Cannabidiol Does Not Convert to $\Delta^9$-Tetrahydrocannabinol in an In Vivo Animal Model

Louise Wray,* Colin Stott, Nicholas Jones, and Stephen Wright

Abstract
Introduction: Cannabidiol (CBD) can convert to $\Delta^9$-tetrahydrocannabinol (THC) in vitro with prolonged exposure to simulated gastric fluid; however, in vitro conditions may not be representative of the in vivo gut environment. Using the minipig, we investigated whether enteral CBD converts to THC in vivo.

Materials and Methods: Synthetic CBD (100 mg/mL) was administered orally in a sesame oil formulation twice daily to minipigs ($N=3$) in 15 mg/kg doses for 5 consecutive days. Blood samples were taken before and 1, 2, 4, and 6 h after morning doses on Days 1 and 5. Six hours after the final dose on Day 5, the animals were euthanized, and samples of gastrointestinal (GI) tract contents were obtained. Liquid chromatography with tandem mass spectrometry analysis determined CBD, THC, and 11-hydroxy-THC (11-OH-THC) concentrations. Lower limits of quantification: plasma CBD = 1 ng/mL, plasma THC and 11-OH-THC = 0.5 ng/mL, GI tract CBD = 2 ng/mL, and GI tract THC and 11-OH-THC = 1 ng/mL.

Results: THC and 11-OH-THC were undetectable in all plasma samples. Maximum plasma concentrations ($C_{\text{max}}$) of CBD were observed between 1 and 4 h on Days 1 and 5. CBD was present in plasma 6 h after administration on Days 1 (mean 33.6 ng/mL) and 5 (mean 98.8 ng/mL). Mean $C_{\text{max}}$ CBD values, 328 ng/mL (Day 1) and 259 ng/mL (Day 5), were within range of those achieved in clinical studies. Mean CBD exposure over 6 h was similar on Days 1 (921 h·ng/mL) and 5 (881 h·ng/mL). THC and 11-OH-THC were not detected in all GI tract samples. Mean CBD concentrations reached 84,500 ng/mL in the stomach and 43,900 ng/mL in the small intestine.

Conclusions: Findings of the present study show that orally dosed CBD, yielding clinically relevant plasma exposures, does not convert to THC in the minipig, a species predictive of human GI tract function.

Keywords: cannabidiol; conversion; minipig; tetrahydrocannabinol
to that of THC (i.e., patients treated with oral formulations of CBD do not experience the same psychoactive adverse effects, with the exception of somnolence observed in some patient populations). A randomized, double-blind, cross-over, placebo-controlled trial revealed that, in 16 healthy male human participants, the effects of oral CBD were the same as placebo, whereas oral THC was associated with acute behavioral and physiological effects. Levels of 11-hydroxy-Δ⁹-tetrahydrocannabinol (11-OH-THC) and carboxy-THC (THC-COOH) were clearly elevated after administration of THC (but not CBD or placebo) and followed a similar time course. These findings support the hypothesis that the conversion of CBD to THC reported in in vitro models is not representative of clinical scenarios. At present, a limited number of studies have been completed investigating the potential for CBD to convert to THC in vivo following dosing of oral formulations of CBD. Bode et al. proposed that the minipig is a suitable animal model for the human gastrointestinal (GI) tract and gut metabolic capability. Both humans and pigs have omnivorous diets, and their digestive tracts have similarities with regard to pH values, transit times, and drug absorption. In addition, THC is a known substrate of human CYP2C9 and CYP3A4; both of these isoenzymes have analogs present in the minipig.

### Table 1. Pharmacokinetic Parameters of CBD, THC, and 11-OH-THC in Male Minipigs

| Day 1<sup>a</sup> | Concentrations of CBD in plasma<sup>b</sup> (ng/mL) | Minipig 1 | Minipig 2 | Minipig 3 | Mean | SD |
|-------------------|--------------------------------------------------|-----------|-----------|-----------|------|----|
| 0                 | BLQ                                              | BLQ       | BLQ       | BLQ       | BLQ  | NA |
| 1                 | 9.80                                            | 217       | 283       | 250       | 206  | 46.7 |
| 2                 | 2.74                                            | 163       | 189       | 176       | 181  | 18.4 |
| 4                 | 3.21                                            | 373       | 73.5      | 223       | 212  | 7.4 |
| 6                 | 3.83                                            | 40.8      | 26.3      | 33.6      | 30.6 | 10.3 |
| AUC<sub>0-6</sub> (h·ng/mL) | 23.4                                           | 1130      | 711       | 921       | 852  | 296 |
| AUC<sub>0-t</sub> (h·ng/mL) | 23.4                                           | 1130      | 711       | 921       | 852  | 296 |
| AUC<sub>0-inf</sub> (h·ng/mL) | NR                                             | NR        | 764       | 764       | 764  | NA  |
| C<sub>max</sub> (ng/mL) | 9.80                                           | 373       | 283       | 328       | 311  | 63.6 |
| T<sub>max</sub> (h) | 1                                               | 4         | 1         | Median: 1  | 1    | Range: 1-4 |
| C<sub>last</sub> (ng/mL) | 3.83                                           | 40.8      | 26.3      | 33.6      | 30.6 | 10.3 |
| T<sub>last</sub> (h) | 6                                               | 6         | 6         | Median: 6  | 6    | Range: 6-6 |
| t<sub>1/2</sub> (h) | NR                                              | NR        | 1.41      | 1.41      | 1.41 | NA  |
| V<sub>F</sub>/F (mL/kg) | NR                                             | NR        | 40,000    | 40,000    | 40,000 | NA |

