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# Development of a Novel Nanoemulsion Formulation to Improve Intestinal Absorption of Cannabidiol

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**Keywords**
Cannabidiol · Nanoemulsion · Pharmacokinetics · Absorption

**Abstract**
***Background:*** Cannabidiol (CBD) is highly lipophilic, and its oral bioavailability is known to be very low in humans. In this study, we developed a novel nanoemulsion preparation of CBD (CBD-NE) to improve the poor solubility and absorption of CBD. The pharmacokinetic profiles of CBD in rats were evaluated after oral administrations of CBD oil and CBD-NE, and the effect of bile secretion on CBD absorption was also evaluated. ***Methods:*** The CBD-NE formulation developed in this study consisted of vitamin E acetate, ethanol, Tween-20, and distilled water (1.7/3.8/70/24.5, w/w%). A CBD oil formulation (CBD oil, control) 100 mg/kg or CBD-NE 50 mg/kg was orally administered to rats, and the blood samples were collected over time. Moreover, the CBD oil or CBD-NE was orally administered to bile-fistulated rats, and the pharmacokinetic profiles of CBD were also evaluated. CBD concentrations in plasma were measured using LC-MS/MS. ***Results:*** The particle size of CBD-NE was 35.3 ± 11.8 nm. Mean $T_{max}$ of CBD-NE was shortened significantly by the factor of 3 (from 8.00 to 2.40 h, $p < 0.001$) and $AUC_{0-\infty}$/dose increased by 65% (from 0.272 ± 0.045 to 0.448 ± 0.087 h L/kg) compared with CBD oil. $AUC_{0-\infty}$/dose and $C_{max}$/dose after oral administration of CBD oil were significantly reduced by the factor of 27 and 23 ($p < 0.05$ and $p < 0.01$), respectively, in bile-fistulated rats compared with the untreated rats. In contrast, all pharmacokinetic parameters after oral administration of CBD-NE were not significantly different between the untreated and bile-fistulated rats. Therefore, these results demonstrated that conventional CBD oil formulation but not CBD-NE requires bile-mediated micelle formation. ***Conclusions:*** The novel NE formulation developed in this study successfully improved the absorption of CBD regardless of bile secretion. The newly developed oral CBD-NE preparation could be useful to achieve a more stable and quicker onset of action by CBD.



## Introduction

Cannabidiol (CBD) is one of the major cannabinoid constituents of marihuana obtained from *Cannabis sativa* L. Although CBD has a similar chemical structure to that of tetrahydrocannabinol, it has no psychotropic ac-

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tivity [1–5]. CBD is known to modulate the activity of many cellular effectors including CB1 and CB2 receptors [2, 6], 5HT1A receptors [7], GPR55 [8], μ- and δ-opioid receptors [9], TRPV1 cation channels [10], PPARγ [11], and FAAH [10].

CBD has been reported to have several effects such as prolongation of sleep, anti-inflammation, anticonvulsant, anxiolytic, and relief of neuropathic pain [1, 3, 12, 13]. Many kinds of formulations containing CBD have been widely distributed for these effects [14]. Moreover, in June 2018, highly purified CBD Epidiolex®, a CBD oil preparation, was approved by the US FDA for the treatment of seizures associated with Lennox-Gastaut syndrome or Dravet syndrome [15, 16].

CBD is highly lipophilic, and it is usually supplied as an oil preparation. Since the oral bioavailability of CBD is known to be approximately 6% in humans [1], CBD is commonly administered via the sublingual route. CBD is metabolized after administration, and several previous studies have identified CYP3A4 and CYP2C19 as the major isoforms mediating the metabolism of CBD [1, 17]. Therefore, the oral bioavailability of CBD is affected by both the poor solubility, i.e., low absorption, and the large first-pass effect.

Self-micro- or self-nano-emulsifying drug delivery systems can produce nanoemulsion (NE) and improve the oral bioavailability of highly lipophilic compounds [18–20]. NE formulations comprise ternary phases: oil, water, and surfactant. NE is a thermodynamically stable system used as a vehicle to deliver highly lipophilic drugs [21, 22].

