

Maternal and Fetal Pharmacokinetic Analysis of Cannabidiol during Pregnancy in Mice[§]

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ABSTRACT

Cannabidiol (CBD), a major component of cannabis, has various effects, such as antiemetic and anxiolytic activities, and has recently been marketed as a supplement. The number of people using CBD during pregnancy is increasing, and there are concerns about its effects on the fetus. In addition, the scientific evidence supporting the fetal safety of CBD use during pregnancy is insufficient. To investigate CBD transfer from the mother to the fetus, a single intravenous dose of CBD was administered to pregnant mice in this study, and fetal pharmacokinetics (distribution and elimination) was analyzed. The transfer of CBD from the maternal blood to the fetus was rapid, and the compound accumulated in the fetal brain, liver, and gastrointestinal tract. Conversely, little CBD was transferred from the mother to the amniotic fluid. We analyzed the pharmacokinetics of CBD using a two-compartment model and found that the maternal and fetal half-lives of CBD were approximately

5 and 2 hours, respectively. Furthermore, we performed a moment analysis of the pharmacokinetics of CBD, observing a mean residence time of less than 2 hours in both the mother and fetus. These results suggest that once-daily CBD intake during pregnancy is unlikely to result in CBD accumulation in the mother or fetus.

SIGNIFICANCE STATEMENT

CBD is currently marketed as a supplement, and despite its increasing use during pregnancy, little information concerning its fetal effects has been reported. In the present study, CBD was administered to pregnant mice, and the pharmacokinetics in the fetus was investigated using a two-compartment model and moment analysis. The results of these analyses provide important information for estimating the risk to the fetus if CBD is mistakenly consumed during pregnancy.

Introduction

The major cannabinoids present in cannabis are Δ^9 -tetrahydrocannabinol (THC) and cannabidiol (CBD) (Fernández, 2016; Lucas et al., 2018; MacCallum and Russo, 2018; Perry et al., 2018; Amin and Ali, 2019). The use of THC is regulated in many countries because it causes psychologic dependence. Meanwhile, CBD is marketed as a supplement based on its effects, which include stress reduction, sleep promotion, and fatigue recovery (Guimarães et al., 1990; Russo et al., 2007; Kaplan et al., 2017). Currently, in the United States and Japan, CBD is readily available in forms including oils, creams, and tobacco without any special regulations. Recently, it was reported that CBD is effective against nausea, pain, inflammation, and anxiety (Abuhasira et al., 2018).

Concerning metabolism, CBD is oxidized in the adult liver by CYP3A at the 6 β , 2', and 4' positions (Jiang et al., 2011; Ujváry and Hanuš, 2016).

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However, the metabolic activity of CYP3A is significantly lower in the fetal liver than in the adult liver (Ochiai et al., 2016; Kitaoka et al., 2018a,b). Therefore, it is considered that CBD is distributed throughout the entire fetal body with little metabolism in the liver. There are two main routes for drug elimination in the fetus. One route is the return of the drug to maternal blood through the placenta. The second route is the excretion of drugs from the fetal kidneys into the urine.

The human gestation period is divided into three trimesters. The first trimester (early pregnancy) is an important stage in which fetal organs and organs are formed. Therefore, if a pregnant woman uses a drug during this trimester, it may be transferred to the fetus, potentially affecting development. Conversely, after the second trimester, the effects of most drugs on the fetus are small, and drugs with confirmed safety profiles can be administered. However, even after the second trimester, the fetal organs are not completely formed, and therefore, some drugs may stunt fetal growth or cause functional abnormalities. The judgment of drug safety during pregnancy depends on the results of reproductive and developmental toxicity studies using experimental animals such as mice. However, the effects of drugs in such studies are judged by morphologic abnormalities in the offspring. Therefore, if no morphologic abnormality is found in the newborn, its effects (toxicity) cannot be clarified.

ABBREVIATIONS: AUC₀₋₁₂, area under the curve (time 0–12 h); AUC_{inf}, area under the curve (time 0 to infinity); CBD, cannabidiol; CL_{tot}, total body clearance; GI, gastrointestinal; LC-MS, liquid chromatography-mass spectrometry; MRT₀₋₁₂, mean residence time (time 0–12 h); MRT_{inf}, mean residence time (time 0 to infinity); $t_{1/2\alpha}$, distribution phase half-life; $t_{1/2\beta}$, elimination phase half-life; THC, Δ^9 -tetrahydrocannabinol; Vd _{α} , volume of distribution at the distribution phase; Vd _{β} , volume of distribution at the elimination phase.

Generally, the effects of drugs occur in the organs or tissues in which they are distributed. The placental route is the most important route of drug transfer from the mother to the fetus. The placenta acts as a barrier that prevents the transfer of drugs to the fetus. However, low-molecular-weight compounds (molecular weight < 600 g/mol) and highly lipophilic drugs easily penetrate the placenta (Bain et al., 1990; Leslie et al., 2005; Holland et al., 2007; Mao, 2008; Joshi et al., 2016). CBD (molecular weight = 314) is highly lipophilic, and it is classified as a low-molecular-weight compound, suggesting that it easily penetrates the placenta. In addition, the placental permeability of the drug depends on the presence of a transporter. Currently, the existence of transporters for CBD has not been reported. Therefore, CBD appears to penetrate the placenta primarily via passive diffusion. After passing through the placenta, CBD travels through the umbilical cord to the fetal liver.

Meanwhile, amniotic fluid is composed of urine from the fetus and liquid exuded from the maternal blood. Therefore, drugs transferred to fetal urine will be accumulated again by the fetus via amniotic fluid. This circulation between the fetus and amniotic fluid delays the elimination of the drug from the fetus.

