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The Effect of Route of Administration and Vehicle on the Pharmacokinetics of THC and CBD in Adult, Neonate, and Breastfed Sprague-Dawley Rats

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Abstract

Introduction: Basic pharmacokinetic (PK) and pharmacodynamic models of the phytocannabinoids Δ-9-tetrahydrocannabinol (THC) and cannabidiol (CBD) are critical for developing translational models of exposure and toxicity. The neonatal period is a particularly important time to study the effects of cannabinoids, yet there are few studies of cannabinoid PKs by different routes such as direct injection or breast milk ingestion. To study this question, we have developed a translationally relevant rodent model of perinatal cannabinoid administration by measuring plasma levels of THC and CBD after different routes and preparations of these drugs.

Materials and Methods: Adult animals and pups were injected with THC or CBD either intraperitoneally or subcutaneously, and plasma was analyzed by liquid chromatography–tandem mass spectrometry to measure cannabinoid levels collected at specified intervals. We also tested the effect of preparation of the drug using an oil-based vehicle (sesame oil) and an aqueous vehicle (Tween). Finally, we measured the plasma levels of cannabinoids in neonatal pups that were transmitted through breast milk after intraperitoneal injection to nursing dams.

Results: We observed differences in the PK profiles of cannabinoids in adults and neonatal pups that were dependent on the route of administration and type of vehicle. Cannabinoids prepared in aqueous vehicle, injected intraperitoneally, resulted in a high peak in plasma concentration, which rapidly decreased. In contrast, subcutaneous injections using sesame oil as a vehicle resulted in a slow rise and low plateau in plasma concentration. Intraperitoneal injections with sesame oil as a vehicle resulted in a slower rise compared with aqueous vehicle, but an earlier and higher peak compared with subcutaneous injection. Finally, the levels of THC and CBD that were similar to direct subcutaneous injections were measured in the plasma of pups nursing from intraperitoneally injected dams.

Conclusions: The route of administration and the preparation of the drug have important and significant effects on the PK profiles of THC and CBD in rats. These results can be used to create different clinically relevant exposure paradigms in pups and adults, such as short high-dose exposure or a low-chronic exposure, each of which might have significant and varying effects on development.

Keywords: cannabinoids; Δ-9-tetrahydrocannabinol (THC); cannabidiol (CBD); liquid chromatography–tandem mass spectrometry (LC-MS/MS)

Introduction

Evolving changes in perceptions about cannabinoids have led to dramatic changes in access stemming from recent legalization and decriminalization at the state and municipality level in the United States.[1–3] One of the consequences is an increased incidence of both recreational and medicinal uses. Although the pharmacology and toxicology of cannabinoids in adulthood have been widely studied, many questions remain regarding exposure during the perinatal period.[4–6] The

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perinatal period is a critical time in neurodevelopment during which cell division, differentiation, and synaptogenesis are all tightly regulated and susceptible to toxicological insult. Recent reports have shown an increase in cannabinoid use by pregnant and breastfeeding women as cannabinoids are sometimes used to relieve nausea and postpartum depression,[7–10] despite a lack of established safety thresholds for infants.[11]

Although early-life cannabinoid toxicity has not been explicitly defined, there is evidence that it can have deleterious effects on neurodevelopment. For example, Astley and Little reported decreased motor development at 1 year of age in infants exposed to cannabis perinatally.[12] A series of longitudinal cohort studies on human subjects reported low birth weight[13] and temperament issues in infants.[14] Other studies have also found an association with perinatal cannabinoid exposure and delinquent behavior at age 14, cognitive deficits, higher rates of depression, anxiety, and substance use in the teens and young adults.[15–17]

Among the 400 active chemicals in the cannabis plant, the 2 most prominent cannabinoids based on activity and abundance are trans-Δ-9-tetrahydrocannabinol (THC) and cannabidiol (CBD).[18] THC is the primary psychoactive molecule in cannabis and functions as a partial agonist of cannabinoid receptors (CBRs) including CB1R and CB2R, which are localized in the central nervous system.[19] In contrast to THC, CBD is not psychoactive but is hypothesized to act as a negative allosteric modulator for the CBRs and can modulate THCs psychoactive effects.[20,21] CBD also is a modulator of several noncanonical receptors (including serotonin, glycine, peroxisome proliferator-activated receptors [PPARs], and opioid receptors), transporters, and enzymes, which may account for its complex and highly variable pharmacology.[22] CBD can alter THC pharmacokinetics (PKs) by inhibiting cytochrome P450s enzyme and potentiate THC-associated negative behavioral outcomes in rodents.[23] *In vitro* exposure to CBD can cause neurotoxicity for perinatal neurons and astrocytes leading to apoptotic death.[24]

