Development of Δ⁹-Tetrahydrocannabinol Prodrug with Improved IOP Lowering Efficacy

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PURPOSE

The aim of this study is to evaluate the intraocular pressure lowering activity of a relatively hydrophilic Δ⁹-Tetrahydrocannabinol (THC) prodrug, Δ⁹-THC-valine-hemisuccinate (THC-Val-HS) in a α-chymotrypsin induced rabbit glaucoma model and to compare the IOP lowering activity with that of THC, WIN-55,212-2 (WIN-55), Timolol maleate and Pilocarpine eye drops and controls.

INTRODUCTION

- Tetrahydrocannabinol (THC) has anti-glaucoma activity: IOP lowering and an independent neuroprotective activity.
- Delivery of THC to the ocular tissues is limited by its poor physicochemical properties, in addition to the formidable ocular barriers to drug penetration following topical application.
- In the present study, the pharmacological activity of a promising, relatively hydrophilic prodrug of THC, THC-valine-hemisuccinate (THC-Val-HS), formulated in nanoemulsion and micellar ophthalmic solutions, was evaluated.
- The IOP lowering activity of THC-Val-HS was evaluated in the α-chymotrypsin induced rabbit glaucoma model and was compared with that of THC, WIN-55, Timolol maleate and Pilocarpine eye drops.
- *In vitro* receptor binding studies were carried out to investigate the affinity of the prodrug for the cannabinoid receptors (CB1 and CB2).
- *In vivo*, THC-Val-HS converts into the parent moiety THC by the actions of esterases and peptidases, which then exerts the anti-glaucoma activity. Bio-reversion rates of THC-Val-HS to THC in the ocular tissue homogenates was, thus, also studied.

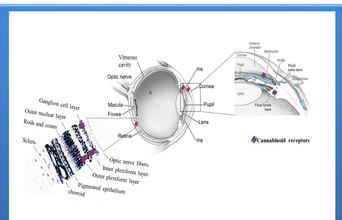

Figure 1: Anatomical features of the eye, depicting localization of cannabinoid receptors in the cornea (corneal epithelium and endothelium), ciliary body (ciliary muscle, non-pigmented ciliary epithelium, trabecular meshwork and ciliary body) and layers of retina (outer plexiform layer, inner plexiform layer and ganglionic cell layer)

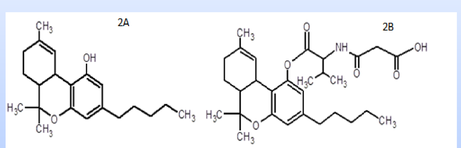

Figure 2: Chemical Structures of A) THC B) THC-Val-HS

METHODS

Preparation of nanoemulsions and micellar solution formulations

- Nanoemulsions of THC-Val-HS (THC Eq: 0.6 %w/v), WIN-55 (0.8 %w/v), THC (0.8 %w/v) were prepared in Tocrisolve™ 100 emulsion (Tocris Bioscience).
- A micellar solution of THC-Val-HS (THC Eq: 0.55 %w/v) was also prepared. The formulation composition was as follows: HPβCD (15 %w/v); Cremophor® RH40 (0.25 %w/v); HPMC (0.5 %w/v); benzalkonium chloride (0.01 %w/v); EDTA (0.2 %w/v) in IPBS (pH 7.4). The THC solution formulations containing HPβCD (2.5 % and 5 %w/v) and HPMC (0.5 %w/v) were also prepared.

Development of rabbit glaucoma model

All animal experiments conformed to the tenets of the Association for Research in Vision and Ophthalmology (ARVO) statement on the Use of Animals in Ophthalmic and Vision Research and followed the University of Mississippi Institutional Animal Care and Use committee approved protocols (UM IACUC Protocol No # 14-005 & 13-004).

