain	0.55 (0.37, 0.84)	**0.16 (0.10, 0.25)**	**0.0010**	0.65 (0.56, 0.75)	0.9348	0.39 (0.20, 0.76)	0.5718
Placenta	0.42 (0.27, 0.67)	0.37 (0.26, 0.53)	0.9644	0.64 (0.55, 0.75)	0.4616	0.82 (0.49, 1.39)	0.1199
Fetal remains	0.30 (0.12, 0.80)	0.17 (0.13, 0.22)	0.1939	0.36 (0.25, 0.51)	0.9246	0.29 (0.17, 0.49)	0.9952
Fetus	0.23 (0.09, 0.59)	0.21 (0.17, 0.25)	0.9658	0.26 (0.13, 0.52)	0.9731	0.22 (0.17, 0.29)	0.9965
11-OH-THC	WT	*P-gp*$^{-/-}$		*Bcrp*$^{-/-}$		*P-gp*$^{-/-}$/*Bcrp*$^{-/-}$	
Maternal brain	1.18 (1.00, 1.40)	0.94 (0.66, 1.34)	0.5721	1.44 (0.96, 2.17)	0.6695	**0.67 (0.48, 0.94)**	**0.0302**
Placenta	1.49 (1.28, 1.73)	1.34 (0.73, 2.48)	0.9327	1.54 (1.34, 1.77)	0.9967	1.08 (0.82, 1.43)	0.3175
Fetal remains	1.38 (1.16, 1.63)	0.91 (0.08, 10.11)	0.1441	1.19 (0.79, 1.80)	0.8318	**0.67 (0.51, 0.86)**	**0.0037**
Fetus	1.45 (1.03, 2.05)	**0.52 (0.44, 0.61)**	**<0.0001**	1.09 (0.65, 1.83)	0.3958	**0.48 (0.37, 0.63)**	**<0.0001**
THC-COOH	WT	*P-gp*$^{-/-}$		*Bcrp*$^{-/-}$		*P-gp*$^{-/-}$/*Bcrp*$^{-/-}$	
Maternal brain	0.32 (0.23, 0.43)	0.43 (0.18, 1.01)	0.6948	0.20 (0.14, 0.28)	0.3225	0.21 (0.14, 0.32)	0.4710
Placenta	1.34 (0.91, 1.99)	1.11 (0.85, 1.45)	0.8866	0.95 (0.65, 1.38)	0.5840	0.79 (0.71, 0.89)	0.2620
Fetal remains	1.04 (0.58, 1.85)	1.13 (0.45, 2.84)	0.9874	0.57 (0.36, 0.92)	0.1873	**0.42 (0.35, 0.50)**	**0.0223**
Fetus	1.00 (0.49, 2.05)	**0.38 (0.24, 0.59)**	**0.0136**	0.48 (0.18, 1.29)	0.0830	**0.37 (0.31, 0.44)**	**0.0106**

Data shown are the bootstrapped tissue/maternal plasma $AUC_{0-\infty}$ GMRs of THC, 11-OH-THC, THC-COOH after retro-orbital injection of THC (3 mg/kg) to pregnant mice on gestation day 18 over 240 min. Data are reported as geometric mean ratio (GMR) (95% confidence interval) (n = 3). Differences in $AUC_{0-\infty}$ GMRs between WT and *P-gp*$^{-/-}$, *Bcrp*$^{-/-}$, or *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ pregnant mice were analyzed by two-way ANOVA followed by the Dunnett's *post hoc* test. Differences indicated in bold were statistically significant with p values of < 0.05 that were adjusted for multiple comparisons.

