The synthetic cannabinoid URB447 exerts antitumor effect in colon carcinoma and reduces liver metastasis in mice

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Background

The endocannabinoid system represents a ubiquitous receptor family in the body, with a wide spectrum of different functions. We aim to find out the involvement of CB1 and CB2 receptors in the malignant phenotype of colon carcinoma cells and subsequent liver metastasis using the URB447 synthetic cannabinoid, which plays a dual role as CB1 antagonist and CB2 agonist.

Methods

Murine colon carcinoma MCA38 cells were treated with different concentrations of URB447, ranging from 10 µM up to 50 µM. Tumor cell viability, apoptosis, cell cycle and cell migration were analyzed *in vitro*. An *in vivo* orthotropic liver metastasis model was carried out to uncover the role of CB1-antagonism/ CB2-agonism in the metastatic growth in this organ.

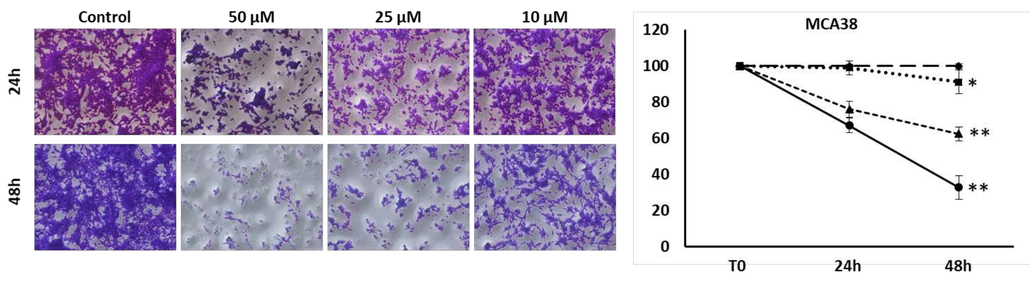


Figure 1: Antitumor effect of URB447 in cancer cell viability. The synthetic cannabinoid URB447 showed cytotoxicity in colon carcinoma cells MCA38. In detail, 10 µM did not affect cell viability after 24 hours, while 25 and 50 µM exerted antitumor effect at this time point. However, 10µM reduced cancer cell viability around 10 % after 48 hours in MCA38, while 25 µM and 50µM reduced up to 40% and 67% respectively cancer cell viability. Statistically significant differences are shown using Student *t* test.* p<0.05, ** p<0.01.

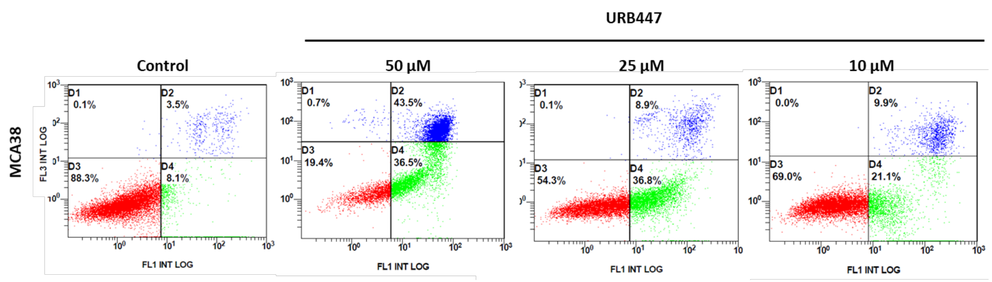
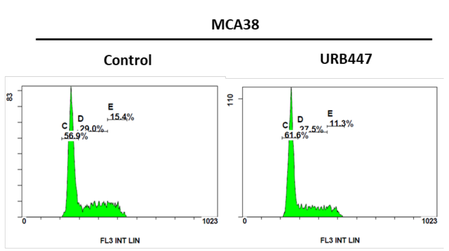


Figure 2: Apoptotic effect of URB447 in cancer cells.. Here we show that the observed reduced viability is partly mediated by apoptosis. CB2 synthetic agonist URB447 promoted apoptotic cell death in MCA38 colon carcinoma cells in a dose-dependent manner after 24h. To his regard, 10 µM increase 2,5-fold the percentage of early and late apoptotic cells. Moreover, 25 and 50 µM led to 4-fold augmentation in early apoptotic cell numbers and 3 and 10 fold increase in late apoptotic cell death.



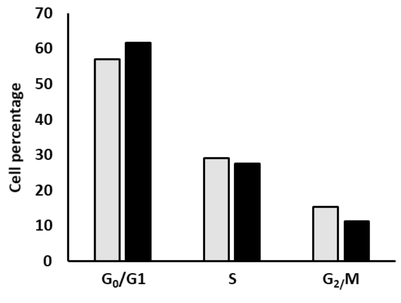


Figure 3: URB447 effect in cancer cell cycle. To further explore the antitumor mechanisms of URB447, we analyzed cell cycle regulation upon URB447 treatment. We observed that URB447 slightly impairs MCA38 colon carcinoma cell cycle through G0/G1 phase arrest. In detail, the percentage of cells in G0/G1 phase increased 5% when treated with URB447 for 24 hours compared to control cells. Slight reduction was detected in cell percentaje in S and G_2/M phases, even though no significant changes were reported, suggesting a different antitumor mechanism for URB447.