In this study, we developed a novel NE formulation containing CBD (CBD-NE) to improve the intestinal absorption of CBD and evaluated its pharmacokinetic profiles in rats. Moreover, we evaluated the effect of bile secretion on the intestinal absorption of CBD from oil and NE formulations using bile-fistulated rats.

## Materials and Methods

*Materials*

CBD powder (purity: 99%) was provided by ENDOCA (Hoofddorp, The Netherlands). Clobetasol propionate, as the internal standard (IS), and acetic acid were obtained from Sigma-Aldrich (St. Louis, MO, USA). Polyoxyethylene sorbitan monolaurate (Tween-20) was purchased from MP Biomedicals, Inc. (Solon, OH, USA). Acetonitrile was obtained from Honeywell International, Inc. (Seelze, Gemany). Other reagents such as ammonium formate, olive oil, (±)-α-tocopherol acetate (vitamin E acetate), and sodium acetate were purchased from FUJIFILM Wako Pure Chemical Corporation (Osaka, Japan).

**Table 1.** Ingredients and each phase volume (w/w%) for the final CBD-NE formulation

| Ingredients | Phase volume, w/w% |
|---|---|
| Vitamin E | 1.70 |
| Ethanol | 3.80 |
| Tween-20 | 70.0 |
| Water | 24.5 |

The CBD formulation was prepared to contain 30 mg CBD/mL.

*Preparation of CBD-NE*

Emulsion formulations are generally composed of ternary phases: oil, water, and surfactant. We used vitamin E acetate, Tween-20, and ethanol as the oil phase, surfactant, and co-surfactant, respectively. In a preliminary study, we optimized the ratios of vitamin E acetate, Tween-20, and water. When the prepared mixture became transparent and clear, we determined that the CBD-NE had been successfully formulated. The particle size of CBD-NE was also measured using a laser nanoparticle analyzer (SALD-7100, Shimadzu, Kyoto, Japan).

The CBD-NE analyzed in the subsequent pharmacokinetic studies was finally prepared as follows. CBD powder (30 mg) was dissolved with vitamin E acetate and gently stirred using a magnetic stirrer in ethanol for 10 min. Then, the mixture was stirred for 10 min after adding Tween-20 and agitated at room temperature (23 ± 5 °C) for 10 min, followed by the addition of distilled water. Ingredients and each phase volume (w/w%) for preparing the CBD-NE formulation are summarized in Table 1 (total volume was 1 mL for 30 mg CBD). The prepared CBD-NE formulation was kept at 4 °C and diluted 3-fold with distilled water just before administration to the rats.

*Animals*

Male Wistar rats (Sankyo Labo Service Corporation, Tokyo, Japan) weighing approximately 240 g were allowed food and water under standard conditions (temperature-controlled facility) on a 12-h light/dark cycle. All animal studies were carried out according to the guidelines for animal experimentation of Showa University, Tokyo, Japan.

*Animal Experiments*

The rats were fasted overnight (approximately 12 h). Under isoflurane inhalation anesthesia, the left femoral artery of each rat was cannulated with silicon tubing (SP-31, Natsume Seisakusho Co., Ltd., Tokyo, Japan) to facilitate blood sampling. The common bile duct was also cannulated using silicon tubing (SP-10, Natsume Seisakusho Co., Ltd.) to prepare the bile-fistulated model as described previously [23]. After the operations, each rat was kept in a Bolman cage and in vivo experiments were carried out after recovery from anesthesia.

*Absorption Profiles of CBD from Oil and NE Formulations*

CBD oil (olive oil solution, 100 mg/kg/2.5 mL) or CBD-NE (50 mg/kg/5 mL) was orally administered to rats, to compare the ab-

sorption profiles of CBD oil and CBD-NE. Approximately 200 μL blood was collected from the femoral artery into heparinized tubes at 0.5, 1, 2, 4, 8, 12, and 24 h (CBD oil) or 0.25, 0.5, 1, 2, 4, 8, 10, and 24 h (CBD-NE) after administration. Plasma samples were obtained by centrifuging (3,000 *g*, 15 min) the blood at 4 °C and kept at –23 °C until analysis.