Although CBD is increasingly used during pregnancy to control nausea and anxiety, little information is available regarding its fetal effects. Considering that the market size of CBD-containing health products continues to expand in areas such as Europe and the United States, it is estimated that a large number of pregnant women will use these products. In this study, CBD was intravenously administered once to mice during a period corresponding to the second trimester of pregnancy in humans, and the distribution of CBD transferred from the mother to the fetus and its pharmacokinetics were analyzed. We also analyzed changes in the CBD concentration in amniotic fluid. We believe that by clarifying the distribution and behavior of CBD in the fetus, we can obtain information on its developmental and toxic effects even when morphologic changes are not observed.

Materials and Methods

Materials. CBD was purchased from Abcam Inc. (Tokyo, Japan). CBD-D3 solution was purchased from Sigma-Aldrich Corp. (St. Louis, MO). All other reagents were of the highest commercially available grade.

Animal Handling. Pregnant ICR mice (gestational day 12.5) were purchased from Japan SLC, Inc. (Tokyo Laboratory Animals Science Co. Ltd., Tokyo, Japan). The mice were kept at room temperature ($24 \pm 1^\circ\text{C}$) and $55\% \pm 5\%$ humidity with 12 hours of light (artificial illumination; 08:00–20:00). Food and water were available ad libitum. Each animal was used only once.

The present study was conducted in accordance with the Guiding Principles for the Care and Use of Laboratory Animals, as adopted by the Animal Care Committee of Hoshi University (Tokyo, Japan). All procedures using animals were carried out according to protocols approved by the Animal Care Committee of Hoshi University (approval number 28-094).

Drug Preparation and Administration. The dose of CBD (10 mg/kg) was determined from previous studies showing that intravenous administration of CBD was effective in relieving epilepsy and chronic stress in mice (Guimaraes et al., 1990; Patra et al., 2019; Xu et al., 2019).

A stock solution of 50 mg/ml CBD was prepared by dissolution in Tween 80. Stock solution was diluted 50-fold with physiologic saline to prepare a CBD solution (1.0 mg/ml) (Murase et al., 2014). Mice were intravenously administered 100 μl of CBD solution per 10 g body weight under isoflurane anesthesia. Preparation of the CBD solution was performed immediately before administration.

Measurements of CBD in Maternal Blood and Fetal Tissue. CBD solution (1.0 mg/ml) was administered via the tail vein to mice on day 14.5 of pregnancy. After CBD administration, at each period (0.25, 0.5, 1, 2, 4, 8, and 12 hours) blood samples (about 1 ml per pregnant mouse) were collected from the inferior vena cava of five pregnant mice for each time point under isoflurane anesthesia. Next, uterine blood vessels were cauterized with an electro-surgical knife to prevent the attachment of the maternal blood to fetus. Amniotic fluid was collected from the uterus with the use of a 26-gauge \times 0.5-inch needle and syringe. When fetuses for

each pregnant mice were removed from the uterus, the umbilical cord was cauterized with an electro-surgical knife.

Sampling was performed from five pregnant mice at each time point. Six fetuses were collected per pregnant mouse. Three fetuses were used for whole-body CBD concentration measurement. The remaining three fetuses were used to measure CBD levels in the brain, liver, and gastrointestinal (GI) tract. The remaining fetuses were stored as spares without measurement at each time point. All samples were stored at -80°C until use.

Liquid Chromatography-Mass Spectrometry Sample Preparation. The fetus (whole body) was homogenized with 4 ml of 2% phosphoric acid solution and vortexed for 1 minute. At that time, 100 ng of CBD-D3 was dissolved in 10 μl of methanol as an internal standard and spiked into the homogenate. The homogenate solution was subjected to centrifugal separation at 9000g for 10 minutes at room temperature.

Solid-phase extraction columns [Oasis HLB PRiME 3 cc (60 mg); Waters, Milford, MA] were preconditioned with 1 ml of methanol and equilibrated with 500 μl of ultrapure water and 2% phosphoric acid solution. After preconditioning, the columns were filled with 3.8 ml of supernatant. Washing was performed with 1 ml of methanol:ultrapure water (5:95, v/v%). The sample was eluted with 1 ml of acetonitrile:methanol (90:10, v/v%). The eluate was removed by blowing nitrogen gas onto the solvent at 50°C . The eluate was redissolved in 100 μl of mobile phase at the start of the gradient and used as the LC-MS sample.

Each fetal organ (brain, liver, and GI tract) was homogenized in 1 ml of a 2% phosphoric acid solution and pretreated in the same manner as the whole fetus.