- Open angle glaucoma was induced in New Zealand white rabbits with a single intravitreal injection of a freshly prepared solution of α-chymotrypsin (50 µL, 20 mg/mL) in water for injection.
- Once the IOP stabilized (constant IOP for three successive days), studies evaluating IOP lowering effect were initiated.
- Fifty microlitres of each of the formulations (THC and THC-Val-HS nanoemulsions, and micellar solutions, WIN-55 nanoemulsions and marketed Timolol and Pilocarpine eye drops) were instilled topically into the lower *cul de sac* of the test eye, while the other eye served as control.
- IOP was measured before instillation (baseline IOP) and every 30 min till the IOP returned to 90 % of the baseline IOP, using Tonovet® tonometer (Reichert Inc.)
- The average percent change in IOP from the baseline IOP was calculated and expressed as %ΔIOP ± SEM.
- The IOP lowering effect of THC-Val-HS was compared against THC, WIN-55, Timolol maleate and Pilocarpine (marketed) eye drops, in terms of %ΔIOP for each of the formulations.
- All the animals were also observed for allergic reactions such as inflammation or redness or excessive tearing throughout the duration of the study.

In vivo bioavailability studies[1]

- Ocular bioavailability was determined in Male New Zealand albino rabbits weighing between 2-2.5 Kg under anesthesia throughout the experiment.
- The rabbits were placed on one side and 50 µL of the formulations (compositions as shown in Table 3) was placed in the *cul-de-sac*.
- At predetermined time intervals, rabbits were sacrificed with an overdose of pentobarbital administered through the marginal ear vein. The surface of the eyes were washed thoroughly with ice cold IPBS and immediately enucleated. Ocular tissues were separated and placed at -80 °C until further analysis. All experiments were carried out in triplicate. and samples were analyzed as per the reported method[1, 2]

Receptor binding studies

- Binding of THC-Val-HS to the cannabinoid receptors (CB1 & CB2) was studied in comparison to CP-55,940, a potent cannabinoid receptor full agonist.

Bio-reversion of the prodrugs in the ocular tissues

- Bio-reversion studies to determine the rate of conversion of THC-Val-HS into the active moiety (THC) was carried out in aqueous humor and samples were analyzed as per the reported method[1]

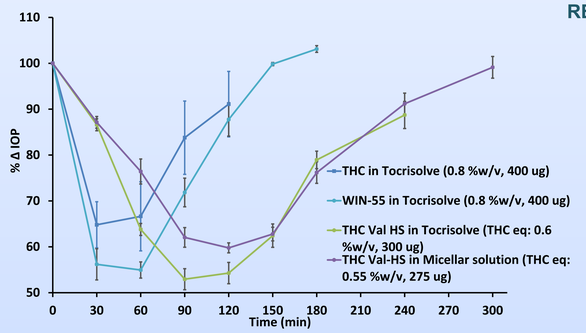

Figure 3: IOP-Time profile for THC-Val-HS in comparison with THC, WIN-55 in rabbit glaucoma model. Data represents Mean ± SEM. Numbers in brackets represent concentration and dose of THC and WIN-55.

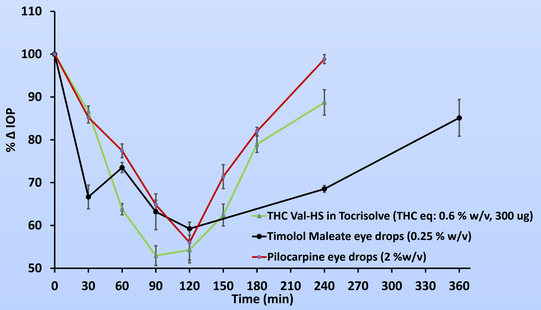

Figure 4: IOP-Time profile for THC-Val-HS in comparison with Timolol maleate and Pilocarpine eye drops (marketed) in rabbit glaucoma model. Data represents Mean ± SEM. Numbers in brackets represent concentration and dose.