*Effect of Bile on Absorption of CBD*

CBD oil (olive oil solution, 50 mg/kg/2.5 mL) or CBD-NE (50 mg/kg/5 mL) was orally administered to bile-fistulated rats, to examine the effect of bile secretion on the intestinal absorption of CBD. Approximately 200 μL blood was collected 0.5, 1, 2, 4, 8, and 10 h after administration. Bile samples were also collected in plastic tubes from 0 to 24 h after oral administration. Plasma samples were obtained by centrifugation (3,000 *g*, 15 min) of the blood at 4 °C and kept at –23 °C until analysis.

*Sample Preparation for Measurement*

The IS solution (clobetasol propionate 10 ng/mL in acetonitrile) was dispensed into microtubes and evaporated under a nitrogen stream at 50–70 °C. Then, 50 μL of the plasma sample was added to each microtube. Solid-phase extraction was performed as described previously [24] with some modifications. Bond Elut Plexa cartridges (60 mg, 3 mL, Agilent Technologies, Santa Clara, CA, USA) were set on vacuum, on which 2 mL each of methanol and water was sequentially added (conditioning). Then, 50 μL blood was applied to the cartridge, which was subsequently washed with 2 mL washing solution (water:acetonitrile:1% glacial acetic acid in acetonitrile, 79:20:1, v/v%). After vacuuming for 50 s, 0.75 mL 1% glacial acetic acid in acetonitrile was added twice, the extract was collected into a sample tube, evaporated under a nitrogen stream, and left to stand at room temperature overnight. The residue was reconstituted in 200 μL acetonitrile:1 mM ammonium formate (80:20, v/v%), vortexed, and centrifuged at 10,000 *g* for 10 min at 4 °C. Subsequently, the supernatant was filtered using a syringe filter unit (Ekicrodisc® 3CR, diameter 3 mm, pore size 0.45 μm, Nihon Pall Manufacturing Ltd., Ibaraki, Japan). The extract solution was stored and kept at –23 °C until analysis.

*Quantification of CBD with LC-MS/MS*

The concentration of CBD in plasma was measured using LC-MS/MS as described previously [25–28] with some modifications. The MS system (QTRAP5500, ABSicex, Framingham, MA, USA) was used in the electrospray ionization mode. The scan type was in the multiple reaction monitoring mode run in the positive mode for the IS (clobetasol) at 0–9 min, and in the negative mode for CBD.

A reversed-phase column, X Bridge®C18 (5 μm, 2.1 × 50 mm, Waters, Milford, MA, USA) with a guard cartridge X Bridge®C18 (5 μm, 2.1 × 10 mm, Waters), was used for the LC. The mobile phases A and B (1 mM ammonium formate in water and acetonitrile, respectively) were used in a gradient mode for the chromatographic elution. Then, each sample was injected 10 μL and measured at a total flow rate of 0.3 mL/min using the following gradient: the ratio of mobile phase B (B-conc) was initially set at 25%, after the measurement commenced it was increased to 50% over 2 min, and then it was held for 6 min. The B-conc was increased again to 65% over 1 min and held for 5 min. Finally, the B-conc was increased to 100% over 1 min and held for 5 min, then it was returned to 25% over 2 min and held for 8 min. The autosampler and the column oven were maintained at 14 and 40 °C, respectively.

**Table 2.** LC-MS/MS conditions for quantification of CBD

| | CBD | IS |
|---|---|---|
| *Mass spectrometry parameters for CBD and IS* | | |
| Polarity | Negative | Positive |
| Q1 mass, Da | 313.085 | 467.161 |
| Q3 mass, Da | 245.033 | 372.900 |
| Declistering potential, V | –30 | 41 |
| Entrance potential, V | –10 | 10 |
| Collision energy, V | –28 | 13 |
| Collision cell exit potential, V | –17 | 10 |
| Retention time, min | 11.8 | 5.71 |
| *Optimized source parameters* | | |
| Curtain gas, psi | 20 | 40 |
| Collision gas, psi | 3 | 5 |
| IonSpray voltage, V | –4,500 | 5,500 |
| Source temperature, °C | 400 | 400 |
| Ion source gas 1 | 40 | 80 |
| Ion source gas 2 | 70 | 70 |

CBD, cannabidiol; IS, internal standard (clobetasol propionate).

