1.052 Antiseizure and Antitumor Effects of Cannabidiol in Preclinical Models of Tuberous Sclerosis Complex Through mTORC1-independent Mechanisms

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Introduction

- Cannabidiol (CBD) oral solution (Epidiolex®) is approved in the US and other countries for the treatment of seizures associated with tuberous sclerosis complex (TSC).[1]
- TSC is a rare genetic disorder, where patients are predisposed to kidney, brain, skin, and heart tumors.
- Genetic mutations in *TSC1* and *TSC2* are associated with TSC. TSC1 and TSC2 function as a protein complex to repress the activity of mechanistic target of rapamycin (mTOR), involved in cell growth.[2] mTOR inhibitors reduce tumor growth,[3] and can further reduce seizures in patients as an adjunctive therapy with standard antiepileptic drugs.[4]
- The anticonvulsive mechanism of action (MoA) of CBD is hypothesized to be mediated, at least in part, via antagonism of GPR55, desensitization of TRPV1 and inhibition of ENT-1.[5] However, CBD's MoA in TSC is yet to be fully elucidated.

Objective

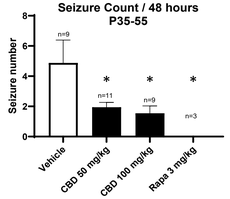
- Explore whether CBD ameliorates the disease state of TSC model systems.
- To explore the effect of CBD on mTOR signaling and other TSC associated pathways.

Methods

- Plant-derived highly purified CBD was supplied by Jazz Pharma. Research UK Ltd.
- Antiseizure and antitumor efficacy of CBD was assessed in TSC in vivo models.
  1. Epilepsy: $Tsc1_{GFAP}$CKO (conditional knockout) mice were dosed i.p. from P10 to P55 with CBD (50 mg/kg/day; or 100 mg/kg/day) or vehicle or the mTORC1 inhibitor rapamycin (3 mg/kg/d) to assess the effects on spontaneous seizure via electroencephalography (EEG), with recordings taken from frontal and parietal cortices and hippocampal local field potentials. Electrode implantation occurred P21-24, recordings taken from P35. Transcriptomic changes were assessed in the right hippocampus using RNA-seq analysis. Gene Set Enrichment Analysis (GSEA) was performed using the ClusterProfiler R package on the full ranked gene list to identify pathway-level changes associated with CBD treatment effects
  2. Oncology: In 7-week-old female BALB/c nude mice injected with *Tsc2*$^{-/-}$ cystadenoma 105K cells, once tumor volume reached 100 mm^3 mice were dosed i.p. for 28 days in 2 separate phases (tumor volume was assessed via callipers):
     - Phase 1: vehicle, CBD (5, 50 or 100 mg/kg BID), rapamycin (0.03, 0.3, or 3 mg/kg 3 days per week)
     - Phase 2: vehicle, CBD 50 mg/kg, rapamycin 0.014 mg/kg or their combination
- In vitro: 1-10 µM CBD, 50 nM rapamycin, CBD + rapamycin, and 30 µM C188-9 (a signal transducer and activator of transcription 3 [STAT3] inhibitor) were tested in TSC cell line models (*Tsc2*$^{-/-}$ mouse embryonic fibroblasts and *TSC2*$^{(-)}$ patient-derived angiomyolipoma (AML) 621-101 cells, with their respective wild-type controls). AML 621-101 cells were treated with either DMSO or 10 µM CBD for 18 h and assessed via RNA sequencing.
- Western blotting of target proteins, cell migration/invasion, transcription of hypoxia inducible factor 1α (HIF-1α) and STAT3, and vascular mimicry were used to assess CBD mechanism.

Results

Figure 1. CBD reduced seizures in a mouse model of TSC



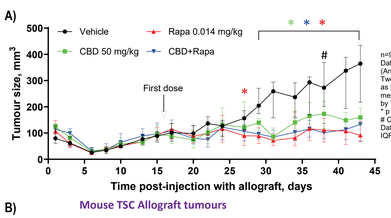
CBD or rapamycin (Rapa) treatment reduced seizure number in TSC mouse model. CBD did not significantly affect survival (data not shown).

