

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)

Xin Chen, Jashvant D. Unadkat, Qingcheng Mao

Department of Pharmaceutics, School of Pharmacy, University of Washington, Seattle,
Washington 98195

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

	AUC _{0-∞} GMR (95% CI)	AUC _{0-∞} GMR (95% CI)	Adjusted <i>P</i> value	AUC _{0-∞} GMR (95% CI)	Adjusted <i>P</i> value	AUC _{0-∞} GMR (95% CI)	Adjusted <i>P</i> value
THC	WT	<i>P-gp</i> ^{-/-}		<i>Bcrp</i> ^{-/-}		<i>P-gp</i> ^{-/-} / <i>Bcrp</i> ^{-/-}	
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	<i>P-gp</i> ^{-/-}		<i>Bcrp</i> ^{-/-}		<i>P-gp</i> ^{-/-} / <i>Bcrp</i> ^{-/-}	
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	<i>P-gp</i> ^{-/-}		<i>Bcrp</i> ^{-/-}		<i>P-gp</i> ^{-/-} / <i>Bcrp</i> ^{-/-}	
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-∞} 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-∞} 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	<i>P-gp</i>^{-/-}	<i>Bcrp</i>^{-/-}	<i>P-gp</i>^{-/-}/<i>Bcrp</i>^{-/-}
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	<i>P-gp</i>^{-/-}	<i>Bcrp</i>^{-/-}	<i>P-gp</i>^{-/-}/<i>Bcrp</i>^{-/-}
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	<i>P-gp</i>^{-/-}	<i>Bcrp</i>^{-/-}	<i>P-gp</i>^{-/-}/<i>Bcrp</i>^{-/-}
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

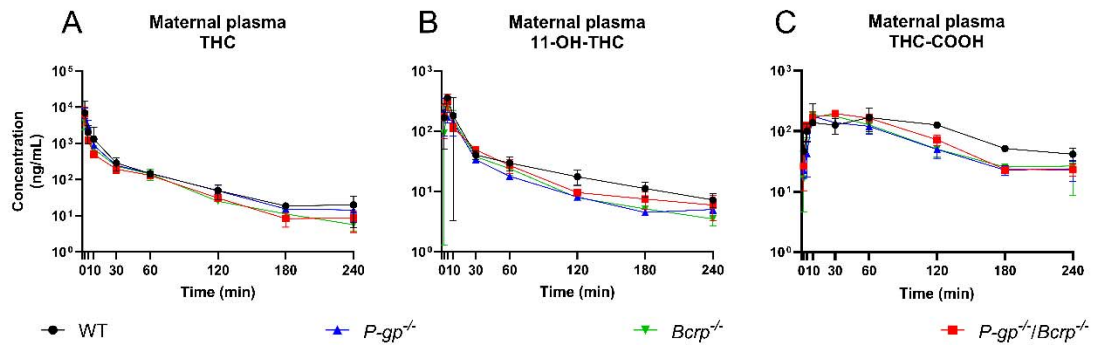
Data shown are the percentages of extrapolation of the tissue AUCs of THC, 11-OH-THC, THC-COOH in WT, *P-gp*^{-/-}, *Bcrp*^{-/-}, and *P-gp*^{-/-}/*Bcrp*^{-/-} pregnant mice. Data are calculated by $1 - (\text{AUC}_{0-240}) / (\text{AUC}_{0-\infty})$, and reported as mean.

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

Molar ratio	WT	<i>P-gp</i>^{-/-}	<i>Bcrp</i>^{-/-}	<i>P-gp</i>^{-/-}/<i>Bcrp</i>^{-/-}
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).