Maternal and fetal pharmacokinetic analysis of cannabidiol during pregnancy in mice

Wataru Ochiai*[1], Satoshi Kitaoka[1], Taisuke Kawamura[1], Jo Hatogai[1], Shohei Harada[1],

Misa Iizuka[1], Mashu Ariumi[1], Seiya Takano[1], Tomomi Nagai[1], Masanaho Sasatsu[2],

Kiyoshi Sugiyama[3]

[1]Department of Pharmacokinetics, School of Pharmacy and Pharmaceutical Sciences,

Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan.

[2]Laboratory of Tissue Regeneration, School of Pharmacy and Pharmaceutical Sciences,

Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan.

[3]Department of Functional Molecule Kinetics, School of Pharmacy and Pharmaceutical

Sciences, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan.

*Corresponding Author: Wataru Ochiai

w-ochiai@hoshi.ac.jp

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ADDRESS MANUSCRIPT CORRESPONDENCE TO: Dr. Wataru Ochiai*,

Department of Pharmacokinetics, School of Pharmacy and Pharmaceutical Sciences,

Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan.

Tel. +81-3-5498-5167

E-mail: w-ochiai@hoshi.ac.jp

*Corresponding Author

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ABBREVIATIONS:

CBD, cannabidiol

THC, Δ9-tetrahydrocannabinol

LC-MS, Liquid chromatography–mass spectrometry

GI, gastrointestinal

SPE, solid phase extraction

AUC_{0-12}, area under the curve (time 0 to 12h)

AUC_{inf}, area under the curve (time 0 to infinity)

MRT_{0-12}, mean residence time (time 0 to 12h)

MRT_{inf}, mean residence time (time 0 to infinity)

CYP, cytochrome P450.

MW: molecular weight

$t_{1/2\alpha}$: distribution phase half-life

$t_{1/2\beta}$: elimination phase half-life

CL_{tot}: total body clearance

Vd_{α}: Volume of distribution at the distribution phase

Vd_{β}: Volume of distribution at the elimination phase

C_o: initial blood concentration (concentration at time 0)

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ABSTRACT

Cannabidiol (CBD), a major component of cannabis, has various effects such as

antiemetic and anxiolytic activities, and it 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 h, respectively.

Furthermore, we performed a moment analysis of the pharmacokinetics of CBD,

observing a mean residence time of less than 2 h 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.

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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.

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Introduction

The major cannabinoids present in cannabis are Δ9-tetrahydrocannabinol (THC) and

cannabidiol (CBD) (Fernandez, 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 psychological dependence. Meanwhile, CBD is marketed as a

supplemented based on its effects including stress reduction, sleep promotion, and

fatigue recovery (Guimaraes 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 cytochrome P450

(CYP) 3A at the 6β, 2′, and 4′ positions (Jiang et al., 2011; Ujvary and Hanus,

2016). 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; Kitaoka et al.,

2018b). 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

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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 when 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 morphological

abnormalities in the offspring. Therefore, if no morphological abnormality is found in

the newborn, its effects (toxicity) cannot be clarified.

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 （MW < 600） and highly

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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 (MW = 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

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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 morphological

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, USA). All other reagents were of

the highest commercially available grade.

Animal handling

Pregnancy 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 ± 1 °C) and 55 ± 5% humidity with 12 h 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].

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Drug preparation and administration

The dose of CBD (10 mg/kg) was determined from previous paper 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 physiological 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. Following CBD administration, at each period (0.25, 0.5, 1, 2, 4, 8 and 12 h) blood samples（about 1 mL/pregnant moue）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 electrosurgical knife to prevent the attachment of the maternal blood to fetus. Amniotic fluid was collected from the uterus with the use of

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a 26G 1/2 needle and syringe. When fetuses for each pregnant mice were removed from

the uterus, the umbilical cord was cauterized with an electrosurgical knife.

Sampling was performed from 5 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℃ until use.

Liquid chromatography-mass spectrometry (LC-MS) sample preparation

The fetus (whole body) was homogenized with 4 mL of 2% phosphoric acid solution

and vortexed for 1 min. At that time, 100 ng of CBD-D3 were dissolved in 10 μL of

methanol as an internal standard and spiked into the homogenate. The homogenate

solution was subjected to centrifugal separation at $9000 \times g$ for 10 min at room

temperature.