Of the potential routes of exposure to cannabinoids in infants which include secondary inhalation exposure and accidental ingestion, breastfeeding represents a significant route, particularly from mothers who are ingesting cannabinoid products.[25] Several studies have detected THC and CBD in human breast milk using liquid chromatography–tandem mass spectrometry (LC-MS/MS).[26–32] However, limited data are available regarding the PKs of cannabinoids transmitted by breast milk in infants. It is important to understand the PK properties in blood plasma, including peak plasma concentration and extent of duration of the exposure, to develop and design clinically relevant rodent models.

To model early-life cannabinoid exposure in rats, we studied the PK signatures of THC and CBD in adult rats and pups. We tested the effect of route of administration (subcutaneous vs. intraperitoneal) as well as the effects of the vehicle into which the drug was dissolved (oil vs. aqueous). Finally, we studied the PKs of dam to pup transmission via breast milk.

Materials and Methods

Animals and housing

Sprague-Dawley rats were obtained from Charles Rivers Laboratories (South San Francisco, CA) either as adults or as pups with nursing dams. Pups arrived in mixed male and female litters ($n = 10$). For PK experiments, animals were grouped in units of three to five animals per time point. Rats were housed in clear polycarbonate cages with in-cage shelters and bedding, as well as *ad libitum* access to water and standard laboratory chow and were acclimated to a standard colony room with reverse 12 h light–dark cycle (temperature of 18–25°C, 45–65% humidity). All animal experiments were carried out in compliance with Animal Research: Reporting of *In Vivo* Experiments guidelines and were approved (AN189143-02B) by the University of California, San Francisco (UCSF) Institutional Animal Care and Use Committee.

THC preparation

THC in acetonitrile was supplied by Cayman Chemical (Cat. No. 12068; Ann Arbor, MI). Acetonitrile was vacuum evaporated, and THC was redissolved in 100% ethanol (Cat. No. 459844; Sigma–Aldrich, St. Louis, MO). Aliquots were stored at −20°C. THC was diluted in either sesame oil (Cat. No. S3547; Sigma–Aldrich) or an aqueous vehicle. Working stocks were prepared on the same day of the experiment. For the sesame oil vehicle preparation, ethanol was vacuum evaporated, and the THC was reconstituted in sesame oil with vigorous vortexing. For the aqueous vehicle preparation, Tween 20 (Cat. No. 37470.01; Serva) was directly mixed with the stock aliquot of THC. Ethanol was vacuum evaporated and sterile saline (0.9% sodium chloride; Cat. No. 1022; Covidien) was added to make 5% of Tween 20 and saline solution.

CBD preparation

CBD powder was procured from Cayman Chemical (Cat. No. 90080) and was stored at −20°C until dissolved

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in sesame oil or aqueous vehicle (6% Tween 80:saline mixture). Tween 80 was purchased from bioWORLD (CAS No. 9005-65-6).

Injections of CBD or THC

Pups were injected on postnatal day 7 and adults between 8 and 12 weeks. Rats received a single injection of CBD (50 mg/kg) or THC (5 mg/kg). Different routes were compared including intraperitoneally or subcutaneously in the scruff of the neck. The needle size used for injecting drugs was 27G × 1/2″ in pups and 22G × 1″ in adults. The injection site was covered with a small amount of Vetbond (3M, St. Paul, MN) to minimize leakage. Following injections, rats were euthanized via decapitation at different time points; blood was collected by cardiac puncture using 25G × 5/8″ and 22G × 1″ needles for pups and adults, respectively. Blood samples were collected in heparin-coated tubes and stored on ice until centrifuged at 1500 *g* for 10 min at 4°C. Plasma was collected and stored at −80°C until analysis.

