Effect of JZL184 on the control of angiogenesis in a murine model of lung carcinoma

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Introduction: angiogenesis, the process of new blood vessel formation, is essential to the growth and dissemination of solid tumors. Tumors trigger the enlargement of their own vessel network, for blood supply, by disrupting the weak balance existing between proangiogenic and antiangiogenic factors (Bergers et al., 2003). These evidences have led to the development of antiangiogenic strategies for cancer treatment (Kerbel et al., 2002). Studies with bevacizumab (Avastin, Roche), a recombinant humanized monoclonal antibody against vascular endothelium growth factor (VEGF) (Presta et al., 1997), have demonstrated that its association with the classical chemotherapics, ameliorates patient's tumor condition and for this reason it was approved in the treatment of several cancer types. Cannabinoids may represent a promising class of anti-angiogenic compound since they modulate various pro-angiogenic factors such as, metallo-proteases, VEGF, Angiopoietins, inducible factor of hypoxia (Freimuth et al., 2009), in different animal model of cancer but their role in lung tumors has not been yet elucidated.
The aim of our study was to evaluate the role and the effect of Endocannabinoid System in a murine model of lung carcinoma. We previously found that in tumor mice CB_2 receptor expression was upregulated and the treatment with CB_2 full agonist, JWH133, was able to slow down tumor condition. Therefore, in this study we used an inhibitor of MAGL, the enzyme degrading 2-AG, the main endogenous ligand at CB_2 sites, trying to up-regulate 2-AG endogen levels where it was, presumably modified, i.e. at tumor level.
Methods: lung tumor was induced in C57/BL6 mice through an intravenously injection of Lewis Lung Carcinoma cells (LLC1) ($2.5x10^5$) (Sorrentino et al., 2010). Animals were treated with JZL184, MAGL inhibitor, at the dose of 5, 10, 20 μg/mouse daily with an intraperitoneally injection. At 17^{th} day, the animal were euthanized and lungs were taken. The left lung lobes were fixed in OCT medium, and the 7-μm cryosections cut for immunoistological analysis to measure the tumour burden, while the right lung lobes were homogenized for the evaluation of VEGF levels and the expression of pro-angiogenic markers.
Results: the administration of JZL184 (10, 20 μg/mouse) significantly reduced the tumor area in LLC-implanted mice compared to PBS and this effect was associated to a significantly reduced VEGF expression in the lung of tumor-bearing mice treated with JZL184 (20 μg/mouse) compared to PBS. To confirm the anti-angiogenic effects of JZL184 we used an array kit for measuring the relative expression levels of 53 angiogenesis related proteins. The array analysis revealed that the treatment with JZL184 (20 μg/mouse) reduced the activation of several pro-angiogenic mediators (Angiopoietin, MMP-9, MMP-3, Endoglin, IGFBP-2, IGFBP-3, Osteopontin), compared to PBS treated mice.
Conclusions: according with our previous data demonstrating, for the first time, that the activation of CB_2 receptor reduces lung tumor burden by altering the angiogenesis process in a mouse model of lung cancer, the treatment with JZL184 reduced lung tumor and the related angiogenesis in mice suffering of lung carcinoma. Our results pointed out definitely that activation of CB_2 pathway during tumor led to an improvement of animal conditions. Therefore, strategy with MAGL inhibitor, can open the way for the use of these molecules as coadjuvant classical anti-cancer therapies with less collateral effects since it possibly acts only where the encocannabinoid tone is deregulated.

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Kerbel et al. (2002) Nat Rev Cancer 2:727 ^ 39.
Presta et al. (1997) Cancer Res 57:4593 ^ 9.
Sorrentino et al. (2010) J Immunol 185(8):4641^50.

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