Data are mean ± SEM. One-way ANOVA with Dunnett's test. *p<0.05 vs vehicle.

Figure 2. CBD attenuated tumor growth in a mouse allograft TSC model via a mTORC1-independent mechanism

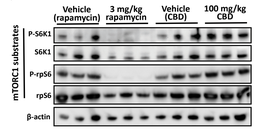
Phase 1: CBD (50 and 100 mg/kg) or rapamycin (0.03, 0.3, or 3 mg/kg) significantly attenuated tumor growth in a dose-dependent manner (p<0.05, data not shown). Both treatments were well-tolerated.
Phase 2: CBD (50 mg/kg) and rapamycin (0.014 mg/kg) as single drug treatments or in combination attenuated tumor growth and were well tolerated (**A**). No additivity or antagonism were observed. All treatments were well tolerated, and no mortality was observed.

A)



n=9-10.
Data not normally distributed (Anderson-Darling test).
Two-way ANOVA (group and day as factors) with repeated measures at each day followed by Tukey's test.
* p < 0.05 vs vehicle
# CBD vs CBD+Rapa.
Data presented as median and IQR.

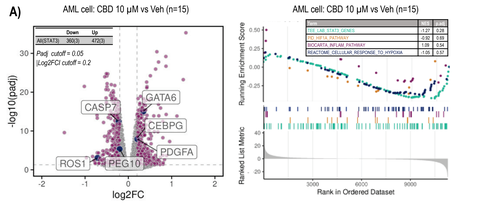
B)



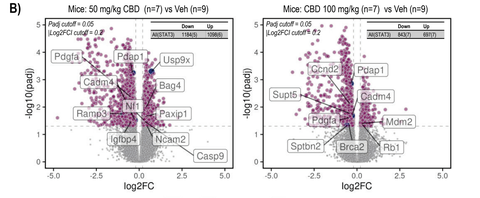
Western blot analysis of tumor samples from the allograft showed that mTORC1 signaling was inhibited with rapamycin 3 mg/kg (p<0.01 to 0.0001) but was unaltered by CBD 100 mg/kg n=5-7 (**B**). Both P-S6K1 and P-rpS6 are surrogate markers of mTORC1.

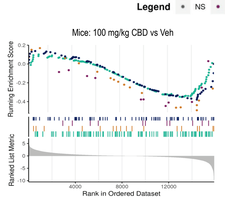
Figure 3. Gene expression analysis of *TSC2*(−) AML cells and *Tsc1*$^{-/-}$ mouse hippocampus samples treated CBD treatment

A)



B)

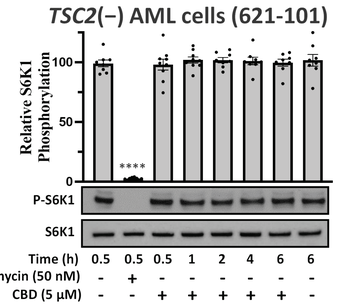




Term	NES	p.adj
TEE_LAB_STAT3_GENES	-1.24	0.40
PID_HIF1A_PATHWAY	-1.33	0.43
BIOCARTA_INFLAM_PATHWAY	-1.15	0.67
REACTOME_CELLULAR_RESPONSE_TO_HYPOXIA	-1.27	0.41

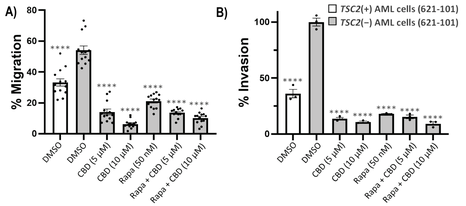
CBD effects are consistent with inhibition of genes associated with HIF-1α and STAT3 signaling in TSC2-deficient AML 621-101 cells (**A**) and in the hippocampus of mice lacking TSC1 (**B**).