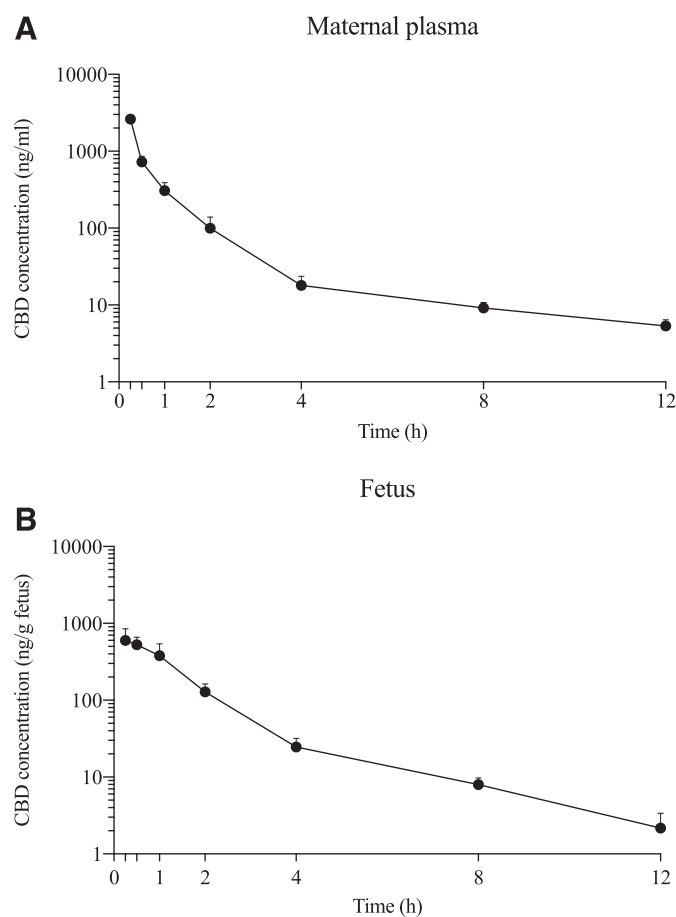


Fig. 1. CBD concentration-time profiles in maternal blood and fetus in pregnant mice. CBD (10 mg/kg) was administered to pregnant mice via the tail vein, and blood was collected via the central vein at various times (0.25, 0.5, 1, 2, 4, 8, and 12 hours). Blood was collected from the central vein. Fetuses were extracted simultaneously. After separation from blood, plasma was analyzed using LC-MS. (A) Maternal plasma ($n = 5$); (B) fetus ($n = 15$). The results of the analysis are displayed as mean \pm S.D. values of the mean levels with respect to individual pregnant mice at each time point (0.25, 0.5, 1, 2, 4, 8, and 12 hours).

TABLE 1
Pharmacokinetic parameters of cannabidiol in the maternal plasma and fetus

	AUC ₀₋₁₂	AUC _{inf}	MRT ₀₋₁₂	MRT _{inf}	<i>t</i> _{1/2α}	<i>t</i> _{1/2β}	CL _{tot}	Vd _d	Vd _β
	Maternal: ng [#] /ml		l/h per kilogram						
	Fetal: ng [#] /g		l/kg						
Maternal sample (plasma) (<i>n</i> = 5)	1295.2 ± 166.0	1692.1 ± 417.5	1.1 ± 0.07	1.7 ± 0.3	0.7 ± 0.1	4.9 ± 1.2	7.7 ± 1.0	2.2 ± 0.1	55.1 ± 2.4
Fetal sample (whole body) (<i>n</i> = 15)	866.4 ± 214.2	874.5 ± 210.0	1.7 ± 0.2	1.8 ± 0.4	0.8 ± 0.2	2.3 ± 0.5	— ^a	— ^a	— ^a

The results of the analysis are displayed as means ± S.D.

^aCan't calculate.

A total of maternal plasma (100 μl) or amniotic fluid (500 μl) and an equal volume of 4% phosphoric acid solution were mixed and vortexed for 1 minute.

At that time, 100 ng of CBD-D3 was dissolved in 10 μl of methanol as an internal standard and spiked into the samples, which were then pretreated using the previously described protocol for whole-fetus samples. The calibration curve was prepared by spiking each control sample with the CBD standard solution and