Standards and reagents for LC-MS/MS

All analytical standards were purchased from Cerilliant (Round Rock, TX), and Fast Red RC Salt (5-chloro-2-methoxybenzenediazonium salt) was purchased from Sigma–Aldrich. LC-MS/MS-grade methanol, acetonitrile, and water were purchased from Honeywell Burdick & Johnson (Muskegon, MI), and ammonium acetate was purchased from Sigma–Aldrich. Drug-free rat plasma used as the sample matrix for calibrators and Quality Controls (QCs) was purchased from Innovative Research (Novi, MI). WAX-S tips (1 mL tip with 20 mg resin and 40 mg salt) were purchased from DPX Technologies (Columbia, SC).

Rat plasma assay

THC and CBD were analyzed in rat plasma using modified versions of the non-derivatized assay and the derivatized assay detailed elsewhere.[33] Modifications for both methods included using rat plasma as a calibrator and QC sample matrix in place of human whole blood or methanol. Derivatization after sample preparation was modified so that the derivatization reaction took place in acetonitrile rather than methanol following the previous method.[34] LC-MS/MS analysis for both assays was performed using an ExionLC™ AD HPLC System (Shimadzu Corp., Kyoto, Japan) and QTRAP® 6500+ triple quadrupole mass spectrometer (AB Sciex, Redwood City, CA).

Statistical analysis

All C_{max} (ng/mL) and AUC_{0-t} (ng·h/mL) data are expressed as geometric mean (95% confidence interval). Statistical differences between data sets were analyzed using two-way analysis of variance (Šídák's multiple comparisons test). To calculate the cannabinoids AUC_{0-t}, we assumed the initial data point (zero time point) as 0 ng/mL concentration to estimate the concentration at the end of the dosing interval ($t = 48$ or 72 h) and used the trapezoidal rule. Data were analyzed and graphed using Prism 9.2 (GraphPad software). Due to the magnitude of differences in cannabinoid concentration in plasma among various injections and vehicles, graphs were presented on a semilogarithmic scale.

Definition of PK parameters

T_{max}: Time to the maximum measured plasma concentration.

C_{max}: Maximum measured plasma concentration over the time span specified.

AUC_{0-t}: The area under the plasma concentration versus time curve, from 0 to t hours.

Results

A brief outline of the experiment design is shown in Figure 1.

THC in adult female plasma

We first measured the plasma levels of THC after intraperitoneal injection in adult female rats. The mean C_{max} value for THC dissolved in sesame oil obtained after intraperitoneal administration at 5 mg/kg dose was 4.12 (34.69–0.49) ng/mL, and the mean time to reach C_{max} (T_{max}) was 1 h (Fig. 2). Since the absorption phase was not captured, the first time point was the C_{max}. T_{max} occurred within 1 h post-dose. After 4 h, THC plasma concentration remained constant. The area under the time curve of plasma concentration (AUC_{0-48h}) from 0 to 48 h after intraperitoneal administration was 147.9 (88.62–207.2) ng·h/mL.

CBD in adult female plasma

We next measured the plasma levels in adult female rats after CBD exposure. After intraperitoneal injection of CBD in sesame oil at 50 mg/kg dose in adult female rats, C_{max} of 284.45 (481.88–163.21) ng/mL and T_{max} of 8 h were detected. CBD remained stable in the plasma for up to 48 h. With an aqueous solution, CBD levels increased significantly; similar to the THC results, the absorption phase was missed, so the C_{max}

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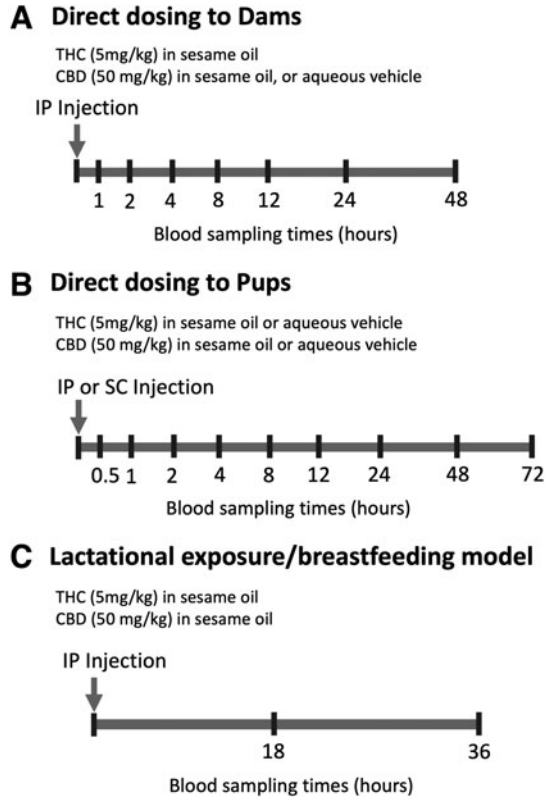