Solid-phase extraction columns (Oasis® HLB PRiME 3cc [60 mg], Waters, Milford,

MA, USA) were preconditioned with 1 mL of methanol and equilibrated with 500 μL of

ultrapure water and 2% phosphoric acid solution. Following preconditioning, the

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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 gastrointestinal [GI] tract) was homogenized in 1

mL of a 2% phosphoric acid solution and pretreated in the same manner as the whole

fetus.

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 min.

At that time, 100 ng of CBD-D3 were 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 pretreating the samples

using the same method. The quantification range of the calibration curve was 1.953–500

ng/fetus for fetuses (whole body), 1.953–250 ng/tissue for each fetal organ, 1.953–1000

ng/mL for maternal plasma, and 1.953–125 ng/mL for amniotic fluid.

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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 HPLC system

(SHIMADZU, Kyoto, Japan) and LCMS-2010 (SHIMADZU). The analytical column

used was an XBridge C18 (Waters, Milford, MA, U.S.A). The analysis was performed

at a column temperature of 40ºC. 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

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condition (% of B); 0.00 min 40%, 8.00 min 80%, 12.00 min 80%, 12.01 min 40%,

17.00 min 40%). The flow rate was constant at 0.2 mL/min.

For the interface, positive mode electrospray ionization (ESI) was used. The

nebulizer gas flow rate was set at 1.5 L/min. The CDL and heat block temperatures

were 250 ℃ and 200 ℃, respectively. The voltage of the detector was set at 1.5 V.

Measurement was performed by the selected ion monitoring (SIM) method based on an

m/z value of 315 for CBD, 318 for CBD-d3 (Internal standard).

The LCMSsolution Ver.3.40 (SHIMADZU) was used for HPLC system control and

MS chromatogram analysis.

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Pharmacokinetic analysis

In this study, pharmacokinetic parameters AUC_{0-12}, AUC_{inf}, MRT_{0-12}, MRT_{inf}, $t_{1/2\alpha,\beta}$

were calculated by fitting the 2-compartment model with calculation software NAPP

（Numeric Analysis Program for Pharmacokinetics）(Hisaka and Sugiyama, 1998).

CL_{tot} was calculated using the following equation:

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

Vd_{α} was calculated using the following equation:

C_0 was extrapolated from the 0.25 and 0.5 hour straight lines to determine the Y-axis

intercept.

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

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:

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

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Statistics

Experimental values are expressed as mean ± standard deviation. 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 ver.9) .

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Results

CBD rapidly reach 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 min 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 (area under the curve [AUC], mean residence time [MRT], half-life [$t_{1/2\alpha, \beta}$],

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 h, whereas that in the fetus was 2.3 ± 0.5 h.

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 min 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 h after maternal administration, after which the decline was more

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gradual. CBD levels in the GI tract were lower than the limit of quantification at 12 h

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 h vs. 4.2 ± 0.4 h). t1/2β 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 ratio of the amount of

CBD transferred to the fetal to the maternal blood CBD concentration was plotted over

time. We have decided to express the "concentration ratio" as the "transfer ratio".

This transfer ratio increased until the first 4 h after administration of CBD and then

decreased (Fig. 3).

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

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increased over the first hour after maternal CBD administration. The transfer ratio was

highest for the liver and lowest for the GI tract. After 1 h, the transfer ratio started to

decrease. However, the transfer ratio in the fetal GI tract increased slightly between 4

and 8 h 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 min after

administration and then rapidly decreased over 4 h following administration (Fig. 5).

Table 3 displays the pharmacokinetic parameters (AUC, MRT, $t_{1/2\alpha, \beta}$) 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 h, which was markedly longer than those in the maternal plasma or

fetal organs.