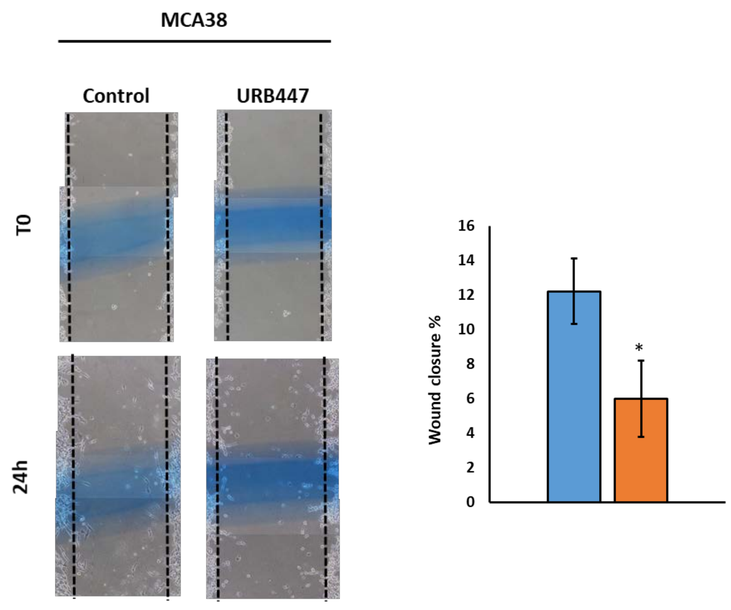


Figure 4: URB447 in tumor cell migration We explored the potential of URB447 to modulate cancer cell migration. Interestingly, URB447 seems to inhibit the migration of colorectal cancer MCA38, as we observed 5-fold reduced ability to close the wound upon treatment of cancer cells with 10 µM of URB447 after 24 hours (Figure 4B). Statistically significant differences are shown using Student *t* test. * p<0.05.

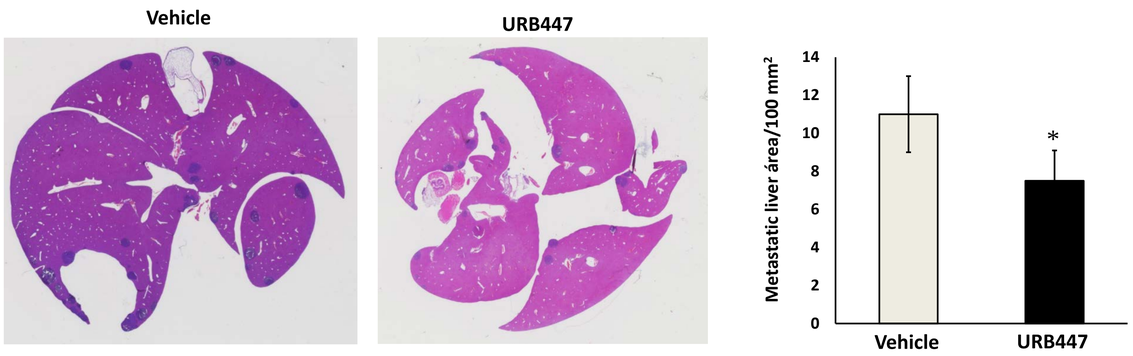


Figure 5: In vivo effect of URB447 in liver metastasis. To study the in vivo growth of colon carcinoma liver metastasis, animals were intrasplenically injected with $2x10^5$ CRC cells and daily treated with intraperiotoneal injections of 1 mg/kg URB447 . 14 days after, mice were sacrificed and liver analyzed for metastatic development. URB447 treatment reduced liver metastasis area around 30% in mice after 14 days of tumor development. Statistically significant differences are shown using Student *t* test. * p<0.05.

Results

URB447 reduced cancer cell viability in a dose-dependent trend, with around 70 % decrease in cells treated with 50 µM, 40% with 25 µM and 10% when stimulated with 10 µM after 48 hours. 50 and 25 µM URB447 boosted cancer cell apoptosis as detected through flow cytometry. URB447 slightly interfered with cell cycle, leading to increased cell counts in G0/G1 phase when treated with 10 µM. Interestingly, cell migration was reduced in 10 µM-stimulated colorectal cancer cells. Finally, URB447 reduced liver metastasis after 14 days of cancer cell inoculation.

Conclusion

The modulation of CB1 and CB2 receptors arises as a potential therapeutic target for the treatment of colon carcinoma liver metastasis.

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