FIG. 1. Diagram depicting the experimental designs used in this study. In the direct dosing experiment, adult females **(A)** and PND 7 pups **(B)** were directly exposed to THC (5 mg/kg) or CBD (50 mg/kg) dissolved in sesame oil or aqueous vehicle via intraperitoneal or subcutaneous injection. Blood was collected at different time points. In the lactational experiments **(C)**, PND 7 pups were exposed to THC or CBD via lactating dams receiving 5 or 50 mg/kg dose in sesame oil, respectively, via intraperitoneal injections. Pup blood was collected at 18 and 36 h of drug exposure to mother. CBD, cannabidiol; PND 7, postnatal day 7; THC, Δ-9-tetrahydrocannabinol.

was 1091.91 (2773.77–429.83) ng/mL and T_{max} was observed within 1 h post-injection, as shown in Figure 3. In contrast to the oil vehicle, aqueous CBD resulted in rapidly decreasing levels of plasma CBD, dropping to 50 ng/mL at 48 h. The AUC_{0-48h} values after intraperitoneal administration in sesame oil and aqueous vehicle were 9501 (4582–14,419) and 7682 (5226–10,139) ng·h/mL, respectively.

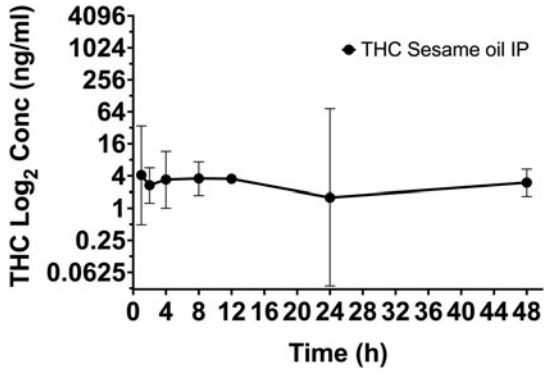

FIG. 2. THC levels in adult female's plasma using sesame oil as a vehicle. Data are presented as geometric mean (95% CI); $n = 2–3$ for each group. THC (5 mg/kg) was administered intraperitoneally. *X*-axis shows the time in hours, and *y*-axis shows the THC concentration in ng/mL in semilogarithmic (Log_2) scale. CI, confidence interval.

THC or CBD in pups' plasma

We next measured the plasma levels after directly injecting pups. Intraperitoneal administration of THC (5 mg/kg) dissolved in sesame oil showed a C_{max} of 15.78 (23.11–10.77) ng/mL and a T_{max} of 1 h (Fig. 4).

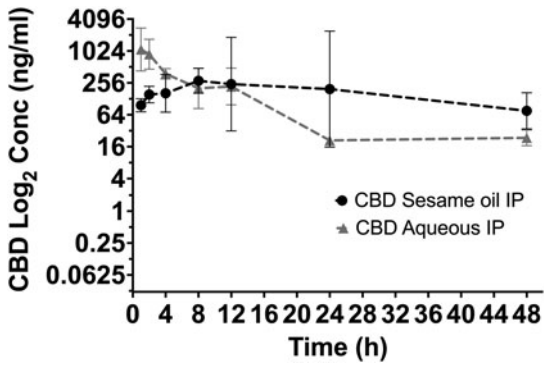

FIG. 3. CBD levels in adult female's plasma using different vehicles. Data are presented as geometric mean (95% CI); $n = 2–3$ for each group. Intraperitoneal administration of CBD in sesame oil (-●-, black color) and aqueous vehicle (-▲-, grey color). *X*-axis shows the time in hours, and *y*-axis shows the CBD concentration in ng/mL in Log_2 scale.