Table 1 : Predicted Physicochemical Properties of THC-Val-HS using ACD-I Lab 2.0

Drug	ACD I-Lab Predicted Values					
	MW	pKa	logP	$logD_{7.4}$	Solubility (µg/mL)	PSA
THC	314.2	9.6	7.68	7.07	1.0	29.46
THC-Val-HS	513.6	4.5 13.3	7.65	3.97	0.96	101.13

Table 2 : The pH Dependent solubility of THC-Val-HS .Results are depicted as mean ± SD (n=3). ND* - Not Detectable

pH	Water	IPBS	pH 3	pH 5	pH 7	pH 9
Solubility (µg/mL)	37.6 ± 6.6	97.3 ± 1.7	ND*	1.3 ± 0.1	76.8 ± 12.9	141.8 ± 32.9

RESULTS

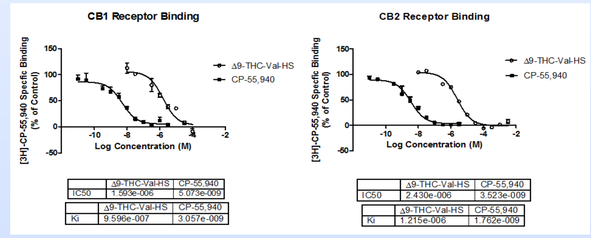

Figure 5 : Results of cannabinoid receptor binding studies.

Bio-reversion of the prodrugs in the ocular tissues:

- The *in vitro* biological half-lives of THC-Val-HS in rabbit aqueous humor was found to be 5.9 ± 0.1 minutes.

Table 3: Total THC concentrations observed in rabbit ocular tissues post topical administration of 50 µL of THC-Val-HS solution formulation. Results are depicted as mean ± SD (n=3)[1]

Tissue	Formulation 1 (2.5 % HPβCD + 0.5% HPMC)		Formulation 2 (5% HPβCD + 0.5% HPMC)	Micellar solution
	1h	3h	1h	1h
Drug Concentration (% w/v)	0.26		0.5	0.25
Cornea (ng/50 mg Tissue)	1677.1 ± 172.1	1142.3 ± 415.9	443.5 ± 152.2	1191. 7 ± 231.1
Aqueous Humor (ng/100 µL)	69.4 ± 16.7	38.3 ± 10.2	31.3 ± 13.5	62.1 ± 12.6
Iris-cilliary Body (ng/50 mg Tissue)	65.8 ± 15.9	57.9 ± 16.1	50.2 ± 9.9	51.44 ± 19.5
Vitreous Humor (ng/mL)	ND*	ND*	ND*	ND*
Retina-Choroid (ng/50 mg Tissue)	ND*	ND*	ND*	ND*
Sclera (ng/250 mg Tissue)	882.2 ± 185.8	241.8 ± 106.6	191.5 ± 50.1	913.4 ± 432.9

ND*- Not detectable

- The *in vivo* bioavailability studies are in agreement with the IOP lowering effect.
- The delay in the onset of IOP reduction can be attributed to the bio-reversion of THC-Val-HS to THC

CONCLUSIONS

- THC-Val-HS showed IOP decrease upon topical administration, as nanoemulsion and micellar solution formulations, in α-chymotrypsin induced glaucoma model, but did not show any effect on normotensive rabbits.
- The IOP lowering effect of THC-Val-HS was more profound than WIN-55 and THC: both in terms of intensity and time for maximum effect (E_{max}) at a lower THC equivalent dose.
- THC-Val-HS produced greater %ΔIOP than Timolol maleate, but a comparatively shorter duration of action. Compared to Pilocarpine, THC-Val-HS had more significant IOP reduction effect.
- *In vitro* receptor binding studies demonstrated that THC-Val-HS does not have any significant affinity for cannabinoid receptors (CB1 and CB2).
- Thus, hydrophilic prodrug derivatization significantly improved ocular penetration and therapeutic potential of THC.

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