The MS/MS parameter settings are shown in Table 2. The retention times of the IS and CBD were 5.71 and 11.77 min, respectively. Data acquisition and analysis were performed using a software implemented in the LC-MS/MS system.

*Calibration Curves for CBD Measurement*

Calibration curves for CBD plasma concentrations were obtained at ranges of 10–500 and 200–2,000 ng/mL. Standard solutions of CBD (10 and 100 ng/mL in acetonitrile) were used for calibration. An appropriate amount of the CBD standard solution was dispensed into each microtube, and 100 μL IS solution (10 ng/mL clobetasol propionate in acetonitrile) was added to the tube. After evaporation under a nitrogen stream at 50–70 °C, 100 μL blank plasma was added and vortexed. A portion (50 μL) of the prepared plasma sample was extracted using the method described above. Moreover, we evaluated the intra-day and inter-day variations at CBD concentrations of 10 and 100 ng/mL in plasma.

*Pharmacokinetic and Statistical Analyses*

Areas under the concentration versus time curve (AUC) from zero to a particular time ($AUC_{0-t}$), AUC from zero to infinity ($AUC_{0-\infty}$), terminal half-life ($T_{1/2}$), total clearance ($CL_{tot}/F$), volume of distribution ($V_{dss}/F$), the maximum blood concentration ($C_{max}$), time to reach $C_{max}$ ($T_{max}$), and mean residence time (MRT) were calculated using the Microsoft Excel software program [29] using a moment analysis. The obtained pharmacokinetic parameters were expressed as the mean ± standard error of the mean. To compare the two formulations or two groups of treatments, parameter values with equal variance were tested using Student's *t* test and the others using the Welch test. Differences were considered as statistically significant at $p < 0.05$.

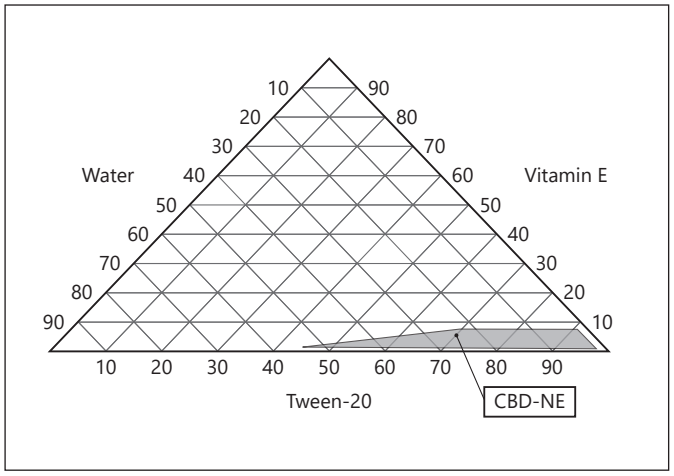


**Fig. 1.** The ternary phase diagram for the optimization of the cannabidiol nanoemulsion (CBD-NE) system (Tween-20/vitamin E/water).