| Day 5<sup>d</sup> | Concentrations of CBD in plasma (ng/mL) | Minipig 1 | Minipig 2 | Minipig 3 | Mean | SD |
|-------------------|--------------------------------------------------|-----------|-----------|-----------|------|----|
| 0                 | 25.5                                            | 17.8      | 94.7      | 46.0      | 42.4 | 42.4 |
| 1                 | 21.4                                            | 25.4      | 194       | 80.3      | 98.5 | 98.5 |
| 2                 | 329                                             | 234       | 207       | 257       | 235  | 64.1 |
| 4                 | 98                                              | 161       | 215       | 158       | 181  | 58.6 |
| 6                 | 58.4                                            | 132       | 106       | 98.8      | 90.0 | 37.3 |
| AUC<sub>0-6</sub> (h·ng/mL) | 733                                           | 834       | 1080      | 881       | 881  | 176 |
| AUC<sub>0-t</sub> (h·ng/mL) | 733                                           | 834       | 1080      | 881       | 881  | 176 |
| AUC<sub>0-inf</sub> (h·ng/mL) | NR                                             | NR        | NR        | NR        | NR   | NA  |
| C<sub>max</sub> (ng/mL) | 329                                           | 234       | 215       | 259       | 259  | 61.1 |
| T<sub>max</sub> (h) | 2                                               | 2         | 2         | Median: 2  | 2    | Range: 2-4 |
| C<sub>last</sub> (ng/mL) | 58.4                                           | 132       | 106       | 98.8      | 98.8 | 37.3 |
| T<sub>last</sub> (h) | 6                                               | 6         | 6         | Median: 6  | 6    | Range: 6-6 |
| t<sub>1/2</sub> (h) | NR                                              | NR        | NR        | NR        | NR   | NA  |
| V<sub>F</sub>/F (mL/kg) | NR                                             | NR        | NR        | NR        | NR   | NA  |

<sup>a</sup>Day 1: following a single 15 mg/kg oral dose of CBD.

<sup>b</sup>THC and 11-OH-THC were not detectable in all samples (LLOQ = 0.5 ng/mL).

<sup>c</sup>A partial regurgitation of minipig 1’s dose was observed on the morning of Day 1. Therefore, data from this minipig have been excluded from Day 1 calculations (italicized).

<sup>d</sup>Day 5: following 4 days of twice-daily oral doses and one final dose of CBD (total of nine doses, each 15 mg/kg).

AUC, area under the plasma concentration-time curve; BLQ, below limit of quantification; CBD, cannabidiol; Cl/F, total body clearance for the fraction of absorbed dose; C<sub>max</sub>, maximum plasma concentration; C<sub>last</sub>, time of the last measurable concentration; LLOQ, lower limit of quantification; NA, not applicable; NR, no result (no clear terminal elimination phase); 11-OH-THC, 11-hydroxy-Δ⁹-tetrahydrocannabinol; SD, standard deviation; THC, Δ⁹-tetrahydrocannabinol; t<sub>1/2</sub>, terminal elimination phase half-life; T<sub>max</sub>, time of maximum concentration profile; t<sub>last</sub>, time of the last measurable concentration; V<sub>F</sub>/F, volume of distribution for the fraction of absorbed dose.
Taking into account the similarity of the pH of the minipig stomach to that of humans, and the reported in vitro conversion of CBD to THC occurring in an acid medium, the minipig was determined to be an appropriate in vivo model for the present research initiative. To assess the potential conversion of CBD to THC in the stomach, the plasma and GI tract samples of minipigs were assayed for the presence of THC and the first metabolite of THC, 11-OH-THC, after oral CBD administration. Outcomes included plasma pharmacokinetics of CBD, THC, and 11-OH-THC, and concentrations of CBD, THC, and 11-OH-THC in GI tract content samples.

**Materials and Methods**

The study was undertaken at Covance Laboratories in Harrogate, United Kingdom, in July and August 2016 and funded by GW Research, Ltd. The study was in full compliance with local, national, ethical, and regulatory principles, in addition to local licensing arrangements.

