Cannabidiol as Potential Glioblastoma Multiforme Treatment: *in vitro* Assessment of Anti-Cancer Properties and *in vivo* Delivery

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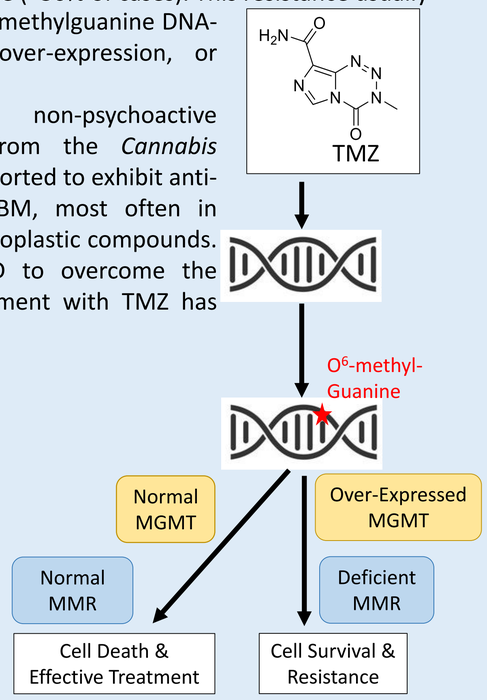
Background

Glioblastoma Multiforme (GBM) is the most aggressive brain cancer, with a poor prognosis of only 12 months survival post diagnosis and <5% 5 year survival. The standard chemotherapy for GBM, with temozolomide (TMZ), leads to high levels of resistance (~50% of cases). This resistance usually occurs as a result of either O^6-methylguanine DNA-methyltransferase (MGMT) over-expression, or MMR deficiency.

Cannabidiol (CBD) is a non-psychoactive phytocannabinoid derived from the *Cannabis Sativa* plant. CBD has been reported to exhibit anti-cancer properties against GBM, most often in combination with other antineoplastic compounds. However, the ability of CBD to overcome the common resistances to treatment with TMZ has not yet been investigated.



Aims

- Determine the ability of CBD to overcome the common resistances to treatment with TMZ (MGMT over-expression and MMR deficiency).
- Investigate CBD delivery to specific anatomical regions within the brain after oral administration.

Methods

In vitro:

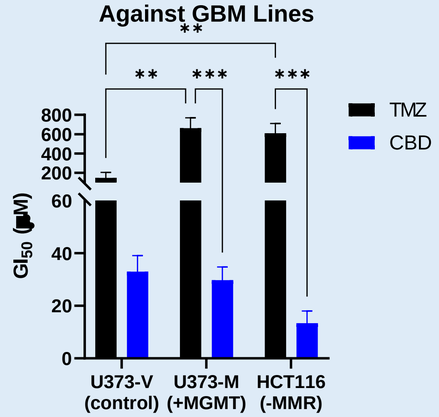
The anti-cancer assessment of CBD and TMZ against human GBM cell lines U373-V (non-resistant control), U373-M (MGMT over-expression), and colorectal cancer cell line HCT116 (MMR deficiency) were investigated by 3-(4,5-dimethylthiazol-2-yl (MTT) and confirmed by cell count assays, after both 72 and 144 hrs dosing.

In vivo:

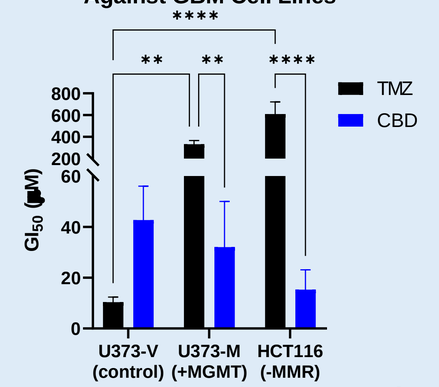
CBD brain distribution was assessed in male Sprague-Dawley rats. CBD was administered orally in either sesame oil or a lipid free vehicle (80:10:10 propylene glycol:ethanol:water) and administered at 12 mg/Kg (1 mL/Kg of 12 mg/mL). Rats were euthanised by CO_2 inhalation at predetermined end points and brain tissue was collected and dissected.[1] CBD concentration was determined by a validated HPLC-UV analytical method.[2]