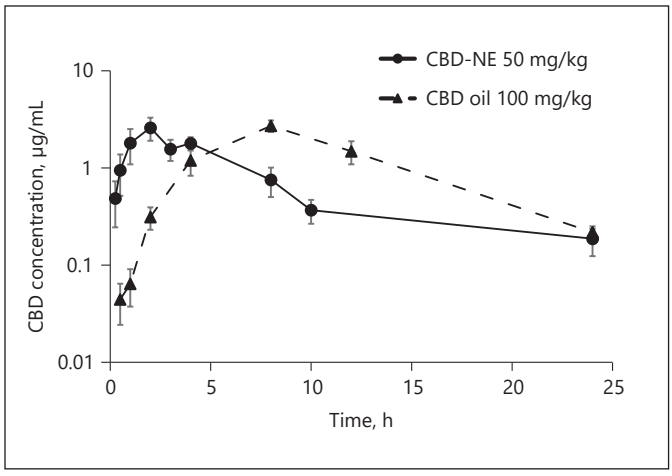


**Fig. 2.** Plasma concentration-time curves of cannabidiol (CBD) oil and CBD nanoemulsion (CBD-NE) administered orally to rats at doses of 100 mg/kg (CBD oil) and 50 mg/kg (CBD-NE). Each symbol with bars represents the mean ± SEM ($n$ = 5 for CBD oil and $n$ = 6 for CBD-NE).

## Results

*Quantification of CBD Using LC-MS/MS*

Linear calibration curves for CBD were obtained at ranges of 10–500 and 200–2,000 ng/mL. Their correlation coefficients ($R^2$) were >0.985 and 0.998, respectively. The lower limit of detection was 10 ng/mL. In the intra-day analysis, precision and accuracy were 15.8 and 3.82% at 10 ng/mL and 3.58 and 3.62% at 100 ng/mL, respectively. In the inter-day analysis, precision and accuracy were 8.20 and 16.2% at 10 ng/mL and 2.88 and 1.83% at 100 ng/mL, respectively. These results satisfied the acceptance criteria (<20 and 15% for the lower and higher concentrations, respectively).

*Characteristics of CBD-NE*

The ternary phase diagram of the CBD-NE system containing Tween-20, vitamin E acetate, and distilled water is shown in Figure 1. In the shaded area of the diagram, the appearance of the mixture became clear and transparent, indicating the formation of a monophasic liquid. The mean ± SD particle size of this transparent formulation immediately after the preparation, on the following day, and 1 week after was determined to be 35.3 ±11.8 nm ($n$ = 12), 43.8 ± 22.0 nm ($n$ = 7), and 44.5 ± 14.0 nm ($n$ = 4), respectively. One week later, the liquid still appeared clear and transparent.

The mean $T_{max}$ of CBD-NE was shortened significantly by the factor of 3 (from 8.00 to 2.40 h) ($p < 0.001$) and $AUC_{0-\infty}$/dose increased by the factor of 1.65 (from 0.272 ± 0.045 to 0.448 ± 0.087 h L/kg) as compared with CBD oil.

**Table 3.** Pharmacokinetic parameters of CBD oil (100 mg/kg) and CBD-NE (50 mg/kg) administered orally to rats

| Parameter | CBD oil ($n$ = 4) | CBD-NE ($n$ = 5) | $p$ |
|---|---|---|---|
| Dose, mg/kg | 100 | 50 | |
| $AUC_{0-24}$, mg h/L | 25.3±4.4 | 16.2±2.7 | |
| $AUC_{0-24}$/dose, h kg/L | 0.253±0.044 | 0.324±0.053 | 0.409 |
| $AUC_{0-\infty}$, mg h/L | 26.7±4.5 | 22.4±4.3 | |
| $AUC_{0-\infty}$/dose, h kg/L | 0.272±0.045 | 0.448±0.087 | 0.172 |
| $T_{1/2}$, h | 4.68±0.31 | 2.40±3.49 | 0.069 |
| $CL_{tot}$/F, L/h/kg | 4.29±1.67 | 2.81±0.67 | 0.258 |
| $V_{dss}$/F, L/kg | 46.7±9.6 | 36.0±7.4 | 0.456 |
| $T_{max}$, h | 8.00±0.00 | 2.40±0.46 | <0.001*** |
| $C_{max}$, µg/mL | 2.72±0.37 | 3.23±0.80 | |
| $C_{max}$/dose, kg/L | 0.0272±0.0037 | 0.0646±0.0159 | 0.103 |
| MRT, h | 10.8±0.4 | 15.10±3.7 | 0.356 |

Each value is expressed as mean ± SEM.

AUC, area under the concentration versus time curve; $T_{1/2}$, terminal half-life; $CL_{tot}$/F, total clearance; $V_{dss}$/F, volume of distribution; $C_{max}$, maximum blood concentration; $T_{max}$, time to reach $C_{max}$; MRT, mean residence time.