**Subjects**

Three naïve male Ellegaard Göttingen minipigs (born in February 2016), without external signs of ill health, were used in the study. Each animal was kept in a separate solid floor pen, given a nominal amount of SDS SMP (E) (Special Diets Services Ltd., Witham) diet twice daily, and allowed free access to water. For 2 weeks before the first dose administration, each animal was acclimatized daily to the bleed cradle and bite bar used for blood sampling and was sham dosed with 10 or 20 mL of water each day.

**CBD formulation**

The CBD oral solution (GW Pharmaceuticals, London, United Kingdom) contained 100 mg/mL of synthetic CBD as the botanically occurring (–)-isomer. Purified, plant-derived CBD is known to contain small traces of THC; therefore, synthetic CBD was dosed in this study to avoid the generation of false positive results.

**Protocol**

**CBD administration.** Oral doses were administered twice per day (morning and afternoon), by gavage, for 4 days; on Day 5, a single dose was administered in the morning. The doses were administered, by weight, at ~0.15 mL/kg, to give a dose equivalent to 15 mg/kg of CBD. After each dose, the gavage was flushed with ~10 mL of water. Dosing levels were determined and justified on the basis of clinical dosing and previous findings that revealed an adequate safety margin based on toxicology studies in dogs.*

**Sampling.** On Days 1 and 5, blood samples were collected from a jugular vein in all animals before dose administration and 1, 2, 4, and 6 h after dose administration. Blood samples were centrifuged (3000 rpm, 10 min, 4°C) to isolate plasma.

Six hours after the final dose (Day 5), the animals were humanely euthanized and contents of the stomach and small intestine were harvested.

Plasma samples and contents of the stomach and small intestine were stored (less than –50°C) and submitted for bioanalysis. Analysis was performed using liquid chromatography with tandem mass spectrometry.

**Outcome measures.** Outcome measures included the following:

- Presence of THC and the first metabolite of THC (11-OH-THC) in plasma, with a lower limit of quantification (LLOQ) of 0.5 ng/mL

*GW Research Ltd., unpublished data, April 2017.
Plasma levels of CBD, with an LLOQ of 1 ng/mL. Concentrations of CBD, THC, and 11-OH-THC in GI tract content samples (CBD LLOQ = 2 ng/mL, THC and 11-OH-THC LLOQ = 1 ng/mL) were determined by the bioanalysis laboratory as the lowest quantifiable concentrations within linear range for the analytes. The 11-OH-THC levels were quantified to allow detection of THC that has already undergone phase I metabolism.

Data analysis
Pharmacokinetic analyses were completed using Phoenix WinNonLin version 6.4 software (Pharsight Corporation, Sunnyvale, CA). Parameters calculated included area under the plasma concentration-time curve calculated from 0–, where is the time of the last measurable concentration (AUC0–), maximum plasma concentration (Cmax), time of maximum concentration profile (tmax), the last measurable concentration (Clast), time of the last measurable concentration (tlast), terminal elimination phase half-life (t1/2), volume of distribution for the fraction of absorbed dose (V/F), and total body clearance for the fraction of absorbed dose (Cl/F).

Results
Plasma samples
Individual plasma concentrations and pharmacokinetic parameters of CBD, THC, and 11-OH-THC are shown in Table 1. THC and 11-OH-THC were below LLOQ in all plasma samples (Fig. 1). On Day 1, a partial regurgitation of one minipig’s dose was observed. As a result, this animal is likely to have received a reduced dose on this day, indicated by concentrations of CBD that were much lower than expected when compared with those of the other two animals. Therefore, data from this minipig have been excluded from Day 1 calculations. All subsequent doses were successfully received by all three animals, so results following the Day 5 final dose are for all three animals.

| Tissue                  | Concentrations of CBD (ng/mL) | Minipig 1 | Minipig 2 | Minipig 3 | Mean  | SD   |
|-------------------------|-------------------------------|-----------|-----------|-----------|-------|------|
| Stomach contents        |                               | 108,000   | 66,700    | 78,900    | 84,500| 21,200|
| Small intestine contents|                               | 43,100    | 56,600    | 32,000    | 43,900| 12,300|

aAll measurements were taken after 4 days of twice-daily oral doses and one final dose of CBD (total of nine doses, each 15 mg/kg).
bTHC and 11-OH-THC were not detectable in all samples (LLOQ = 1 ng/mL).

- Plasma levels of CBD, with an LLOQ of 1 ng/mL
- Concentrations of CBD, THC, and 11-OH-THC in GI tract content samples (CBD LLOQ = 2 ng/mL, THC and 11-OH-THC LLOQ = 1 ng/mL)

LLOQs were determined by the bioanalysis laboratory as the lowest quantifiable concentrations within linear range for the analytes. The 11-OH-THC levels were quantified to allow detection of THC that has already undergone phase I metabolism.