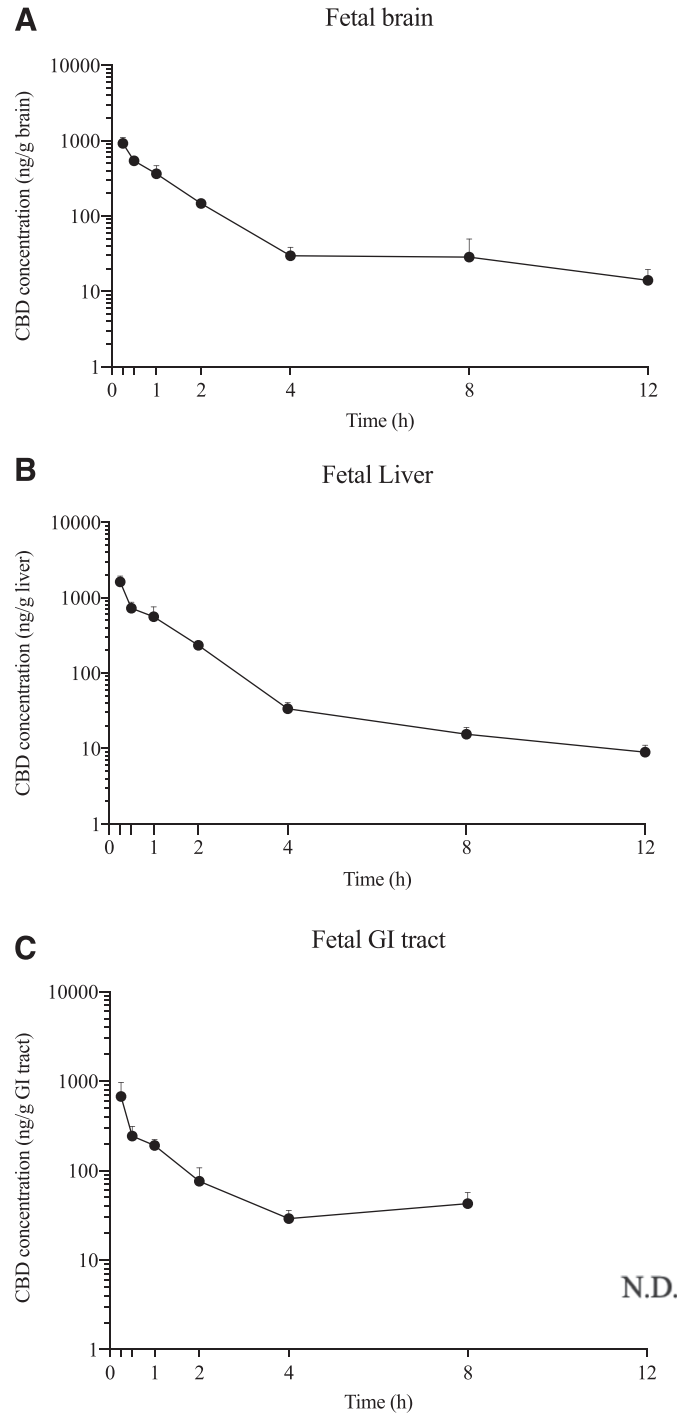


Fig. 2. CBD concentration-time profiles in fetal tissue. CBD (10 mg/kg) was administered to pregnant mice via the tail vein, and fetal tissues were dissected at various times (0.25, 0.5, 1, 2, 4, 8, and 12 hours). The homogenate solutions of the organs were analyzed via LC-MS. (A) Fetal brain (*n* = 15); (B) fetal liver (*n* = 15); (C) fetal GI tract (*n* = 15). The results of the analysis are displayed as mean ± S.D. values of the mean levels with respect to individual pregnant mice at each time point (0.25, 0.5, 1, 2, 4, 8, and 12 hours). N.D., not detected.

pretreating the samples using the same method. The quantification range of the calibration curve was 1.953–500 ng per fetus for fetuses (whole body), 1.953–250 ng per tissue for each fetal organ, 1.953–1000 ng/ml for maternal plasma, and 1.953–125 ng/ml for amniotic fluid.

Under acidic conditions, CBD is transformed to THC and other THC isomers. Therefore, the stability of CBD in the phosphoric acid solution used in this study was analyzed. As a result, the stability of CBD was 89% in the 2% phosphoric acid solution and 74% in the 4% phosphoric acid solution. There was no significant difference between these values (Supplemental Fig. 1). In other words, the decomposition of CBD during the pretreatment was limited. In this study, a calibration curve was created using the matrix matching method. In other words, even in the calibration curve, the decomposition of CBD occurred at the same rate, and thus, it can be said that the quantification results are also accurate.

Liquid Chromatography-Mass Spectrometry Analysis. LC-MS analysis was performed using a combination of an LC-20A high performance liquid chromatography system (SHIMADZU, Kyoto, Japan) and LCMS-2010 (SHIMADZU). The analytical column used was an XBridge C18 (Waters). The analysis was performed at a column temperature of 40°C. Ultrapure water containing 0.1% formic acid was used as mobile phase A. Acetonitrile containing 0.1% formic acid was used as mobile phase B. The analysis was performed in binary gradient mode, as shown below [gradient condition (percentage of B): 0.00 minutes, 40%; 8.00 minutes, 80%; 12.00 minutes, 80%; 12.01 minutes, 40%; 17.00 minutes, 40%]. The flow rate was constant at 0.2 ml/min.

For the interface, positive mode electrospray ionization was used. The nebulizer gas flow rate was set at 1.5 l/min. The curved desolvation line (CDL) and heat block temperatures were 250 and 200°C, respectively. The voltage of the detector was set at 1.5 V. Measurement was performed by the selected ion monitoring method based on a charge/mass ratio (*m/z*) value of 315 for CBD and 318 for CBD-D3 (Internal standard).

The LCMSsolution version 3.40 (SHIMADZU) was used for high performance liquid chromatography system control and mass spectrometry chromatogram analysis.

Pharmacokinetic Analysis. In this study, pharmacokinetic parameters AUC_{0-12} , AUC_{inf} , MRT_{0-12} , MRT_{inf} , and $t_{1/2\alpha,\beta}$ were calculated by fitting the two-compartment model with the calculation software Numeric Analysis Program for Pharmacokinetics (Hisaka and Sugiyama, 1998).

CL_{tot} was calculated using the following equation:

$$CL_{tot} = \frac{D}{AUC_{0-12}}$$

D and C_0 were defined as the amount of CBD administered to mice and initial blood CBD concentration (concentration at time 0), respectively. Vd_{α} was calculated using the following equation:

$$Vd_{\alpha} = \frac{D}{C_0}$$

C_0 was extrapolated from the 0.25 and 0.5 hour straight lines to determine the y-axis intercept.

Elimination rate constant (β) was calculated using the following equation:

$$\beta = \frac{0.693}{t_{1/2\beta}}$$

Vd_{β} was calculated using the following equation:

$$Vd_{\beta} = \frac{D}{\beta AUC}$$

Statistics. Experimental values are expressed as means \pm S.D. Outliers were calculated using the Smirnov-Grubbs outliers test.

The significance of differences was evaluated using ANOVA (Tukey's test) or Student's *t* test. Calculation and graphical exploration were performed using GraphPad Prism software (Prism version 9).

Results

CBD Rapidly Reaches the Fetus After Maternal Administration.

Maternal blood CBD levels peaked (2615.3 ± 442.3 ng/ml) immediately after administration and decreased rapidly (Fig. 1A). Fetal CBD levels were measured using the same procedure. CBD transfer to the fetus began 15 minutes after maternal administration and peaked at 598.7 ± 251.9 ng/g. The changes in fetal and maternal blood CBD levels were nearly identical (Fig. 1B). Table 1 displays the pharmacokinetic parameters (AUC , MRT , $t_{1/2\alpha,\beta}$, CL_{tot} , $Vd_{\alpha,\beta}$) of CBD in maternal blood and fetus (whole body). The transfer rate of CBD from the mother to the fetus was calculated as 66.9% of the AUC. Furthermore, $t_{1/2\beta}$ in the mother was 4.9 ± 1.2 hours, whereas that in the fetus was 2.3 ± 0.5 hours.

CBD Rapidly Reaches Fetal Organs After Maternal Administration.