*** $p < 0.001$ versus CBD oil.

*Absorption Profiles from CBD Oil and CBD-NE*

Figure 2 shows the absorption profiles of CBD oil (100 mg/kg) and CBD-NE (50 mg/kg) after oral administration to rats. Table 3 presents the pharmacokinetic param-

eters of CBD oil and CBD-NE. $T_{max}$ with CBD-NE indicated a statistically significant ($p < 0.001$) 3.3-fold decrease from 8.00 to 2.40 h compared with CBD oil (control). $AUC_{0-\infty}$/dose and $AUC_{0-24}$/dose increased by 65% (from 0.272 ± 0.0445 to 0.448 ± 0.0866 h L/kg) and by 28% (from 0.253 ± 0.0440 to 0.324 ± 0.532 h L/kg), respectively, with the self-emulsifying NE formulation of CBD compared with CBD oil. Therefore, the relative bioavailability of the CBD-NE formulation compared with that of CBD oil was calculated to be 1.65. On the other hand, the value of AUC/dose obtained after an intravenous bolus administration of CBD was reported to be 0.345 kg/h [30]. Using this literature data and the equation $(AUC_{po}/dose_{po})/(AUC_{iv}/dose_{iv})$, the absolute bioavailability of CBD oil and CBD-NE was estimated to be 73.3 and 93.9%, respectively.

*Effect of Bile on the Absorption of CBD*

Figure 3 shows the absorption profiles of CBD from oil and NE formulations in the bile-fistulated rats. The pharmacokinetic parameters of CBD oil and CBD-NE are presented in Table 4. $AUC_{0-\infty}$ in the CBD-NE group was significantly higher ($p < 0.001$) than that in the CBD oil group, whereas the $V_{dss}/F$ was significantly lower ($p < 0.001$). On the other hand, the $CL_{tot}/F$, $T_{1/2}$, $C_{max}$, MRT, and $AUC_{0-10}$ were not significantly different between the CBD oil and CBD-NE groups. Mean $AUC_{0-\infty}$ and $AUC_{0-10}$ of CBD-NE were markedly higher by the factor of 21.5 and 19.7, respectively, than those of the CBD oil. The $T_{max}$ values were completely the same among all rats (4 h).

In the CBD oil group, $AUC_{0-\infty}$/dose and $AUC_{0-24}$/dose of the bile-fistulated rats were significantly decreased by the factor of 0.058 and 0.020, respectively, compared with those of the untreated rats ($p < 0.05$, Tables 3 and 4). $C_{max}$ of the bile-fistulated rats was also significantly decreased by the factor of 0.022 ($p < 0.01$). In contrast, all pharmacokinetic parameters after oral administration of CBD-NE were not significantly different between the untreated and bile-fistulated rats.

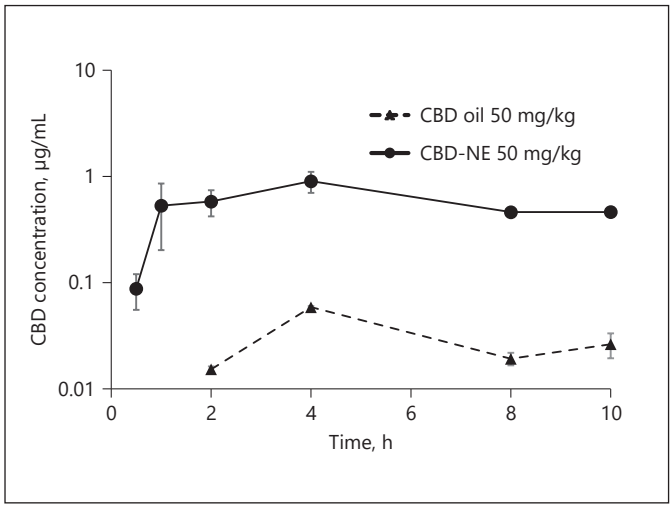


**Fig. 3.** Plasma concentration-time curves of cannabidiol (CBD) oil and CBD nanoemulsion (CBD-NE) administered orally to bile-fistulated rats at doses of 50 mg/kg (CBD oil) and 50 mg/kg (CBD-NE). Each symbol with bars represents the mean ± SEM ($n = 3$). CBD was not detected at 0.5 and 1 h after the administration of CBD oil.