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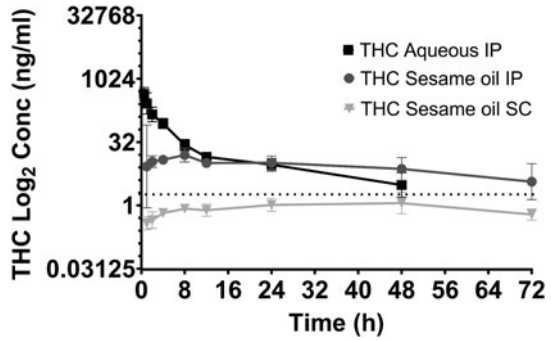

FIG. 4. THC plasma levels in PND 7 pups with different vehicles and route of administration. Data are presented as geometric mean (95% CI); $n = 4–7$ for each group. The subcutaneous administration in sesame oil is separated by a dashed line. Sesame oil subcutaneous administration (-▼-, light grey color), intraperitoneal administration (-●-, grey color), and aqueous vehicle intraperitoneal administration (-■-, black color). *X*-axis shows the time in hours, and *y*-axis shows the THC concentration in ng/mL in Log_2 scale.

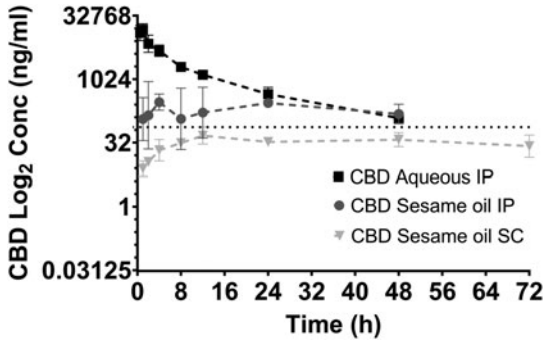

FIG. 5. CBD plasma levels in PND 7 pups with different vehicles and route of administration. Data are presented as geometric mean (95% CI); $n = 4–6$ for each group. The subcutaneous administration in sesame oil is separated by a dashed line. Sesame oil subcutaneous administration (-▼-, light grey color), intraperitoneal administration (-●-, grey color), and aqueous vehicle intraperitoneal administration (-■-, black color). *X*-axis shows the time in hours, and *y*-axis shows the CBD concentration in ng/mL in Log_2 scale.

THC concentration remained stable at 3.67 (9.83–1.37) ng/mL in pups' plasma for 72 h. In contrast, intraperitoneal injection of THC (5 mg/kg) in aqueous vehicle increased the THC C_{max} to 446.94 (637.19–313.49) ng/mL and reduced its T_{max} at 0.5 h, as shown in Figure 4. For both sesame oil and aqueous intraperitoneal administration, the absorption phase was missed, so the T_{max} was under 1 and 0.5 h, respectively. The plasma levels of THC after subcutaneous administration of the same dose (5 mg/kg) dissolved in sesame oil were significantly lower than either of the intraperitoneal injections with a C_{max} of 1.14 (2.05–0.63) ng/mL at 48 h. The area under the time curve of plasma concentration ($AUC_{0–72h}$) obtained was 659.9 (500.6–819.2) ng·h/mL THC intraperitoneally in sesame oil, which is approximately two times lower than THC intraperitoneally in aqueous vehicle ($AUC_{0–48h} = 1396$ [1148–1643]) and nine times higher than THC subcutaneously in sesame oil ($AUC_{0–72h}$ 69.02 [48.15–89.88]).

In a parallel set of studies, CBD injected at 50 mg/kg in sesame oil intraperitoneally had a C_{max} of 293.04 (456.94–187.93) ng/mL and T_{max} of 4 h (Fig. 5). The plasma levels of CBD remained stable up to 48 h. In contrast, intraperitoneal injection of CBD (50 mg/kg) with an aqueous vehicle increased the C_{max} to 15,406.33 (21,147.68–11,223.69 ng/mL) and decreased the T_{max} to 1 h. Subcutaneous injection of CBD in sesame oil at 50 mg/kg administration showed a C_{max} of 47.11 (74.85–29.65) ng/mL for CBD at 12 h (T_{max}), and the levels remained unchanged even at 72 h (26.42 [48.59–14.37]). The average area under the curve ($AUC_{0–48h}$) value obtained was 11,183 (8233–14,132) ng·h/mL for CBD intraperitoneally in sesame oil, which is approximately six times lower than aqueous intraperitoneal administration ($AUC_{0–48h} = 73,721$ [61,705–85,736]) and four times higher than sesame oil subcutaneous administration ($AUC_{0–72h} = 2504$ [1954–3055]). PK parameters are summarized in Table 1.