CBD levels in the fetal brain, liver, and GI tract were measured to identify the fetal organs in which CBD accumulated. CBD transfer to the fetal brain, liver, and GI tract began 15 minutes after maternal CBD administration (Fig. 2). CBD levels were highest in the liver (1627.5 ± 3126 ng/g, Fig. 2B). CBD levels in these organs rapidly decreased over the first 4 hours after maternal administration, after which the decline was more gradual. CBD levels in the GI tract were lower than the limit of quantification at 12 hours after maternal CBD administration (Fig. 2C). The pharmacokinetic parameters (AUC , MRT , $t_{1/2\alpha,\beta}$) of CBD in the fetal organs are presented in Table 2. The AUC of CBD was highest in the liver and lowest in the GI tract. $t_{1/2\beta}$ was longer in the brain than in the liver (7.2 ± 2.4 vs. 4.2 ± 0.4 hours). $t_{1/2\beta}$ could not be measured for the GI tract.

Estimation of CBD Transfer to and Distribution in the Fetus. To assess the extent of maternal-to-fetal transfer of CBD, the fetal CBD concentration was divided by the maternal blood drug concentration and plotted over time. We have decided to express the "concentration ratio" as the "transfer ratio."

This transfer ratio increased until the first 4 hours after administration of CBD and then decreased (Fig. 3).

TABLE 2

Pharmacokinetic parameters of cannabidiol in the fetal brain, liver, and GI tract

The results of the analysis are displayed as means \pm S.D. Numerical data are expressed as means \pm S.D. The significance of differences was evaluated using ANOVA, followed by Tukey's test (AUC_{0-12} , MRT_{0-12} , $t_{1/2\alpha}$). The Student's *t* test was used for the significant difference in $t_{1/2\beta}$ between brain and liver.

Fetal Sample	AUC_{0-12}	AUC_{inf}	MRT_{0-12}	MRT_{inf}	$t_{1/2\alpha}$	$t_{1/2\beta}$
	<i>ng*h/g tissue</i>		<i>h</i>			
Brain (<i>n</i> = 15)	1078.4 ± 72.3	1224.3 ± 138.9	2.2 ± 0.3	4.7 ± 1.4	0.8 ± 0.1	7.2 ± 2.4
Liver (<i>n</i> = 15)	1519.2 ± 154.3	1572.3 ± 164.7	1.6 ± 0.04	2.2 ± 0.08	0.7 ± 0.1	4.2 ± 0.4
GI tract (<i>n</i> = 15)	668.8 ± 52.1	1150.7 ± 218.6	2.2 ± 0.2	10.5 ± 4.5	1.1 ± 0.1	— ^a
Brain \times liver	$P < 0.0001$	— ^b	N.S.	— ^b	N.S.	$P < 0.0001$
Brain \times GI tract	$P = 0.0001$	— ^b	N.S.	— ^b	N.S.	
Liver \times GI tract	$P < 0.0001$	— ^b	N.S.	— ^b	N.S.	

^aCan't calculate.

^bNot analyzed.

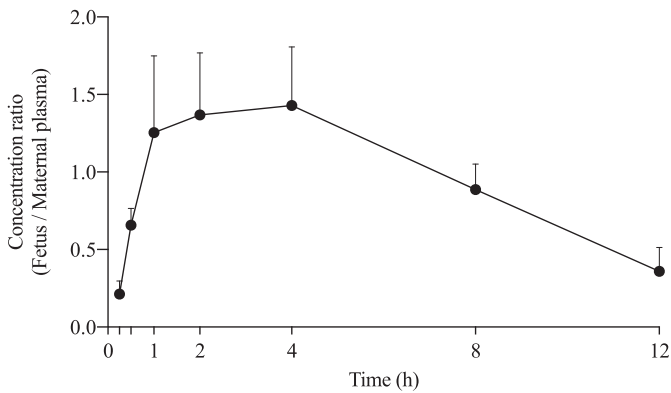


Fig. 3. Estimation of the CBD transfer ratio from mothers to fetuses. A time-course plot of the ratio of the fetus CBD concentration to the maternal plasma CBD concentration was created [maternal plasma ($n = 5$), fetus ($n = 15$)]. The results of the analysis are displayed as mean \pm S.D. values of the mean levels with respect to individual pregnant mice at each time point (0.25, 0.5, 1, 2, 4, 8, and 12 hours) Concentration ratio = (CBD concentration of fetus (Fig.1B)) / (Maternal plasma CBD concentration (Fig.1A))

Estimation of CBD Transfer to and Distribution in the Fetal Brain, Liver, and GI Tract. To assess the transferability and accumulation of CBD in fetal organs (brain, liver, and GI tract), the ratio of the amount of CBD transferred to each organ of the fetus to the maternal blood CBD concentration was plotted over time. In these organs, the ratio increased over the 1st hour after maternal CBD administration. The transfer ratio was highest for the liver and lowest for the GI tract. After 1 hour, the transfer ratio started to decrease. However, the transfer ratio in the fetal GI tract increased slightly between 4 and 8 hours after maternal CBD administration (Fig. 4).

CBD Transfer to Maternal Amniotic Fluid Is Small. We measured CBD levels in amniotic fluid because fetuses ingest amniotic fluid in the second trimester. CBD was detected in amniotic fluid immediately after maternal administration. CBD levels in amniotic fluid peaked (74.4 ± 12.8 ng/ml) 30 minutes after administration and then rapidly decreased over 4 hours after administration (Fig. 5).

Table 3 displays the pharmacokinetic parameters (AUC, MRT, $t_{1/2\alpha}$, β) of CBD in amniotic fluid. The AUC of CBD in amniotic fluid was 23.9% of that in maternal blood. $t_{1/2\beta}$ was 51.5 ± 6.4 hours, which was markedly longer than those in the maternal plasma or fetal organs.

