THE EFFECTS OF CBD AND TMP COADMINISTRATION IN CANCER

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Introduction:

Cannabidiol (CBD) and tetramethylpyrazine (TMP) have anti-cancer properties in vitro and in vivo. However, both suffer from poor oral bioavailability. Co-crystallisation of CBD and TMP (ART12.11, Artelo Biosciences, https://cbdcocrystal.com/) could confer the new crystal form with a more desirable pharmacokinetic profile, with the added potential for positive pharmacodynamic interactions. In this initial study, the effects of CBD and TMP co-administration were investigated in cancer cell lines.

What is a cocrystal?

Pharmaceutical co-crystals are crystalline solids that complex two or more molecules together by non-covalent bonds at stoichiometric ratios, designed to improve the physicochemical properties of either or both coformers.

Methods:

Experiments were performed in proliferating and confluent CaCo2 (colorectal), SKOV-3 (ovarian) and DU145 (prostate) cancer cells. The metabolism of resazurin to resorufin was measured as a marker of cell viability. Scratch assays were performed on 24h serum starved (1%) SKOV-3 and DU145 cells at 70% confluence as an assay of cell migration. Percentage area closure at 24 and 48h was calculated as 100*value/baseline. Data was analysed using an unpaired t-test for IC_{50} values or one-way ANOVA with multiple comparison for all other data.

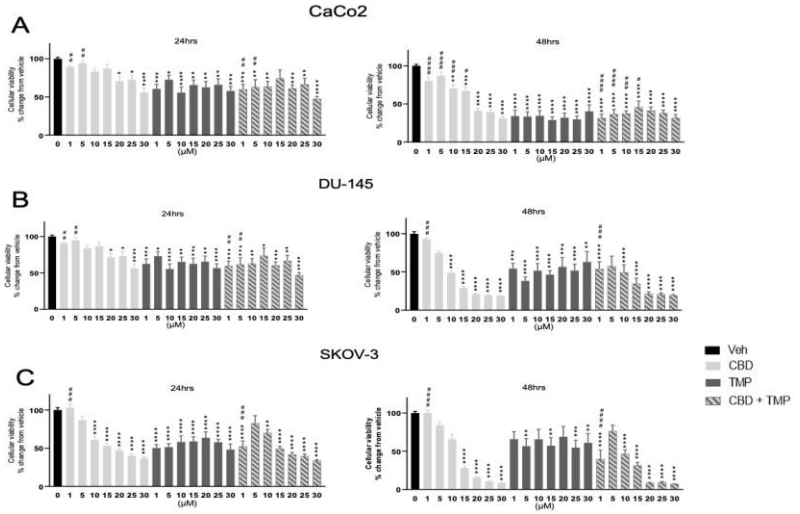

Figure 1. Effects of CBD, TMP and CBD + TMP on proliferating CaCo2 cells (p49-50, A), DU-145 cells (p12-13, B) and SKOV-3 cells (p36-37, C) 48 and 72hrs following drug application. n= 8, from two separate experiments. Data is presented as percentage change from vehicle S.E.M and was compared for statistical significance against vehicle (*) or between CBD and CBD + TMP at their respective concentrations and time-points (#) using a one way ANOVA.*/# P ≤ 0.05, **/## P ≤ 0.01, ***/### P ≤ 0.001, ****/#### P ≤ 0.0001.

Results:

1. In proliferating CaCo2, DU145 and SKOV-3 cells, CBD caused a concentration-dependent decrease in resazurin metabolism (48h; IC50 = 18.5μM ±1.2, 9.5μM ±0.2 and 11μM ±0.4 respectively), indicating reduced cancer cell proliferation. TMP also reduced the metabolism of resazurin in all cell types. CBD in combination with TMP increased the potency of CBD, reducing the IC50 by -6.μM ±1.3 (p<0.001) in DU145 and by -9μM ±1.1 (p<0.001) in SKOV-3 cells (Figure 1).
2. In confluent cancer cells, CBD reduced resazurin metabolism (144h, repeated treatments, 10-20 μM). This cytotoxic effect of CBD was not affected by TMP, except at 15 μM, where the effects of CBD were enhanced in DU145 by 5% ±1.7 (p<0.01) and in SKOV-3 cells by 27% ±8.1 (p<0.01) (Figure 2).
3. In scratch assays, CBD at 10μM and 20μM (48h) caused a reduction in the rate of wound closure in DU145 cells (Figure 3) and SKOV-3 cells (Figure 4), indicating reduced migratory effects of the cancer cells. The presence of TMP had no effect on the anti-migratory effect of CBD at 10μM, but caused a small but significant reduction in the effect of 20μM CBD, by 23% ±2.6 (p<0.05) in DU145 cells and by 28% ±3.67(p<0.0001) in SKOV-3 cells. CaCo2 cells did not possess enough migratory capacity to carry out this assay.