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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 (NAPP) (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 CBD pharmacokinetics. Since the Vd_α 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 2-compartment model. However, these parameters change depending on the number of compartments in compartment

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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 (Table 1 & 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; Kitaoka et al.,

2018b). 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

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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 to 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 transiently high, 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 (Fig. 4 & 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 CDB in amniotic fluid is low, the low concentration of CDB 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 the

fetus is exposed to a low CBD concentration following 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, morphological 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 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

Participated in research design: Ochiai and Kitaoka

Conducted experiments: Ochiai, Kitaoka, Hatogai, Harada, Iizuka, Ariumi, Takano,

Nagai

Performed data analysis: Ochiai, Kitaoka, Kawamura, Sasatsu,

Wrote or contributed to the writing of the manuscript: Ochiai, Kitaoka, Kawamura,

Sugiyama

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Footnotes

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Conflict of Interest

The authors declare no conflict of interest.

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Figure Legends

Fig. 1. Cannabidiol (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 h). 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 ± SD values of the mean levels with respect to individual pregnancy mice at each time point (0.25, 0.5, 1, 2, 4, 8 and 12 h).

Fig. 2. Cannabidiol (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 h). 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 ± SD values of the mean levels with respect to individual pregnancy mice at each time point (0.25, 0.5, 1, 2, 4, 8 and 12 h). N.D. , not detected.

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Fig. 3. Estimation of the cannabidiol (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 ± SD values of the mean levels with respect to

individual pregnancy mice at each time point (0.25, 0.5, 1, 2, 4, 8 and 12 h).

$$\text{Concentration ratio} = \frac{CBD\ concentration\ of\ fetus\ (Fig.1B)}{Maternal\ plasma\ CBD\ concentration\ (Fig.1A)}$$

Fig. 4. Estimation of the cannabidiol (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 (n = 15). Values are displayed as mean ± SD.

N.D. , not detected.

$$\text{Concentration ratio} = \frac{CBD\ concentration\ of\ each\ fetal\ organs\ (Fig.2A,B,C)}{Maternal\ plasma\ CBD\ concentration\ (Fig.1A)}$$

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Fig. 5. Cannabidiol (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 ± SD values of the mean levels with

respect to individual pregnancy mice at each time point (0.25, 0.5, 1, 2, 4, 8 and 12 h).

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Table 1.   Pharmacokinetic parameters of cannabidiol in the maternal plasma and fetus.

Maternal Sample	AUC_{0-12} (ng*h/mL)	AUC_{inf} (ng*h/mL)	MRT_{0-12} (h)	MRT_{inf} (h)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	CL_{tot} (L/h/Kg)	Vd_{α} (L/Kg)	Vd_{β} (L/Kg)
Plasma (n=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	AUC_{0-12} (ng*h/g fetus)	AUC_{inf} (ng*h/g fetus)	MRT_{0-12} (h)	MRT_{inf} (h)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)	CL_{tot} (L/h/Kg)	Vd_{α} (L/Kg)	Vd_{β} (L/Kg)
Whole body (n=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	-	-	-

The results of the analysis are displayed as mean ± SD.

Table 2. Pharmacokinetic parameters of cannabidiol in the fetal brain, liver and GI tract.

Fetal Sample	AUC_{0-12} (ng*h/g tissue)	AUC_{inf} (ng*h/g tissue)	MRT_{0-12} (h)	MRT_{inf} (h)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)
Brain (n=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 (n=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 (n=15)	668.8 ± 52.1	1150.7 ± 218.6	2.2 ± 0.2	10.5 ± 4.5	1.1 ± 0.1	-
Brain × Liver	P<0.0001	-	N.S.	-	N.S.	P<0.0001
Brain × GI tract	P=0.0001	-	N.S.	-	N.S.	
Liver × GI tract	P<0.0001	-	N.S.	-	N.S.	

The results of the analysis are displayed as mean ± SD. Numerical data are expressed as the mean ± standard deviation. 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. N.S.; not significant

Table 3. Pharmacokinetic parameters of cannabidiol in amniotic fluid

in utero Sample	AUC_{0-12} (ng*h/mL)	AUC_{inf} (ng*h/mL)	MRT_{0-12} (h)	MRT_{inf} (h)	$t_{1/2\alpha}$ (h)	$t_{1/2\beta}$ (h)
Amniotic Fluid (n=5)	309.9 ± 5.8	1658.6 ± 173.2	6.4 ± 3.8	71.0 ± 8.9	2.6 ± 0.2	51.5 ± 6.4

The results of the analysis are displayed as mean $\pm$ SD.