Discussion

CBD is reported to have pharmacological effects such as antiemetic and anxiolytic activities. Pregnant women are increasingly using CBD in anticipation of nausea and anxiety. However, the safety of CBD in the fetus has not been clarified. Therefore, we analyzed the transfer of CBD from the mother to the fetus and the pharmacokinetics of CBD in the fetus using mice in the second trimester of pregnancy.

When we input the raw data into the pharmacokinetic analysis software (Numeric Analysis Program for Pharmacokinetics) (Hisaka and Sugiyama, 1998), we obtained analysis results in which the distribution phase (α) was 4 hours after the administration of CBD, and the elimination phase (β) was after that. Although it is possible to analyze with multiple compartments, we decided that it would not be useful, so we decided to analyze with a two-compartment model (Fig. 1; Table 1). We calculated maternal CBD clearance and volume of distribution to validate the fetal

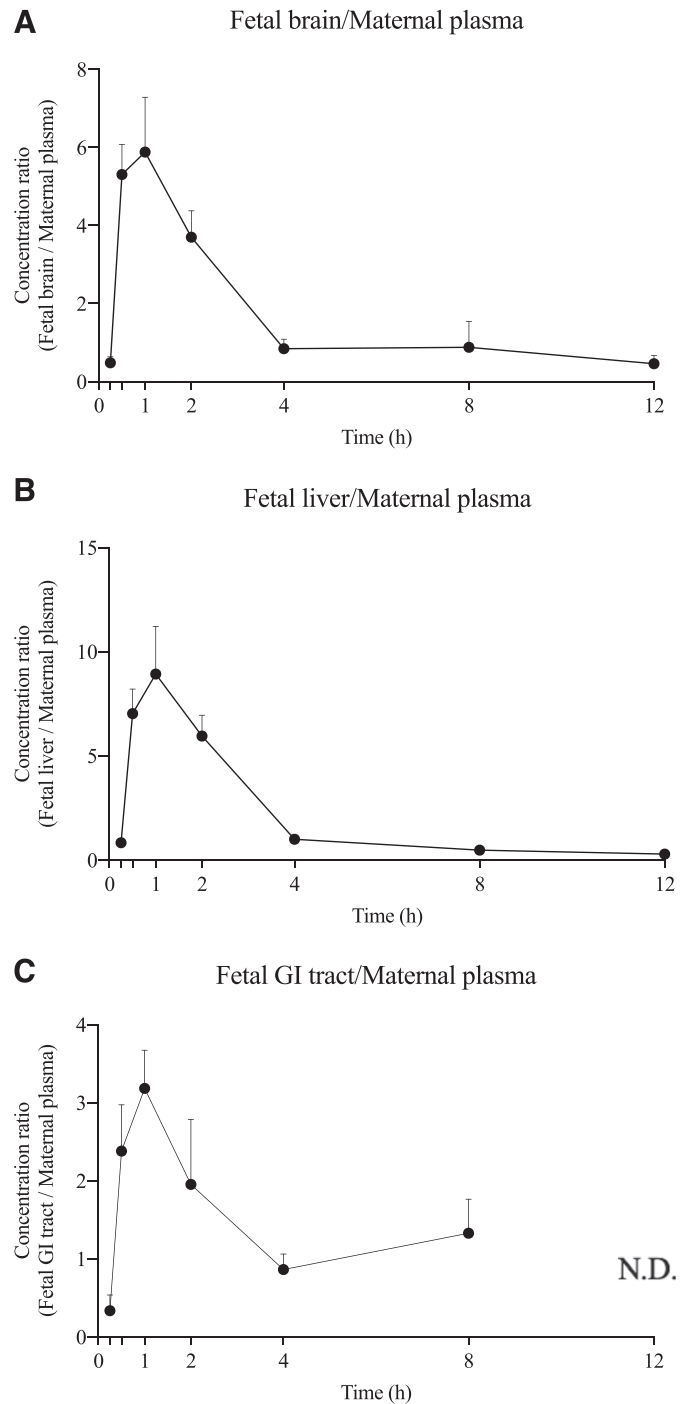


Fig. 4. Estimation of the CBD transfer ratio from maternal blood to fetal tissue. A time-course plot of the ratio of the fetal tissue CBD concentration to maternal plasma CBD concentration was created [(A) fetal brain/maternal plasma; (B) fetal liver/maternal plasma; (C) fetal GI tract/maternal plasma ($n = 15$)]. Values are displayed as means \pm S.D. N.D., not detected. Concentration ratio = (CBD concentration of each fetal organs (Fig.2A, B, C)) / (Maternal plasma CBD concentration (Fig.1A))

CBD pharmacokinetics. Since the $V_{d\alpha}$ of CBD in the mother's body is small and the CL_{tot} is several times larger, it is clear that the disappearance of CBD from the mother's body is rapid. Therefore, it is unlikely that CBD that has transferred to the fetus will continue to remain.

In this study, we analyzed AUC and half-life using a two-compartment model. However, these parameters change depending on the number of

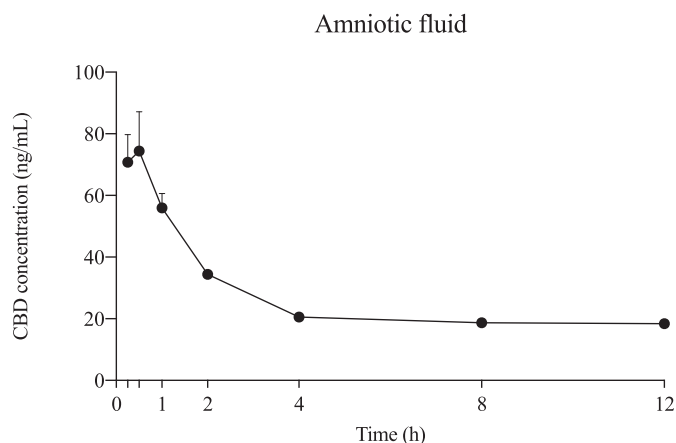


Fig. 5. CBD concentration-time profiles of amniotic fluid in pregnant mice. CBD (10 mg/kg) was administered by tail vein injection, and its levels in amniotic fluid were measured at various times via LC-MS [amniotic fluid ($n = 5$)]. The results of the analysis are displayed as mean \pm S.D. values of the mean levels with respect to individual pregnant mice at each time point (0.25, 0.5, 1, 2, 4, 8, and 12 hours).

compartments in the compartment model. Therefore, we provided the validity of the data on the pharmacokinetics of CBD by using MRT analysis for the relationship between AUC and time (Tables 1 and 2).