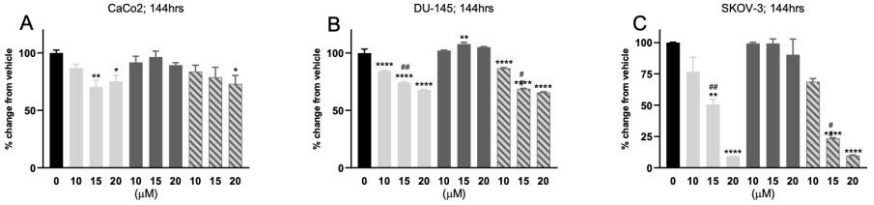

Figure 2. The effects of CBD, TMP and CBD + TMP on cellular viability in CaCo2 (p45-47, A), DU-145 (p35-38, B) and SKOV-3 (p23-24, C) cells. Fresh media containing drugs or vehicle was applied at 0hrs and then again at 72hrs. n=6, from two separate experiments. Data is presented as percentage change from vehicle S.E.M and was compared for statistical significance against vehicle (*) or between CBD and CBD + TMP at their respective concentrations and time-points (#) using a one way ANOVA.*/# P ≤ 0.05, **/## P ≤ 0.01, ***/### P ≤ 0.001, ****/#### P ≤ 0.0001.

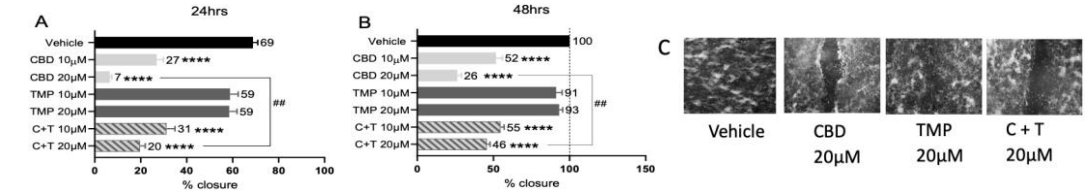

Figure 3. The effects of CBD, TMP and CBD + TMP on serum starved DU-145 cells (p27-28) on wound closure rate at A) 24hrs or B) 48hrs. n= 6, from two separate experiments. C) Data is presented as mean percentage closure from baseline S.E.M and was compared for statistical significance against vehicle or between CBD and CBD + TMP at their respective concentrations and time-points using a one way ANOVA.* P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, **** P ≤ 0.0001.

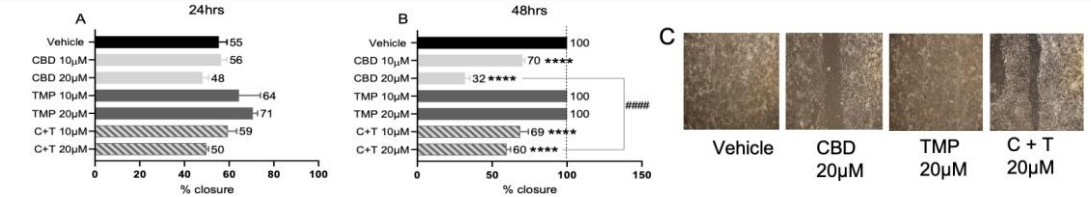

Figure 4. The effects of CBD, TMP and CBD + TMP on serum starved SKOV-3 cells (p27) on wound closure rate at A) 24hrs or B) 48hrs. n= 6, from two separate experiments. Data is presented as mean percentage closure from baseline S.E.M and was compared for statistical significance against vehicle or between CBD and CBD + TMP at their respective concentrations and time-points using a one way ANOVA.* P ≤ 0.05, ** P ≤ 0.01, *** P ≤ 0.001, **** P ≤ 0.0001.

Conclusion: In proliferating and confluent cancer cells, the combination TMP and CBD increases their anti-cancer effects, particularly in ovarian cancer cells. However, CBD's anti-migratory effects were slightly attenuated by TMP at high concentrations. Future work is required to test the CBD:TMP co-crystal *in vivo* in relevant cancer models to establish whether further potential pharmacodynamic and pharmacokinetic interactions occur.

Acknowledgements:

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