## Discussion

To improve the absorption and bioavailability of CBD, we developed a new CBD-NE formulation and examined the differences in the absorption profiles between the CBD-NE preparation and conventional CBD oil solution. For the CBD-NE preparation, we used the shaded area of the ternary phase diagram system (Fig. 1), which is similar to that used for cyclosporine A microemulsion [19]. The appearance and particle diameter of CBD-NE were the same during storage at 4 °C for at least 6 months. The appearance of the CBD-NE preparation developed in this study was transparent and its particle diameter was small enough to be evaluated as an NE (<45 nm as the mean value). The NE formulation solubilized CBD and remained transparent even after extensive dilution with water (e.g., >100 times diluted), indicating that this was a self-emulsifying system. To the best of our knowledge, this is the first study to develop a self-emulsifying NE for CBD to date.

In this study, we used four ingredients, i.e., water, ethanol, Tween-20, and vitamin E acetate to emulsify CBD for CBD-NE. Ethanol was used as a co-solvent to dissolve CBD powder in the lipid phase, which was also used for Neoral® [31], the commercially available microemulsion formulation of cyclosporine A. Tween-20, which was used as a surfactant, is generally known to be a non-toxic and non-irritant material [32, 33]. However, the Joint FAO/WHO Expert Committee on Food Additives (JECFA) recommended the acceptable daily intake of Tween-20 without any side effects to be 25 mg/kg body weight. Since the effective CBD dose used to treat chronic pain in humans is 100 mg/day [34], the dose of Tween-20 contained in the prepared CBD-NE (70% ratio for Tween-20) can be calculated to be 20.2

mg/kg/day for a 70-kg human, which is less than the above-mentioned acceptable daily intake value (25 mg Tween-20/kg), assuming that the bioavailability of CBD was increased by the NE formulation by 65%.

The volume of oral administration for CBD-NE was chosen to be 5 mL/kg, since this is the optimal volume [35]. On the other hand, an oral administration of CBD oil at 5 mg/kg induced diarrhea in most of the rats used; therefore, the volume was reduced to 2.5 mL/kg for the CBD oil formulation.

In this study, $AUC_{0-\infty}$ and $C_{max}$ following oral administration of CBD oil were similar to the results of previously published studies [26, 27, 30, 36]. The present results demonstrated that the novel CBD-NE formulation improved the bioavailability of CBD ($AUC_{0-\infty}$/dose) by approximately 65%, although this change was not significant. In contrast, $T_{max}$ with CBD-NE was significantly lower than with CBD oil (2.4 vs. 8 h, $p < 0.001$), as shown in Figure 2 and Table 3, indicating an extensively (3.3-fold) enhanced intestinal absorption of CBD using the NE formulation. A cross-sectional study of CBD users [37] reported that >60% intended to treat pain, followed by arthritis pain, anxiety, and depression. Therefore, the rapid absorption of CBD from NE formulations would be preferable for the main purposes of CBD intake, especially pain relief and depression.

Intestinal absorption of a highly lipophilic drug is generally affected by bile production and high-fat meals [38]. In fact, $C_{max}$ and AUC of Epidiolex® with a high-fat/high-calorie meal were reported to increase by the factor of 5 and 4, respectively, compared to normal meals [39]. This suggests that after placing the oil formulation of Epidiolex® inside the cheek, it is largely transferred into the intestine and absorbed via the bile-mediated micelle formation with lipids (released from fat by lipase), but not via oral mucosal absorption.