A   Maternal plasma

B   Fetus

Fig.1

A   Fetal brain

B   Fetal Liver

C   Fetal GI tract

N.D.

Fig. 2

Fig. 3

A Fetal brain/Maternal plasma

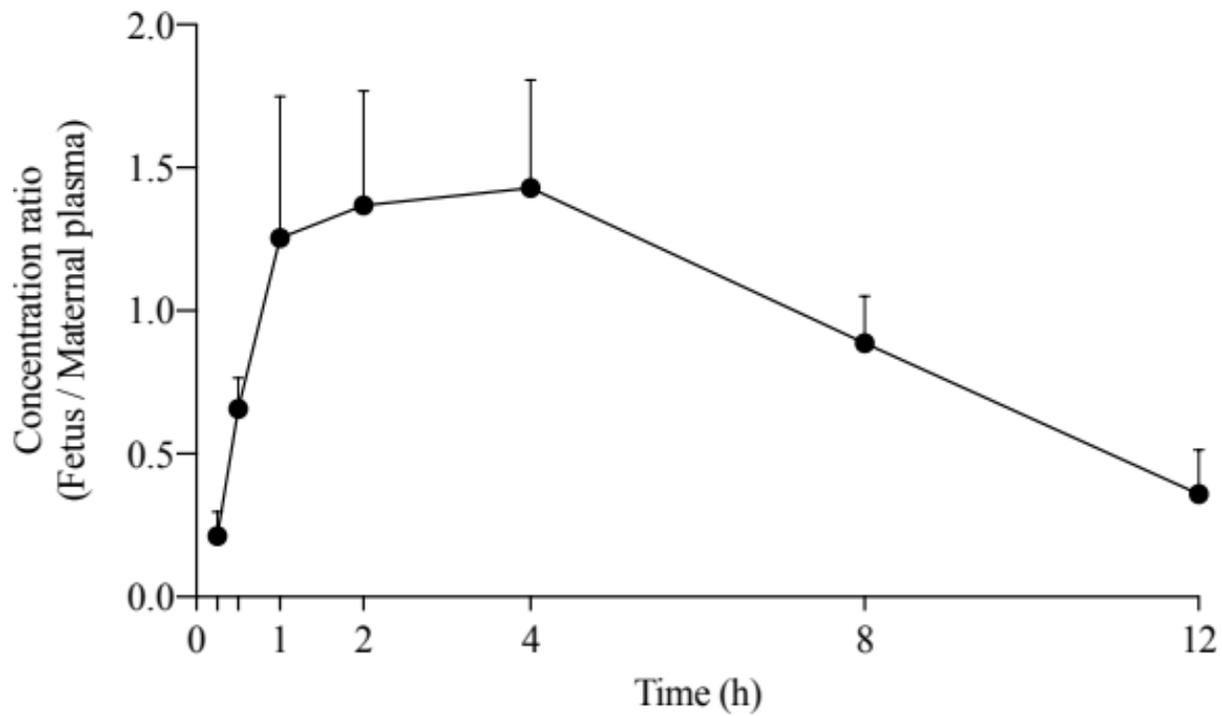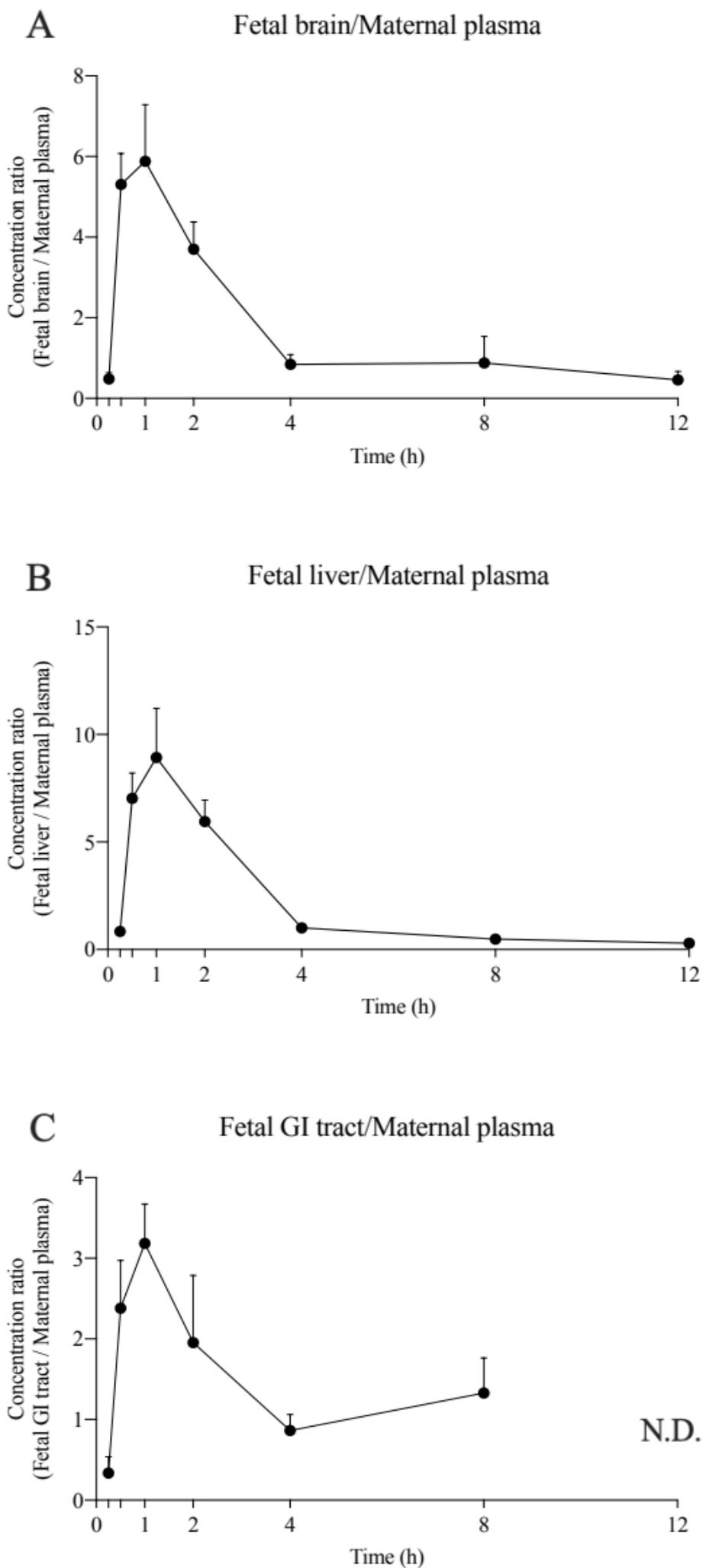B Fetal liver/Maternal plasma