Supplementary Table 2. The percentages of extrapolation of tissue AUCs

THC Extrapolation%	WT	*P-gp*$^{-/-}$	*Bcrp*$^{-/-}$	*P-gp*$^{-/-}$/*Bcrp*$^{-/-}$
Maternal brain	6.65	3.29	2.31	11.35
Placenta	19.05	26.66	5.88	36.70
Fetal remains	37.80	20.88	24.89	32.41
Fetus	31.81	22.12	17.67	29.64

11-OH-THC Extrapolation%	WT	*P-gp*$^{-/-}$	*Bcrp*$^{-/-}$	*P-gp*$^{-/-}$/*Bcrp*$^{-/-}$
Maternal brain	5.47	27.26	8.96	11.49
Placenta	17.71	36.58	10.13	10.19
Fetal remains	25.00	30.26	22.03	8.71
Fetus	29.76	8.34	14.87	4.08

THC-COOH Extrapolation%	WT	*P-gp*$^{-/-}$	*Bcrp*$^{-/-}$	*P-gp*$^{-/-}$/*Bcrp*$^{-/-}$
Maternal brain	31.86	57.40	14.38	17.40
Placenta	44.35	44.53	20.37	21.61
Fetal remains	55.46	71.39	40.85	28.53
Fetus	56.57	42.24	43.96	28.25

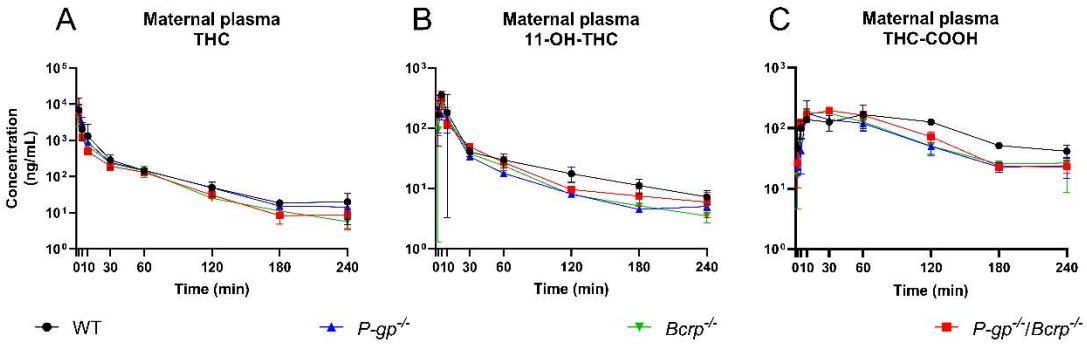
Data shown are the percentages of extrapolation of the tissue AUCs of THC, 11-OH-THC, THC-COOH in WT, *P-gp*$^{-/-}$, *Bcrp*$^{-/-}$, *and P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ pregnant mice. Data are calculated by 1-(AUC_{0-240})/($AUC_{0-\infty}$), and reported as mean.

Supplementary Table 3. Metabolite to parent maternal plasma AUC molar ratios

Molar ratio	WT	*P-gp*$^{-/-}$	*Bcrp*$^{-/-}$	*P-gp*$^{-/-}$/*Bcrp*$^{-/-}$
11-OH-THC/THC	0.14 ± 0.05	0.08 ± 0.01	0.14 ± 0.02	0.14 ± 0.03
THC-COOH/11-OH-THC	2.83 ± 0.40	2.89 ± 0.30	3.02 ± 0.32	2.91 ± 0.27

Data shown are metabolite to parent maternal plasma AUC molar ratios in WT, *P-gp*$^{-/-}$, *Bcrp*$^{-/-}$, and *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ pregnant mice. Data are reported as mean ± SD (n = 3). Differences among the four mouse groups were analyzed by ANOVA and were considered statistically significant with p values of < 0.05. No statistically significant differences were found.

Supplementary Figure 1. Unnormalized maternal plasma concentration-time profiles of THC, 11-OH-THC and THC-COOH.



Maternal plasma concentration-time profiles of THC (**A**), 11-OH-THC (**B**), and THC-COOH (**C**) over 240 min after retro-orbital injection of 3 mg/kg THC to pregnant wild-type (●), *P-gp*$^{-/-}$ (▲), *Bcrp*$^{-/-}$ (▼), and *P-gp*$^{-/-}$/*Bcrp*$^{-/-}$ (■) mice on gestation day 18. Data shown are mean ± SD (n = 3 at each time point).