Suppression of vascular endothelial growth factor expression by cannabinoids in a canine osteosarcoma cell line

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Abstract: Vascular endothelial growth factor (VEGF) is a key regulator in both physiologic and pathologic angiogenesis, and cannabinoids decrease VEGF release in human and murine cancer cells. The aim of this study was to assess the in vitro effects of a synthetic cannabinoid, WIN-55,212-2, on the expression of the proangiogenic factor VEGF-A in the canine osteosarcoma cell line 8. After analysis of gene expression by quantitative real-time polymerase chain reaction, the compound decreased VEGF-A expression by 35% ± 10% (P, 0.0001) as compared with the control. This synthetic cannabinoid shows promise as a potential inhibitor of angiogenesis, and further studies are warranted to investigate its in vivo effects and to explore the potential of this and related compounds as adjuvant cancer therapy in the dog.

Keywords: dog, cancer, angiogenesis, cannabinoids

Introduction

Vascular endothelial growth factor (VEGF) is a proangiogenic signaling protein that induces proliferation and migration of vascular endothelial cells. The VEGF family comprises several glycoproteins, including VEGF-A, which is a major regulator of normal and abnormal angiogenesis.1 It plays a key role in tumor angiogenesis and has been found to be overexpressed in malignant tumors.2 Neoplastic tumors are dependent on angiogenesis because the formation of new blood vessels is required for delivery of the necessary nutrients and oxygen. In the absence of angiogenesis, tumors cannot grow larger than approximately 2 mm in diameter. Cells in rapidly growing masses are triggered by hypoxia to increase cellular VEGF expression in order to induce new vessel growth.3

Endocannabinoids, phytocannabinoids, and synthetic cannabinoids bind to cannabinoid 1 (CB1) and 2 (CB2) receptors,4 and can inhibit tumor growth in several cancers, including mouse glioma,5 and human pancreatic,6 the uterine,7 and prostatic cancer.8 CB1 and CB2 receptors are found in many tissues in mammals,4 including the bone cells of human2 and mouse10 species. WIN-55,212-2 is a synthetic cannabinoid that binds to both CB1 and CB2 receptors and had been demonstrated to impede angiogenesis, induce apoptosis in mouse cancer cells,7 and inhibit VEGF expression in both human and mouse cancer cells.11,12

Cancer biology and tumor behavior have been shown to be similar in humans and dogs for several common cancers,13 and the dog is emerging as a valuable naturally occurring cancer model.14 Examples of these similarities are the canine and human leukemias and lymphomas, that have a similar clinical presentation, tumor biology, and response to therapy, and have been demonstrated to share evolutionary conserved
had the same dimethylsulfoxide content of 0.1% (v/v). Three independent experiments were performed.

After total RNA extraction (RNaseq Mini Kit, Qiagen, Valencia, CA, USA), RNA quality and concentration was evaluated by spectrophotometry. Real-time quantitative polymerase chain reaction was performed by a one-step reaction with a primer pair and labeled probe specific for canine VEGF-A (TaqMan Gene Expression Assay, Invitrogen, Carlsbad, CA, USA).

A specific canine GAPDH primer pair and labeled probe (Eurofins MWG Operon, Huntsville, AL, USA) was designed for normalization purposes. It was demonstrated to be a stable gene for normalization under the experimental conditions after following procedures described elsewhere. GAPDH was considered to be stably expressed during the experiment on the basis of a −1.068-fold change because of treatment and checked by the Student’s t-test with a P value of 0.48.

The comparative quantification method was used for relative quantification and all samples were run in triplicate. The amplification efficiencies were analyzed by 10-fold dilution of a series of pooled RNA samples by 96.3% and 99.9% for VEGF-A and GAPDH, respectively.

Data are presented as the mean ± standard deviation. The statistical analysis was done by analysis of variance followed by the Student’s t-test using SAS version 9.2 (SAS Institute Inc, Cary, NC, USA). A value of P < 0.05 was considered to be statistically significant.

Results
As shown in Figure 1, OSA-8 cells incubated with WIN-55,212-2 for 48 hours showed a decrease in VEGF-A expression by 35% ± 10% on average (P < 0.0001) compared with the control (dimethyl sulfoxide), which was 100% ± 23%.

Discussion
In this paper, we detail the use of a highly sensitive and specific quantitative real-time polymerase chain reaction method for initial assessment of VEGF-A mRNA levels in a canine tumor cell line following treatment with a cannabinoid. We have demonstrated that WIN-55,212-2 decreased VEGF-A expression in the cultured OSA-8 cells by 35%. This finding is in accordance with other reports of the antiangiogenic effect of WIN-55,212-2, with a greater than 25% decrease in VEGF release from human and rat glioma cells, a 40% decrease from human prostate cancer cells, and marked inhibition of VEGF expression in human skin carcinoma.

In addition to the antiangiogenic effects of cannabinoids observed here and previously, these compounds have been
and human osteosarcoma, make the canine OSA-8 cell line an attractive in vitro model for assessing the antiangiogenic effects of cannabinoid treatment, and future work will benefit both comparative angiogenesis research and canine patients with cancer.

**Conclusion**

In conclusion, the cannabinoid agonist, WIN-55,212-2, was shown to reduce the expression of VEGF-A in a canine osteosarcoma cell line. On the basis of the results reported here, we suggest that cannabinoid receptor agonists may have the potential for use as clinical angiogenesis inhibitors. Our results suggest a first step toward the use of cannabinoids as potential adjuvants to chemotherapeutics in the treatment of canine cancers.

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**Disclosure**

The authors report no conflicts of interest in this work.

**References**