THC or CBD levels in breastfed pups

To measure the plasma levels of cannabinoids in pups from breast milk transmission, we injected breastfeeding dams with a single intraperitoneal injection of cannabinoids dissolved in oil: THC (5 mg/kg) or CBD (50 mg/kg). Noting that the levels of cannabinoids in adult females' plasma remained elevated after 8 h, we chose to measure the levels in both male and female

Table 1. Pharmacokinetic Parameters: Plasma Concentration (Geometric Mean with 95% CI) and Area Under the Curve Total Peak Area (95% CI) Assessed After Various Route of Administration of THC (5 mg/kg) and CBD (50 mg/kg) Using Different Vehicles in Postnatal Day 7 Pups

		THC (5 mg/kg)			CBD (50 mg/kg)		
Vehicle	Route of administration	T_{max} (h)	C_{max} Geometric mean (95% CI), ng/mL	AUC Total peak area (95% CI), ng·h/mL	T_{max} (h)	C_{max} Geometric mean (95% CI), ng/mL	AUC Total peak area (95% CI), ng·h/mL
Aqueous	IP	0.5	446.94 (637.19–313.49)	1396 (1148–1643)	1	15,406.33 (21,147.68–11,223.69)	73,721 (61,705–85,736)
Sesame	IP	1	15.78 (23.11–10.77)	659.9 (500.6–819.2)	4	293.04 (456.94–187.93)	11,183 (8233–14,132)
Sesame	SC	48	1.14 (2.05–0.63)	69.02 (48.15–89.88)	12	47.11 (74.85–29.65)	2504 (1954–3055)

AUC, area under the curve; CBD, cannabidiol; CI, confidence interval; IP, intraperitoneal; SC, subcutaneous; THC, Δ-9-tetrahydrocannabinol.

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pups at 18 and 36 h. Each time point had five male and five female pups. THC concentrations in plasma at 18 and 36 h in males were 0.40 (0.49–0.33) and 0.49 (0.63–0.38) ng/mL and in females 0.45 (0.60–0.34) and 0.43 (0.54–0.35) ng/mL, respectively (Fig. 6A). No significant differences were observed in sex and time. CBD plasma levels were recorded at 14.54 (35.21–6.01) ng/mL at 18 h in males and dropped to 5.54 (7.03–4.36) ng/mL at 36 h. In females, CBD plasma concentrations were 9.81 (21.95–4.38) and 5.87 (8.04–4.28) ng/mL at 18 and 36 h, respectively (Fig. 6B). Table 2 shows the summarized mean concentration values.

Discussion

Our results show how the PKs of the cannabinoids THC and CBD are significantly affected by two important factors: injection route and vehicle used for dilution. For consistency and comparison purposes, we chose to keep the injection concentrations the same across studies (5 mg/kg for THC and 50 mg/kg for CBD), which are similar to the range of previous studies (10–120 mg/kg for CBD and 0.5–30 mg/kg for THC).[35–42] Cannabinoids in aqueous vehicle delivered by intraperitoneal injection resulted in rapid absorption and a consistent decay for both THC and CBD in pups and adults. By comparison, intraperitoneal injection with an oil vehicle resulted in a lower C_{max} and higher T_{max} but had a slower decay. Finally, subcutaneous injection with an oil vehicle had the lowest C_{max} and highest T_{max} but had the least decay—consistent with a depot injection. Thus, different models of exposure can be replicated by changing the route and vehicle.

These experiments also demonstrate the physiological relevance of mother to pup transmission of cannabinoids via breast milk. In both the CBD and THC studies, there is a consistent and measurable plasma concentration of drug in the pups, which is in a similar range to direct subcutaneous injection. Both breast milk transmission and direct subcutaneous injection