C Fetal GI tract/Maternal plasma
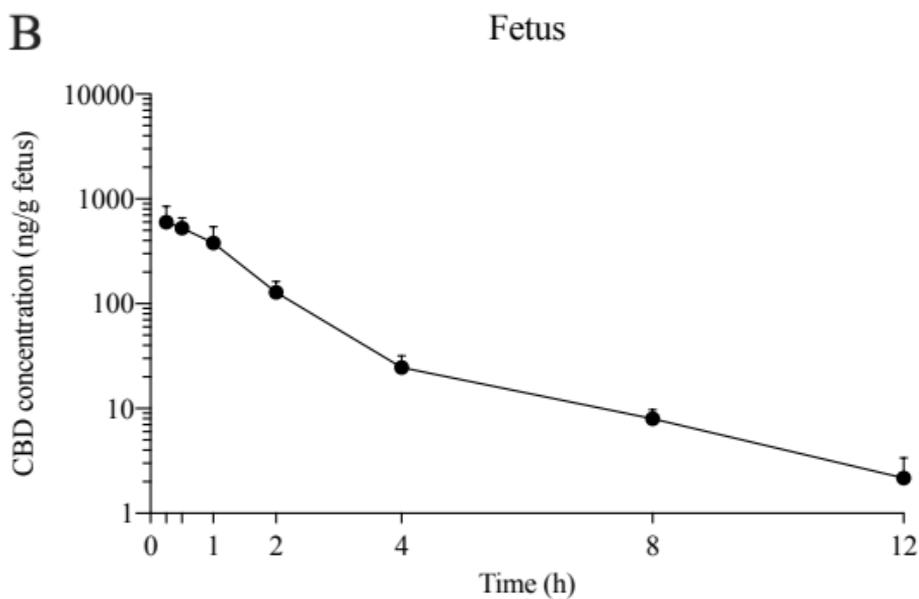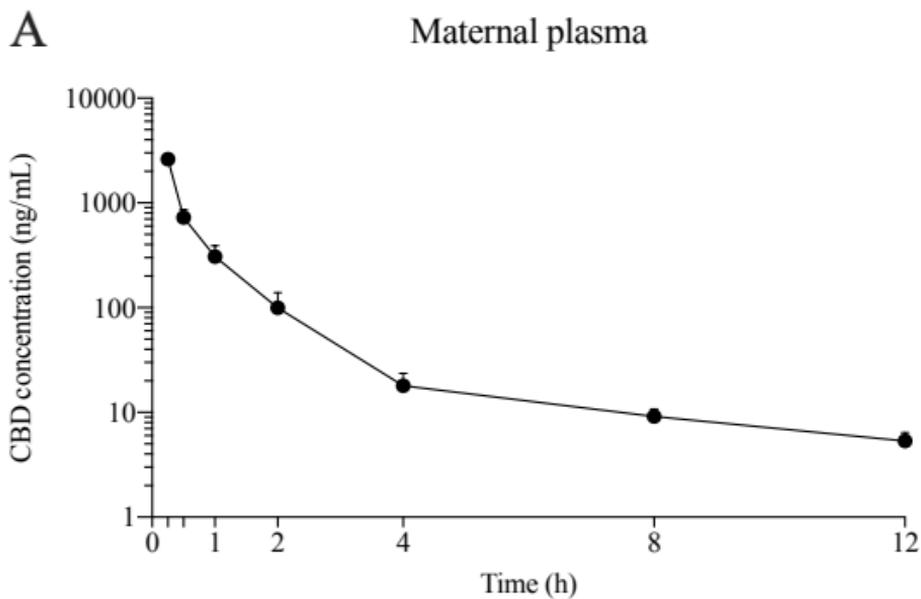