IOP lowering ability of NB-1111 active moiety, THC, in a human tissue model

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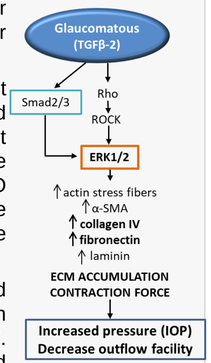
Background/Objective

The human eye has a high density of CB11 and CB22 cannabinoid receptors, especially located in tissues that regulate intraocular pressure (IOP). Both receptors are located along the trabecular outflow pathways of the trabecular meshwork (TM).

Tetrahydrocannabinol (THC) has been shown to lower IOP3,4. Most recently, the University of Mississippi, utilizing a topically administered prodrug (NB1111; THC-valine-hemisuccinate) in a validated rabbit model, demonstrated superior IOP lowering by THC (the active moiety) versus established therapies of latanoprost and timolol (AAO 2019, e-poster 30061347). While THC clearly lowers IOP, the mechanism of action is suspected to be multifactorial and therefore needs further elucidation.

We have previously reported on the development of a bioengineered 3D human trabecular meshwork (3D-HTM™) tissue5 with physiologically relevant characteristics of hypertensive glaucoma6,7. Glaucomatous trabecular meshwork expresses elevated ECM and fibrotic markers which may correlate directly to increased IOP. Using the 3D-HTM tissue model, THC was studied for its potential to mitigate fibrosis, inflammation and IOP elevation.



Methods and Material

The effects of THC treatments on TM fibrosis and outflow physiology were studied in normotensive and glaucomatous (TGFβ-2) tissue models.

- 3D HTM samples were treated with 0.1, 0.5 and 2uM THC for 6 days as shown in the table below.
- After duration of the treatment, the samples were assayed for a series of fibrotic markers at the protein- and gene-level. p42/p44 MAP kinase (ERK1/2) was also studied.
- Pressure modulation was assessed using a microfluidic system. The outflow facility (inverse of IOP) was determined by the ratio of Δ(flow rate)/Δ(pressure).

Group No.	Treatment Type	Tissue Type
1	Vehicle (on day: 0, 3)	healthy
2	Vehicle (on day: 0, 3, 6) + THC (**0.1** μM) (on day: 3, 6)	healthy
3	Vehicle (on day: 0, 3, 6) + THC (**0.5** μM) (on day: 3, 6)	healthy
4	Vehicle (on day: 0, 3, 6) + THC (**2** μM) (on day: 3, 6)	healthy
5	TGFβ-2	diseased
6	TGFβ-2 (on day: 0, 3, 6) + THC (**0.1** μM) (on day: 3, 6)	diseased
7	TGFβ-2 (on day: 0, 3, 6) + THC (**0.5** μM) (on day: 3, 6)	diseased
8	TGFβ-2 (on day: 0, 3, 6) + THC (**2** μM) (on day: 3, 6)	diseased

Key finding:

Extended treatments with low concentrations of THC, increased fluid outflow across 3D trabecular meshwork and reduced biomarkers associated with inflammation and fibrosis mediated via P42/44 MAPK (ERK1/2) protein modulation.

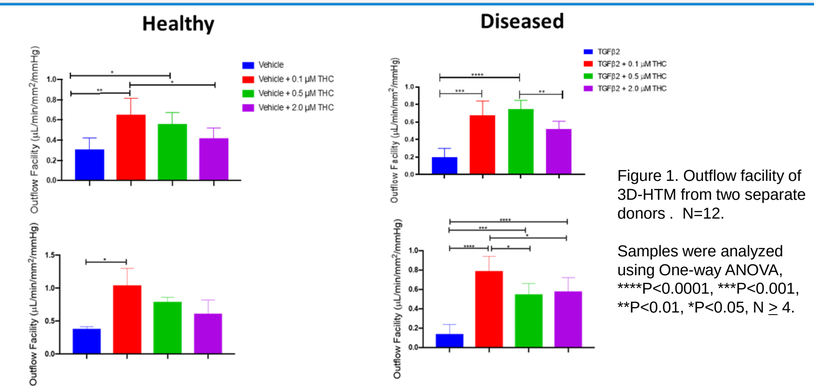


Figure 1. Outflow facility of 3D-HTM from two separate donors . N=12.

