M5100 Analogue Derivatization Of CBD For Improved Ocular Permeation: In Vitro And In Vivo Evaluation

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PURPOSE

- Cannabidiol (CBD) is one of the active components of the plant *Cannabis Sativa* and has been studied in the management of epilepsy, anxiety, sleep and appetite disorders.
- CBD, by virtue of its anti-inflammatory properties, might also be a treatment option for diabetic retinopathy induced pain and inflammation, by modulating the formation of tumor necrosis factor (TNF) and scavenging reactive oxygen species (ROS).
- However, CBD is a lipophilic molecule (log P 5.9) making its topical delivery to target tissues at the back of the eye extremely challenging.
- This work aims at improving ocular penetration of CBD by means of analogue derivatization.

OBJECTIVES

1. Modelling of CBD analogues with desirable physicochemical properties (aiming improved permeation properties).
2. *In-vitro* screening of synthesized analogs in ocular tissue homogenates for determination of stability of the engineered analogues in the ocular tissues.
3. Formulation development of lead candidates for topical ocular therapy.
4. The ocular disposition of optimized formulations in a rabbit model (*in-vivo)*. The tissues analyzed were Aqueous Humor (AH), Vitreous Humor (VH, Retina Choroid (RC), Iris Ciliary bodies (IC).

METHODS

CBD derivatives were prepared using the procedure described in an International patent application # WO2017/132526A1.

Tissue Homogenate Stability (*In-vitro*)

AH, VH, RC, IC bodies used were homogenized in ice cold IPBS

The homogenates were centrifuged at 13000 rpm at 4 °C for 15 mins and equilibrated for 30 min at 37 °C to activate the enzymes.

To the supernatant, the analogues in ethanol were added and mixed.

Samples were withdrawn at specific time intervals up to 6 h.

An equal volume of ice cold acetonitrile was added to the samples and centrifuged at 13000 rpm for 15 min.

The supernatant was analyzed by HPLC-UV.

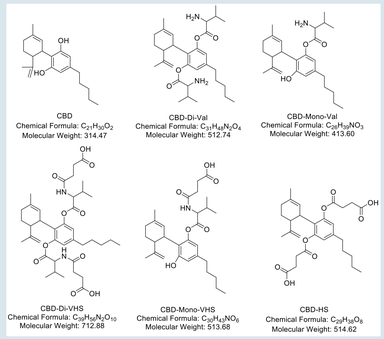

Fig 1. (On the left) CBD and analogues including their physicochemical properties. *All animal studies were conducted as per the University of Mississippi Institutional Animal Care and Use Committee approved protocols.

Formulation Development

CBD and the associated analogues were formulated into a soybean-oil based nanoemulsion composed of a 1:4 ratio of soya oil/water that is emulsified with the block co-polymer Pluronic F68 (Tocrisolve™).

The formulations were prepared by adding the drug/analogue to the blank emulsion and vortexing for five minutes followed by sonication for ten minutes.

The maximum drug loading (drug content) for CBD and the analogues is listed in Table.1.

Ocular Disposition (*In-vivo*)

Selected formulations were tested in a conscious New Zealand Albino rabbit model to determine ocular tissue disposition, following topical application.

Fifty microliters of the formulations were instilled in the conjunctival sac. Dose: 250µg CBD equivalent

The rabbits were sacrificed after 90 minutes. The concentration of CBD and derivatives from the AH, VH, IC, and RC tissues were analyzed by Shimadzu LC with AB Sciex LC-MS/MS.

RESULTS (TABLE)

Drug/ Analogue	Log P	Maximum Solubility Achieved (in terms of CBD)
CBD	5.9	11.9 mg/ml
CBD-Mono-VAL	6.43	14.1 (10.7) mg/ml
CBD-Mono-VHS	5.42	11.8 (6.1) mg/ml
CBD-HS	5.19	11.2 (7) mg/ml
CBD-Di-VAL	6.96	20.4 (10.9) mg/ml
CBD-Di-VHS	5.06	12.1 (5.3) mg/ml

Table.1. Log P and % Solubility of select analogues in Tocrisolve™ emulsion

*Log P was predicted using ChemDraw Professional by Crippen's fragmentation method