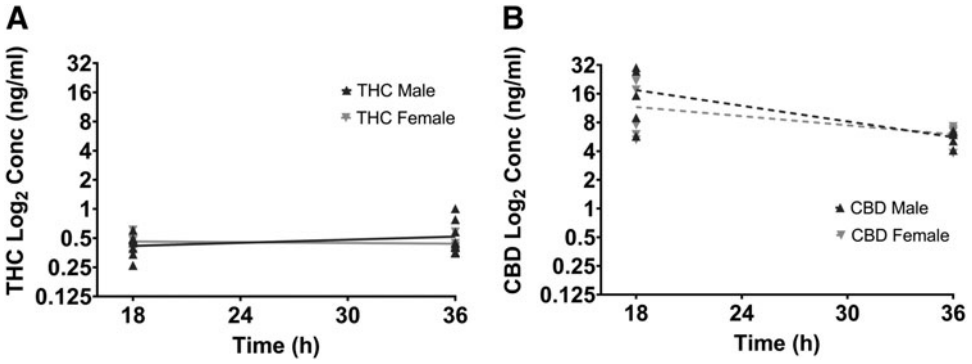

FIG. 6. **(A)** THC (*solid line*) or **(B)** CBD (*dashed line*) plasma levels in breastfed pups. Data are presented as individual data points; $n = 10$ for each group. Males ($n = 5$ per time point; -▲-, black color) and females ($n = 5$ per time point; -▼-, grey color). *X*-axis shows the time in hours, and *y*-axes show the THC concentration and CBD concentration in ng/mL in Log_2 scale.

Table 2. Plasma Concentration of Drugs Found in Plasma at 18 and 36 h in Breastfed Pups

Sex	Time of plasma collection post-dose (h)	Concentration Geometric mean (95% CI), ng/mL	
		THC (5 mg/kg)	CBD (50 mg/kg)
Male	18	0.40 (0.49–0.33)	14.54 (35.21–6.01)
	36	0.49 (0.63–0.38)	5.54 (7.03–4.36)
Female	18	0.45 (0.60–0.34)	9.81 (21.95–4.38)
	36	0.43 (0.54–0.35)	5.87 (8.04–4.28)

Dams were injected intraperitoneally with THC (5 mg/kg) and CBD (50 mg/kg) in sesame oil.

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to pups could serve as an animal model to study this clinically and socially relevant question or toxicity in development.

It is interesting to note that the same injection method (same route and dose) can result in higher plasma levels in pups compared with adults. We observed this phenomenon in both the intraperitoneal injections of CBD dissolved in oil as well as intraperitoneal injections of THC in oil. This could be from physiological differences (such as absorption, excretion, metabolism, and plasma protein binding) between them.[43,44] Alternatively, it may be related to adipose sequestration of these lipophilic drugs, and because neonates have less percentage of adipose tissue compared with adults, they could have less sequestration of drug.[6,43]

A related observation of the general differences in bioavailability between pups and adults is that it is drug dependent. Plasma concentrations of THC in adults versus pups (approximately four times less in adults) were larger than the difference in CBD between adults and pups (approximately two times). While differences between the drugs in terms of metabolism could account for this disparity, there were also differences in the dose injected (THC 10-fold less than CBD), which could affect the peak by saturating metabolic mechanisms or lipophilic storage and may have contributed to technical reasons for an observed difference such as injection variability or batch preparation.

There are several limitations to this study. In many of our experiments, the first measured value (1 h) was the maximum value, which we have labeled as the C_{max} because the absorption phase was not captured due to the rapid increase in plasma bioavailability. The true C_{max} could be < 1 h, but this temporal resolution was beyond the scope of our experiments. There are also technical challenges surrounding the consistent injections in pups. Precise dosing by weight via injection is challenging to achieve in very small pups, and there is always a small but variable amount of leakage of injectate, which we tried to limit by applying a tissue adhesive directly over the injection site. However, overall error values between animals suggest that this represents a viable model for wider use.

Conclusions

In this study, we investigated the effect of different routes of administration and vehicles and determined the PK profiles that result. We also demonstrated the physiologically significant transmission of CBD and THC from nursing dams to pups. These data represent several potential models using aqueous or sesame oil direct exposure to pups via intraperitoneal and subcutaneous administration and lactation exposure of cannabinoids during critical periods of development.

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Authors' Contributions

I.S.: Investigation, methodology, validation, writing—original draft. G.A.C.: Conceptualization, methodology, funding acquisition, supervision, writing—review and editing. J.C.H.: Investigation, validation, writing—review, and editing. K.L.L.: Methodology, supervision, writing—review and editing. J.W.S.: Conceptualization, supervision, funding acquisition, writing—review and editing.

Author Disclosure Statement

The authors declare no conflicts of interest.

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Abbreviations Used

CBD = cannabidiol
CBRs = cannabinoid receptors
LC-MS/MS = liquid chromatography–tandem mass spectrometry
PKs = pharmacokinetics
QCs = Quality Controls
SC = subcutaneous
THC = Δ-9-tetrahydrocannabinol

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