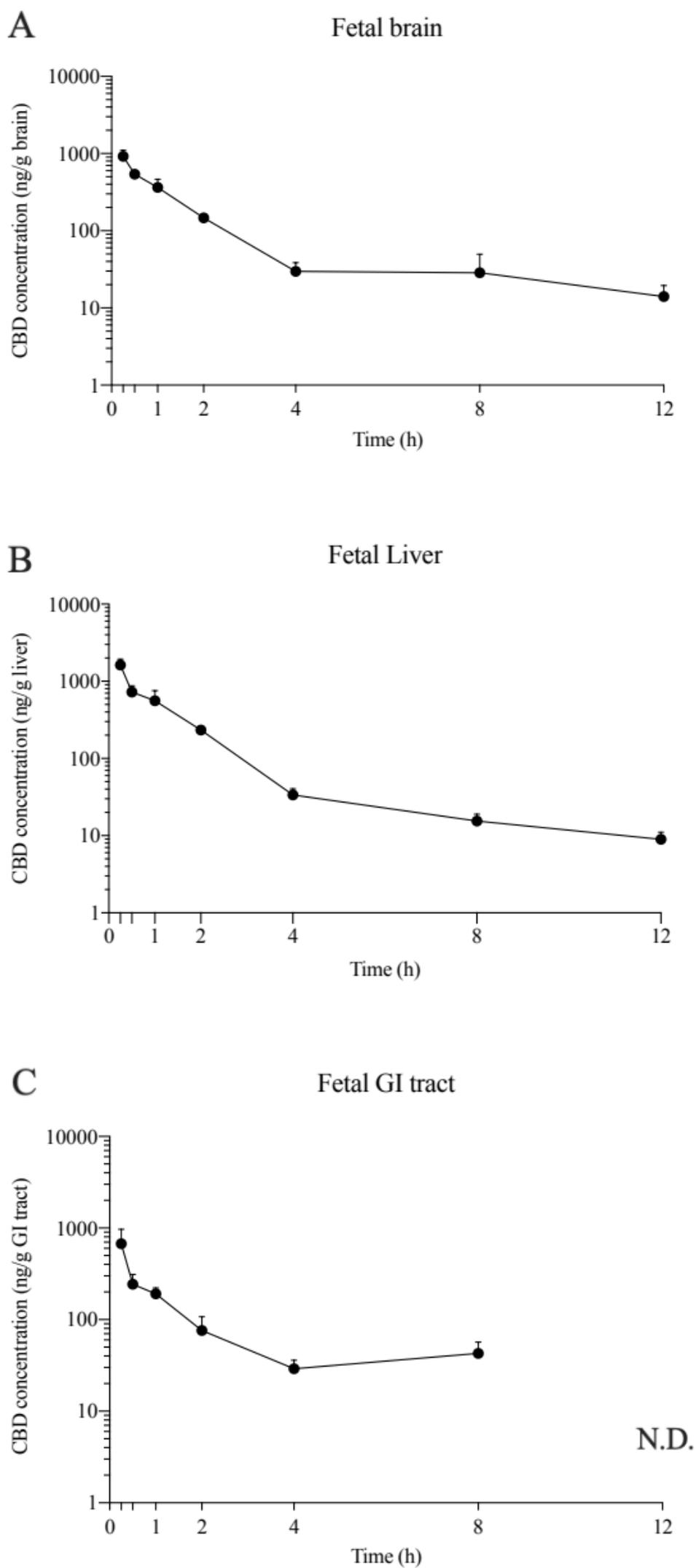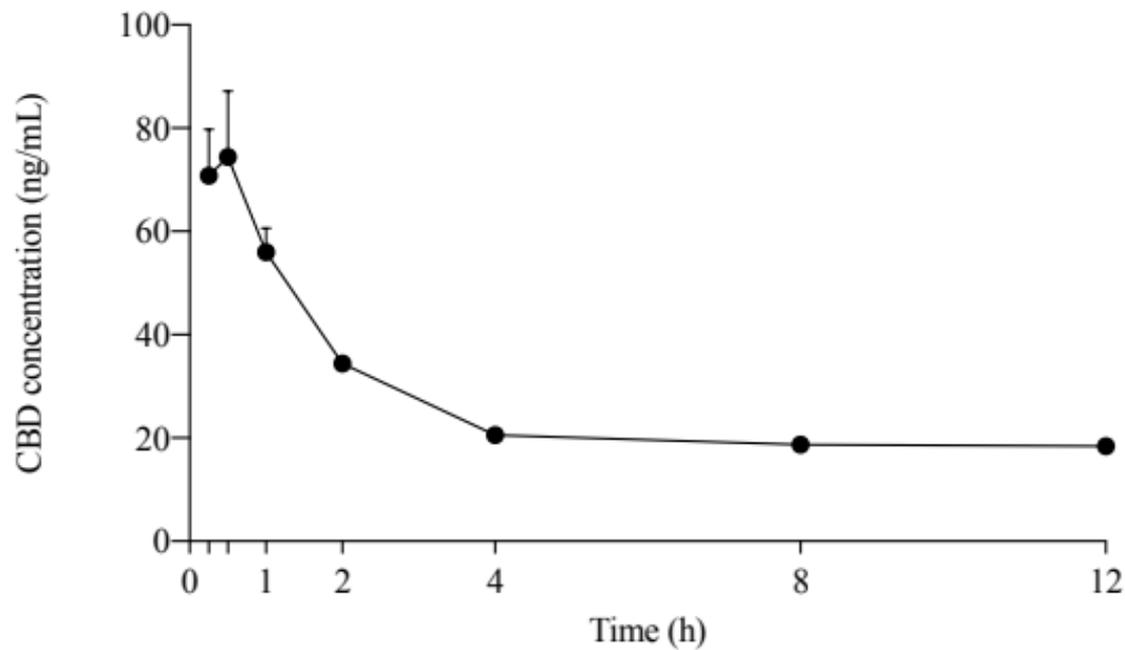
Fig. 4