In Vitro Results

GI_{50} of TMZ and CBD After 72 hrs Dosing Against GBM Lines



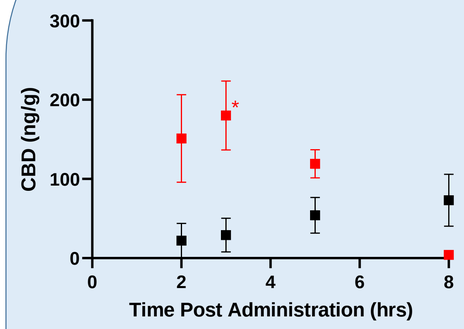
GI_{50} of TMZ and CBD After 144 hrs Dosing Against GBM Cell Lines



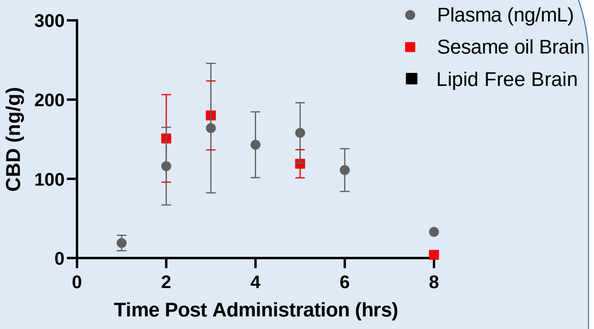
Mean ± SEM, 3 independent repeats of n=5. Two-way ANOVA statistical analysis, α=0.05, **=p<0.01, ***=p<0.001, ****=p<0.0001. Values calculated by MTT and confirmed by cell counts at 72 and 144 hrs after dosing.

In Vivo Results

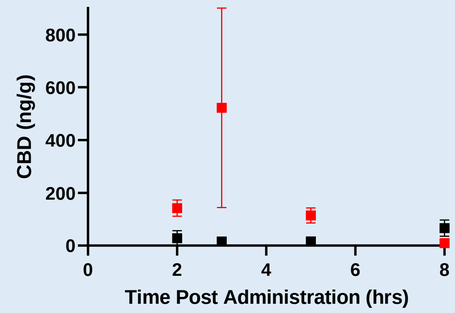
Brain CBD Concentration: Sesame oil v Lipid Free Vehicle



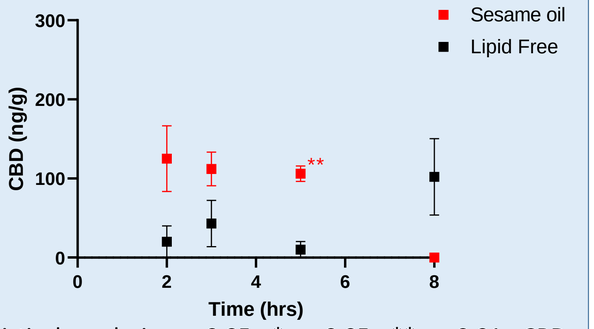
Sesame oil CBD Concentration: Brain v Plasma



Temporal Lobe CBD Concentration: Sesame oil v Lipid Free Vehicle



Frontal Lobe CBD Concentration: Sesame oil v Lipid Free Vehicle



Mean ± SEM, n=3. Multiple t-tests statistical analysis, α=0.05, *=p<0.05, **=p<0.01. CBD concentration in rat brain and plasma after administration in sesame oil and a lipid free vehicle. Data for the plasma concentration taken from Feng et al, 2021.[3] The C_{max} after delivery with sesame oil is 3 hrs, the same as the plasma T_{max}, with a similar trend in other brain regions. Sesame oil shows improved delivery compared to the lipid free vehicle, including to the temporal and frontal lobes, both common GBM locations.

Conclusions

- CBD can overcome the two common resistances of GBM treatment by TMZ (MGMT over-expression and MMR deficiency).
- CBD is successfully delivered to the brain after oral administration in sesame oil.
- Good distribution of CBD to the temporal and frontal lobes was demonstrated after administration in sesame oil, at a relatively low dose of 12 mg/Kg.

Acknowledgements

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References: [1] Spijker, S. Dissection of Rodent Brain Regions. *Neuromethods* **57**, (Human Press, Totowa, N.J., 2011), [2] Zgair, A. *et al.* Development of a simple and sensitive HPLC-UV method for the simultaneous determination of cannabidiol and Δ(9)-tetrahydrocannabinol in rat plasma. *J. Pharm. Biomed. Anal.* **114**, 145-151 (2015), [3] Feng, W. *et al.* Natural sesame oil is superior to pre-digested lipid formulations and purified triglycerides in promoting the intestinal lymphatic transport and systemic bioavailability of cannabidiol. *Eur. J. Pharm. Biopharm.* **162**, 43-49 (2021).

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