**FIG. 2.** Mean gastrointestinal concentrations for CBD, THC, and 11-OH-THC in male minipigs after 4 days of twice-daily oral doses and one final dose of CBD (total of nine doses, each 15 mg/kg; Day 5). Error bars represent standard deviations. *THC and 11-OH-THC levels were not detectable.
The mean CBD $C_{\text{max}}$ values of 328 and 259 ng/mL were observed between 1 and 4 h on both Days 1 and 5, respectively. These values are confirmed as similar to those reported in clinical studies following a 20 mg/kg daily oral dose of CBD (mean plasma concentration, 380 ng/mL).\textsuperscript{16} CBD remained present in plasma 6 h after dosing on Days 1 and 5 at mean concentrations of 33.6 and 98.8 ng/mL, respectively. The mean exposure levels over 6 h were similar on Days 1 and 5 (921 and 881 h·ng/mL, respectively). Only one animal (minipig 3) exhibited a clear terminal phase to allow terminal parameters to be calculated from Day 1 data (Table 1). Terminal phase parameters were not calculable from the Day 5 samples, as no clear terminal phase could be identified in the data for any of the three animals. Overall, the data showed that the metabolic turnover of CBD was consistent following a single dose or multiple twice-daily doses of 100 mg/mL CBD oral formulation.

GI tract samples

Individual concentrations of CBD, THC, and 11-OH-THC in the GI tract contents of all minipigs are shown in Table 2. THC and 11-OH-THC were not detected in any of the GI tract samples. Mean CBD concentrations in GI contents reached 84,500 ng/mL (stomach) and 43,900 ng/mL (small intestine), \~326 times and 169 times the maximum day 5 plasma concentrations, respectively (Figs. 1 and 2).

Discussion

The results show that standard clinical doses of CBD in a sesame oil formulation do not convert to THC in the minipig. These data support previous reports suggesting that caution should be taken when extrapolating \textit{in vitro} results to the \textit{in vivo} situation.\textsuperscript{12,13} Although \textit{in vitro} studies are essential in drug development, absorption of a drug via the GI tract is a multistep, dynamic process that needs to account for physiologic factors, formulation, and influences from the whole organism to yield accurate predictions.\textsuperscript{12,17}

Plasma levels of CBD found in the minipigs were similar to those measured in the clinic (average plasma concentration = 380 ng/mL),\textsuperscript{16} supporting the notion that the CBD plasma levels in the minipig were representative of those observed in a clinical environment. High concentrations of CBD were detected in the stomach and small intestine of the minipigs. However, THC and 11-OH-THC were not found in any of the plasma or GI tract samples across all time points. These results strongly suggest that the conversion of CBD to THC observed \textit{in vitro}\textsuperscript{8,9} is not reproducible \textit{in vivo}, which is in agreement with previous clinical observations from human studies.\textsuperscript{10,11}

Schwilke et al.\textsuperscript{18} found that after multiple high doses of THC to human subjects, the metabolites 11-OH-THC and THC-COOH increased over time, indicating accumulation, whereas THC did not. Therefore, it is likely that if CBD had converted to THC in our study, 11-OH-THC would have accumulated to a detectable level (>0.5 ng/mL) over the 5-day period. However, plasma 11-OH-THC was not detected in this study.

When the findings of the present study are set beside the data that describe the conversion of CBD to THC in simulated gastric juices \textit{in vitro}, it is evident that the use of a complete GI system in a species predictive of the human GI tract can produce markedly different results. We have, therefore, substantially extended our understanding of the likelihood of such a conversion taking place by demonstrating its absence in an intact, \textit{in vivo} mammalian system. We acknowledge that our study had some limitations such as group sample size and end-point analytes. Consequently, supplementary research in human subjects with the measurement of 11-COOH-THC and $\Delta^8$-THC, in addition to validated \textit{in silico} modeling may be required to confirm the hypothesis supported by our results that the conversion of CBD to THC does not occur \textit{in vivo}.

Conclusion

The findings of this study suggest that, when dosed orally in a sesame oil formulation to give CBD plasma exposures similar to those known to be clinically relevant in patients, CBD does not convert to THC in the minipig. This finding significantly advances our understanding of the safety concerns regarding CBD-based therapies beyond \textit{in vitro} systems. Given the randomized controlled clinical trials of oral CBD now published, confirmation of these findings in humans may be warranted.

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Author Disclosure Statement

All authors are employees of GW Research Ltd.
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Abbreviations Used

CBD = cannabidiol
GI = gastrointestinal
LC-MS/MS = liquid chromatography with tandem mass spectrometry
LLOQ = lower level of quantification
THC = Δ⁹-tetrahydrocannabinol
11-OH-THC = 11-hydroxy-Δ⁹-tetrahydrocannabinol

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