Next, we analyzed the pharmacokinetics of CBD in fetal organs because side effects generally occur in the organ (tissue) in which the drug accumulates. In other words, when analyzing the effects of CBD on the fetus, this strategy can analyze the organs (tissues) in which it accumulates in detail.

The drug (compound) administered to the mother reaches the fetal liver via the umbilical vein. Via fetal circulation, the drug transferred from the mother to the fetus is distributed to each organ of the fetus. Because the fetus is not a small adult, the pharmacokinetics of adults cannot be applied to fetuses. Therefore, we analyzed the changes of CBD concentrations in the fetal brain, liver, and GI tract (Fig. 2; Table 2). The reason we measured the fetal brain is that delayed fetal neurogenesis is a serious problem. The CBD levels of the fetal brain were similar to those of other organs. We also report that the CYP3A family involved in CBD metabolism is rarely expressed in the fetal liver during this period (Ochiai et al., 2016; Kitaoka et al., 2018a,b). Therefore, we felt the need to measure CBD levels in the liver. In fact, the CBD concentration in the fetal liver showed the highest value (1627.5 ± 3126 ng/g, Fig. 2B). In addition, it was also found that the AUC_{0-12} of the liver was significantly higher than that of the brain and gastrointestinal tract (Table 2). The finding that the drug concentration was highest in the liver can be explained by the fact that most of the umbilical vein fluid containing CBD is transferred to the whole fetus via the liver (Fig. 2; Table 2). Next, we measured the gastrointestinal tract as a site involved in the absorption of CBD, as the fetus drinks amniotic fluid. The CBD concentration in amniotic fluid remained lower than that in other organs. The low AUC_{0-12} of the gastrointestinal tract compared with the brain and liver is consistent with the low concentration of CBD

in amniotic fluid (Fig. 5; Table 3). There was a significant difference in AUC_{0-12} among the three organs (fetal brain, liver, and GI tract) (Table 2). However, it was considered that there was not much difference when compared with the AUC_{0-12} of the whole fetal body (Table 2). Therefore, it was suggested that CBD was not distributed in high concentrations in organs other than the three organs (fetal brain, liver, and GI tract).

We used the transferability of CBD from the mother to the fetus as an index of the transfer of CBD to the fetus relative to its concentration in the mother (Fig. 3). Up to 4 hours after administration of CBD, it was shown to have increased maternal-to-fetal transfer (Fig. 3). However, the transfer ratio of CBD in fetal organs peaked 1 hour after administration and began to decrease (Fig. 4). We speculate that this is the result of fetal CBD being dispersed in each organ. Therefore, although the transfer ratio of CBD in organs is organ-specific, it is considered that the distribution to specific organs is small.

The placental route and fetal kidney-to-urinary excretion are important for the elimination of drugs from the fetus. However, in mouse fetuses on gestational day 14.5, the kidneys are not completely formed. Furthermore, because CBD readily binds to proteins in plasma, it is considered that unmetabolized CBD is less likely to undergo urinary excretion via glomerular filtration. The reason for the high CBD concentration in amniotic fluid immediately after administration could be the transfer of CBD directly to amniotic fluid through the umbilical artery wall and amniotic membrane. However, the CBD concentration in amniotic fluid was significantly lower than those in other organs (Figs. 4 and 5). Meanwhile, given the longer $t_{1/2\beta}$ in amniotic fluid than in fetal tissues, if pregnant women repeatedly ingest CBD over a long period, it cannot be ruled out that even if the concentration of CBD in amniotic fluid is low, the low concentration of CBD may affect the fetus.

In this study, we analyzed the pharmacokinetics of CBD in the mother and fetus as an index to estimate the effect on the fetus when taking CBD during pregnancy. We also analyzed the pharmacokinetics of the drug in each organ (brain, liver, GI tract) in the fetus and found that it was not accumulative in any particular organ. We illustrated that the fetus is exposed to a low CBD concentration after the ingestion of a small amount of CBD by the mother. The short $t_{1/2}$ and low MRT of CBD in the mother and fetus restrict its accumulation (Table 1). However, because the pharmacokinetics of the fetus differs from that of the adult, it is necessary to evaluate the effects of multiple CBD doses in the future.

In addition, morphologic analytical methods are also important for more accurately clarifying the effects of CBD use during pregnancy on the fetus. Furthermore, it is necessary to analyze the actual effects of different doses on the fetus. Finally, we would like to clarify the relationship between the amount of CBD transferred to each organ in the fetus and the presence or absence of toxicity.

In recent years, various supplements containing CBD as the main component have been marketed. However, the scientific evidence of their efficacy and safety is not sufficient. In particular, the effects on the fetus can result in a significant mental and financial burden on children

TABLE 3

Pharmacokinetic parameters of cannabidiol in amniotic fluid

The results of the analysis are displayed as means \pm S.D.