Samples were analyzed using One-way ANOVA, ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05, N ≥ 4.

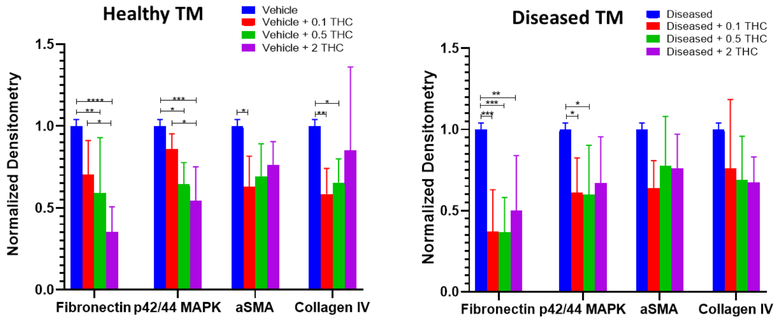


Figure 2. Protein analysis of markers linked to fibrosis at the 3D HTM after 6 days of treatment with THC. All samples of three donors were analyzed using Two-way ANOVA ****P<0.0001, ***P<0.001, **P<0.01, *P<0.05, N ≥ 4 per donor.

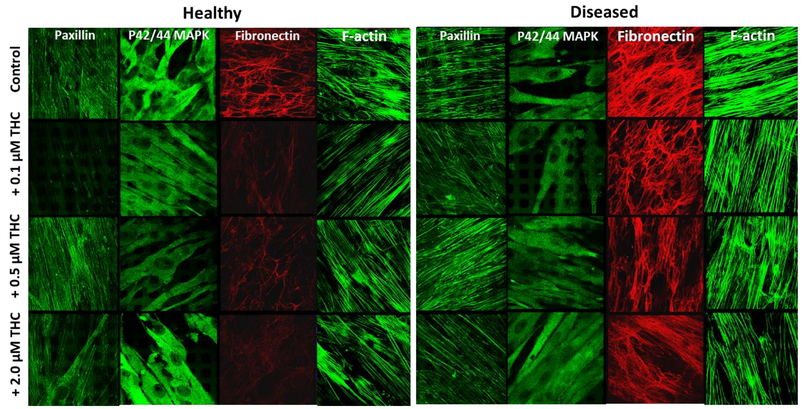


Figure 3. Confocal micrographs of 3D HTM treated with 0.1, 0.5 and 2 μM THC. Immunocytochemistry of paxillin, p42/44 MAPK (ERK1/2), fibronectin and F-actin.

Results

- 0.1 μM THC lowered pressure and increased outflow facility from 0.35 ± 0.07 to 0.85 ± 0.21 mL/min/mm²/mmHg for normotensive and 0.26 ± 0.10 to 0.74 + 0.16 mL/min/mm²/mmHg for glaucomatous samples (N>12, p<0.05 and p<0.001; respectively)
- In glaucomatous tissue constructs, 0.5 μM THC increased outflow facility from 0.26 ±0.10 to 0.63 ±0.10 mL/min/mm²/mmHg (N>12, p<0.001; respectively)
- THC concentration of 2 μM, the highest dose tested, exhibited a more muted response when compared to the lower concentrations of 0.1 and 0.5 μM THC
- In normotensive and glaucomatous samples, 0.1uM THC demonstrated antifibrotic properties by decreasing collagen IV, paxillin, and a-smooth muscle actin

Conclusion & Discussion

- Higher exposure to THC results in a biphasic response that results in less activity, an action seen in the past with other organ systems but by a mechanism not understood at this time.
- THC treatments significantly affected fibronectin expression across the normotensive and glaucomatous TM
- Although p42/44 MAPK has been previously shown to be activated in response to THC, after six days of treatment, a significant decrease in this protein-level was observed on normotensive and glaucomatous samples. This observation suggests the MAPK/ERK pathway is involved in the anti-fibrotic effect seen in this study
- This work suggests that the pressure-lowering ability of THC may be multifactorial, resulting in vasodilatory, antifibrotic and anti-inflammatory responses.
- Further studies are warranted to better understand the interplay between the ocular endocannabinoid system and the cytokine cascade of the inflammatory response. These data support the continued development of THC derivatives as a new class of therapy for the treatment and management of glaucoma and ocular hypertension

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