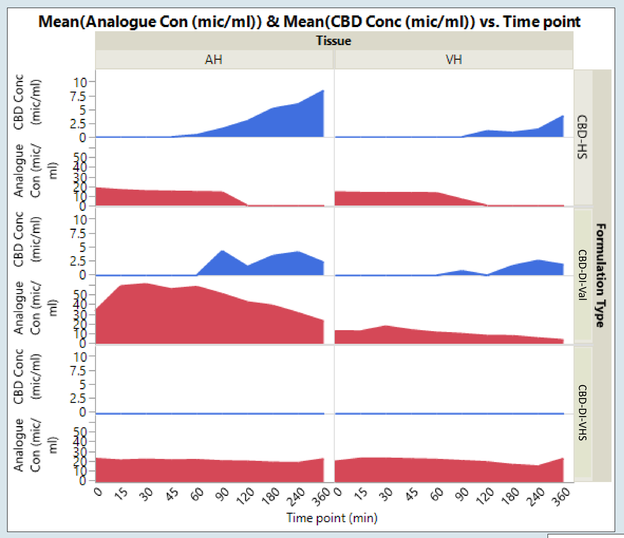

Fig.2. Stability of CBD-HS, CBD-Di-Val, CBD-Di-VHS in Aqueous Humor and Vitreous Humor

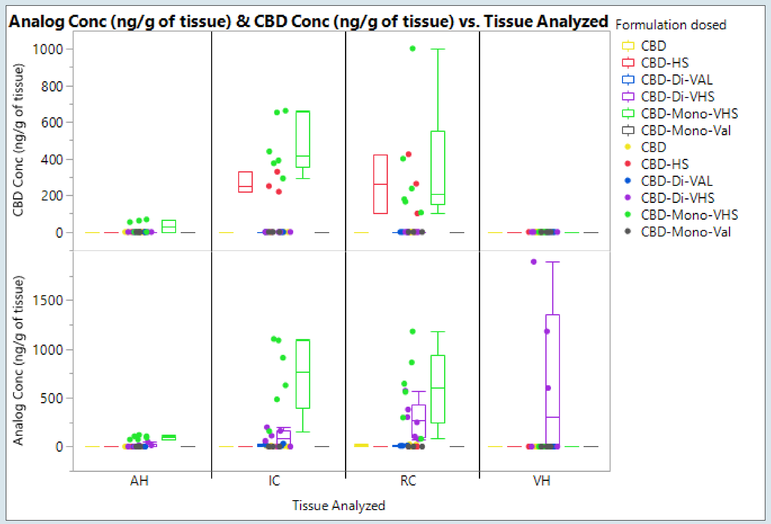

Fig.3. Disposition of CBD and analogs formulated in Tocrisolve™ emulsion 90 minutes post topical administration in AH, IC, RC, VH. CBD, CBD-Di-VHS, CBD-Mono-VHS (n=6); CBD-HS, CBD-Di-Val, CBD-Mono-Val (n=3)

RESULTS

1. In the *in-vitro* setup:
- We observed the bioconversion of CBD-HS into CBD.
- CBD-Di-VHS showed a slow decline over six hours, but we did not observe bioconversion to CBD.
- The amino acid analogue, CBD-Di-Val **retained analogue levels up to six hours** whilst slowly converting to CBD.

2. The **AA** or **DCA** analogues **did not permeate** efficiently to the anterior as well as posterior segments.
3. A **combination amino acid-dicarboxylic acid (AA-DCA) analogue** could impart improved stability and permeation characteristics.
4. In the *in-vivo setup:*
- The mono derivatized form CBD-Mono-VHS and CBD-HS showed a **conversion of Analogue to CBD** indicating slow bioconversion of analogue.
- The AA-DCA analogues, CBD-Di-VHS and CBD-Mono-VHS showed **enhanced permeation to the posterior ocular tissues, IC and RC**.

CONCLUSION

1. Chemical-engineering of analogs, taking into account the microenvironment of the eye and tissue barrier characteristics, is an efficient way of designing molecules with improved permeation profiles.
2. A DCA-AA derivatization protocol resulted in analogs with favorable physico-chemical properties allowing improved permeation (into multiple ocular compartments) and stability.
3. CBD-Mono-VHS demonstrated the best ocular bioavailability.

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