Figure 4. CBD did not inhibit mTORC1 in TSC cell models



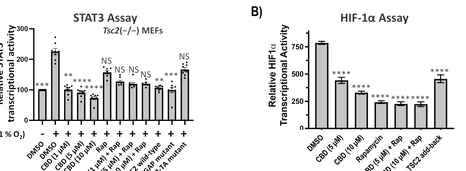
In agreement with the data generated from the mouse allograft, 5 µM CBD treatment does not inhibit mTORC1 in patient-derived *TSC2*(−) cells, i.e., P-S6K1 remains elevated (n=7-8 per treatment, data points shown in bars). mTORC1 is significantly inhibited by 50 nM rapamycin. Data are mean ± SEM. One-way ANOVA with Dunnett's test. ****p<0.0001 vs vehicle.

Figure 5. CBD blocked cell migration and invasion of TSC disease cells

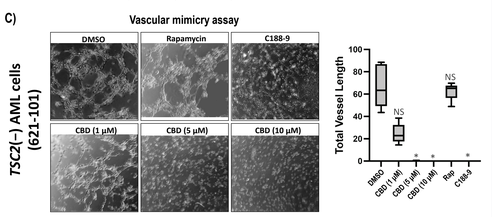


CBD treatment reduces cell migration (n=9) (**A**) and invasion (n3) (**B**) in *TSC2*(−) cells. Data are mean ± SEM. For (**A**), Kruskal-Wallis one-way ANOVA with Tukey's test; (**B**) one-way ANOVA with Dunnett's test. ****p<0.0001 vs vehicle.

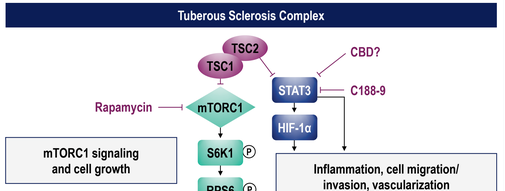
Figure 6. CBD treatment inhibits STAT3, HIF-1α, and vascularisation in TSC cell models



CBD treatment potently blocks hypoxia-induced activation of (**A**) STAT3 (n=8) and (**B**) HIF-1α (n=6) in *Tsc2*(−/−) MEF cells to a level equivalent to TSC2 add-back. Rapamycin did not affect STAT3 activation (**A**) but did reduce activation of HIF-1 α (**B**). For (**A**), Kruskal-Wallis one-way ANOVA with Tukey's test; (**B**) one-way ANOVA with Dunnett's test. **p<0.01, ***p<0.001, ****p<0.0001 vs vehicle.



CBD, but not rapamycin, treatment phenocopies a STAT3 inhibitor to block vascular mimicry in *TSC2*(−) cells, reducing total vessel length (n=5). Kruskal-Wallis one-way ANOVA with Dunn's test. *p<0.05 vs vehicle.



Conclusions

- Data show that CBD treatment inhibits spontaneous seizures and tumor growth in TSC model systems.
- In TSC model systems, CBD treatment reduces STAT3/HIF-1α signaling in an mTORC1-independent manner, showing differentiation from the mechanisms through which rapamycin works.
- The effect of CBD on STAT3/ HIF-1α signaling was confirmed by RNAseq analysis of samples obtained from hippocampus of mice lacking *TSC1* and in *TSC2*-deficient AML 621-101.
- Evidence that CBD can effectively block STAT3 and HIF-1α in *TSC2*-deficient cells warrants further research, as both STAT3 and HIF-1α are drug targets in neuroinflammation and cancer.

References: 1. EPIDIOLEX® (cannabidiol) oral solution [prescribing information]. Jazz Pharmaceuticals, Inc.; 2024. **2.** McEneaney LJ, et al. *Adv Genet*. 2019;103:91–118. **3.** David N. et al. *Neurol Clin Pract* . 2018;8(5):412–420. **4.** Franz DN, et al. *Neurol Clin Pract*. 2018;8:412–420. **5.** Gray RA, Whalley BJ. *Epileptic Disord*. 2020;22(S1):10–15.

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