In this study, an enormous reduction in CBD absorption from the oil preparation was observed in bile-fistulated rats, indicating that the absorption of CBD oil depended on the bile-mediated micelle formation (i.e., solubilization), diffusion of the drug-containing micelles beyond the unstirred water layer at the proximity of intestinal epithelial cells, and subsequent absorption (i.e., translocation) of CBD molecules through the epithelial cell membranes. This notion is the basis of lipid-based formulations of water-insoluble drugs [40, 41]. In contrast, there were no significant changes in the pharmacokinetic parameters between the bile-fistulated and untreated rats administered the CBD-NE formulation, suggesting that CBD-NE rapidly diffused in the intestinal liquids and transversed the unstirred water layer, so that the lipophilic CBD molecules could be efficiently absorbed into the intestinal epithelial cells without the need for bile-mediated micelle formation. Thus, it is reasonable that $T_{max}$ following oral administration of CBD-NE was much shorter than that of CBD oil, which was probably due to bypassing of the micelle formation process in the intestine by formulating CBD into an NE.

**Table 4.** Pharmacokinetic parameters of CBD oil (50 mg/kg) and CBD-NE (50 mg/kg) administered orally to bile-fistulated rats

| Parameter | CBD oil ($n = 3$) | CBD-NE ($n = 3$) | $p$ |
|---|---|---|---|
| Dose, mg/kg | 50 | 50 | |
| $AUC_{0-10}$, mg h/L | 0.300±0.019 | 5.9±1.1 | 0.056 |
| $AUC_{0-\infty}$, mg h/L | 0.502±0.127 | 10.8±0.1 | <0.001*** |
| $T_{1/2}$, h | 5.46±1.81 | 8.3±2.2 | 0.461 |
| $CL_{tot}$/F, L/h/kg | 117±23 | 4.62±0.05 | 0.059 |
| $V_{dss}$/F, L/kg | 992±21 | 63.3±16.0 | <0.001*** |
| $T_{max}$, h | 4.00±0.00 | 4.00±0.00 | |
| $C_{max}$, μg/mL | 0.0588±0.0015 | 0.90±0.20 | 0.077 |
| MRT, h | 10.0±2.6 | 13.7±3.4 | 0.520 |

Each value is expressed as mean ± SEM. For abbreviations, see Table 3. *** $p < 0.001$ versus CBD oil.

This study estimated that the absolute bioavailability of CBD oil in rats is approximately 70%, which is much higher than that in humans (approximately 6%). Several studies showed that the metabolism of CBD in rats was different from that in humans [36, 42, 43], suggesting that the human-rat difference in the absolute bioavailability (i.e., very low in humans but moderate in rats) may be due to the lower first-pass metabolism of CBD in rats. Considering a future clinical study, where the present CBD-NE formulation is orally administered and its pharmacokinetic profile is compared with a conventional oil solution in humans, we could expect the increment of the CBD bioavailability by nanoemulsification to be higher than that observed in the present study (65%). Because the basal bioavailability of CBD in an oil formulation in humans is so low (due to poor absorption and extensive first-pass metabolism), the bioavailability-enhancing effect of NE formulations could be more greatly pronounced than in rats.

Since bile secretion is affected by various factors including food intake, biliary tract infection, and liver function, the anticipated effects of CBD may be more stable and consistent with the formulations than conventional oil formu-

lations. In any case, the novel CBD-NE formulation, which demonstrated a much higher absorption profile of CBD in this study, should be tested in clinical trials and its human bioavailability needs to be clarified in future.

## Conclusion

This study is the first to develop a CBD-NE formulation, which extensively enhanced the absorption of CBD and improved its bioavailability to some extent. We demonstrated that the CBD absorption from the formulation was not affected by bile secretion, whereas that from the conventional oil formulation was greatly reduced by bile fistulation, suggesting a more robust absorption of CBD from NE formulations without food effects. Considering the safety of Tween-20 used as a surfactant in this study, the new NE preparation of CBD might be useful for clinical use in the future.


## Acknowledgement

The authors wish to thank ENDOCA for kindly supplying the pure CBD crystals for our research.


## Statement of Ethics

All animal studies were carried out according to the guidelines for animal experimentation of Showa University, Tokyo, Japan.

## Disclosure Statement

The authors declare no conflicts of interest associated with this manuscript.

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