Amniotic fluid

Fig.5

Supplemental Data

Maternal and fetal pharmacokinetic analysis of cannabidiol during pregnancy in mice

Wataru Ochiai*[1], Satoshi Kitaoka[1], Taisuke Kawamura[1], Jo Hatogai[1], Shohei Harada[1], Misa Iizuka[1], Mashu Ariumi[1], Seiya Takano[1], Tomomi Nagai[1], Masanaho Sasatsu[2], Kiyoshi Sugiyama[3]

[1]Department of Pharmacokinetics, School of Pharmacy and Pharmaceutical Sciences, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan.

[2]Laboratory of Tissue Regeneration, School of Pharmacy and Pharmaceutical Sciences, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan.

[3]Department of Functional Molecule Kinetics, School of Pharmacy and Pharmaceutical Sciences, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan.

w-ochiai@hoshi.ac.jp

ADDRESS MANUSCRIPT CORRESPONDENCE TO: Dr. Wataru Ochiai*, Department of Clinical Pharmacokinetics, School of Pharmacy and Pharmaceutical Sciences, Hoshi University, 2-4-41 Ebara, Shinagawa-ku, Tokyo 142-8501, Japan.

Drug Metabolism and Disposition
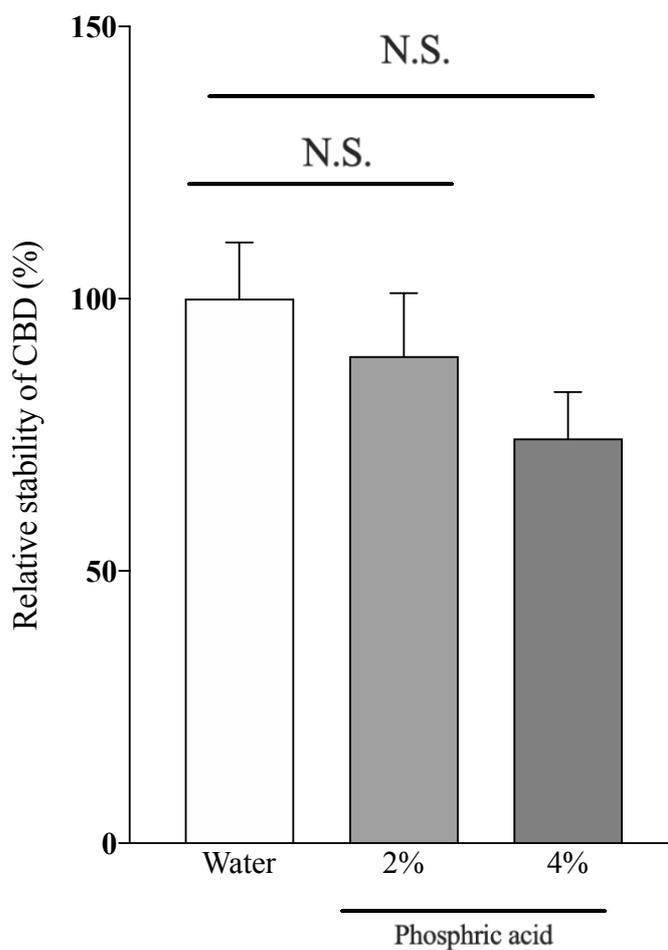
Supplemental Fig. 1   Stability of CBD in phosphoric acid aqueous solution

The stability of CBD during the preparation of LC-MS samples was verified. In

particular, since CBD is unstable under acidic conditions, its stability in a phosphoric

acid aqueous solution was evaluated. Specifically, a CBD solution (10 ng / mL) was

prepared using a 2% or 4% phosphoric acid aqueous solution, and incubated at room

temperature for 10 min. After that, pretreatment was performed according to the

protocol for preparing the LC-MS sample. Then, CBD was quantitatively analyzed by

LC-MS. The analysis results were shown as mean $\pm$ standard deviation (n = 3).

Significant differences were calculated using Student's t-test. N.S., Not significant.