In Utero Sample	AUC_{0-12}	AUC_{inf}	MRT_{0-12}	MRT_{inf}	$t_{1/2\alpha}$	$t_{1/2\beta}$
	$ng \cdot h/ml$		h			
Amniotic fluid ($n = 5$)	309.9 ± 5.8	1658.6 ± 173.2	6.4 ± 3.8	71.0 ± 8.9	2.0 ± 0.2	51.5 ± 6.4

and their families. Therefore, we hope that our study will spur more detailed analyses of the safety of CBD use during pregnancy.

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Authorship Contributions

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References

- Abuhasira R, Shbiro L, and Landschaft Y (2018) Medical use of cannabis and cannabinoids containing products - regulations in Europe and North America. *Eur J Intern Med* **49**:2–6.
- Amin MR and Ali DW (2019) Pharmacology of medical cannabis. *Adv Exp Med Biol* **1162**:151–165.
- Bain MD, Copas DK, Taylor A, Landon MJ, and Stacey TE (1990) Permeability of the human placenta in vivo to four non-metabolized hydrophilic molecules. *J Physiol* **431**:505–513.
- Fernández Ó (2016) THC:CBD in daily practice: available data from UK, Germany and Spain. *Eur Neurol* **75** (Suppl 1):1–3.
- Guimarães FS, Chiaretti TM, Graeff FG, and Zuardi AW (1990) Antianxiety effect of cannabidiol in the elevated plus-maze. *Psychopharmacology (Berl)* **100**:558–559.
- Hisaka A and Sugiyama Y (1998) Analysis of nonlinear and nonsteady state hepatic extraction with the dispersion model using the finite difference method. *J Pharmacokinetic Biopharm* **26**:495–519.
- Holland ML, Lau DT, Allen JD, and Arnold JC (2007) The multidrug transporter ABCG2 (BCRP) is inhibited by plant-derived cannabinoids. *Br J Pharmacol* **152**:815–824.
- Jiang R, Yamaori S, Takeda S, Yamamoto I, and Watanabe K (2011) Identification of cytochrome P450 enzymes responsible for metabolism of cannabidiol by human liver microsomes. *Life Sci* **89**:165–170.
- Joshi AA, Vaidya SS, St-Pierre MV, Mikheev AM, Desino KE, Nyandege AN, Audus KL, Unadkat JD, and Gerk PM (2016) Placental ABC transporters: biological impact and pharmaceutical significance. *Pharm Res* **33**:2847–2878.
- Kaplan JS, Stella N, Catterall WA, and Westenbroek RE (2017) Cannabidiol attenuates seizures and social deficits in a mouse model of Dravet syndrome. *Proc Natl Acad Sci USA* **114**:11229–11234.
- Kitaoka S, Hatogai J, Iimura R, Yamamoto Y, Oba K, Nakai M, Kusunoki Y, Ochiai W, and Sugiyama K (2018a) Relationship between low midazolam metabolism by cytochrome P450 3A in mice and the high incidence of birth defects. *J Toxicol Sci* **43**:65–74.
- Kitaoka S, Hatogai J, Ochiai W, and Sugiyama K (2018b) Zonation of the drug-metabolizing enzyme cytochrome P450 3A in infant mice begins in pre-weaning period. *J Toxicol Sci* **43**:223–227.
- Leslie EM, Deeley RG, and Cole SP (2005) Multidrug resistance proteins: role of P-glycoprotein, MRP1, MRP2, and BCRP (ABCG2) in tissue defense. *Toxicol Appl Pharmacol* **204**:216–237.
- Lucas CJ, Galetti P, and Schneider J (2018) The pharmacokinetics and the pharmacodynamics of cannabinoids. *Br J Clin Pharmacol* **84**:2477–2482.
- MacCallum CA and Russo EB (2018) Practical considerations in medical cannabis administration and dosing. *Eur J Intern Med* **49**:12–19.
- Mao Q (2008) BCRP/ABCG2 in the placenta: expression, function and regulation. *Pharm Res* **25**:1244–1255.
- Murase R, Kawamura R, Singer E, Pakdel A, Sarma P, Judkins J, Elwakeel E, Dayal S, Martinez-Martinez E, Amere M, et al. (2014) Targeting multiple cannabinoid anti-tumour pathways with a resorcinol derivative leads to inhibition of advanced stages of breast cancer. *Br J Pharmacol* **171**:4464–4477.
- Ochiai W, Hirose A, Kawamura T, Komachi K, Yamamoto Y, Kitaoka S, Hatogai J, Kusunoki Y, Kon R, Ikarashi N, et al. (2016) Role of the drug-metabolizing enzyme CYP during mouse liver development. *Biol Pharm Bull* **39**:2015–2021.
- Patra PH, Barker-Haliski M, White HS, Whalley BJ, Glyn S, Sandhu H, Jones N, Bazelot M, Williams CM, and McNeish AJ (2019) Cannabidiol reduces seizures and associated behavioral comorbidities in a range of animal seizure and epilepsy models. *Epilepsia* **60**:303–314.
- Perry D, Ton J, and Allan GM (2018) Evidence for THC versus CBD in cannabinoids. *Can Fam Physician* **64**:519.
- Russo EB, Guy GW, and Robson PJ (2007) Cannabis, pain, and sleep: lessons from therapeutic clinical trials of Sativex, a cannabis-based medicine. *Chem Biodivers* **4**:1729–1743.
- Ujváry I and Hanuš L (2016) Human metabolites of cannabidiol: a review on their formation, biological activity, and relevance in therapy. *Cannabis Cannabinoid Res* **1**:90–101.
- Xu C, Chang T, Du Y, Yu C, Tan X, and Li X (2019) Pharmacokinetics of oral and intravenous cannabidiol and its antidepressant-like effects in chronic mild stress mouse model. *Environ Toxicol Pharmacol* **70**:103202.

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