The Effect of the Acetaminophen Metabolite AM404 on the Viability of Daudi Cells in Culture

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Nine out of ten people worldwide test positive for Epstein-Barr virus (EBV) and it persists silently for life in latently infected cells. EBV has been shown to cause several B cell and epithelial malignancies including Burkitt’s lymphoma, nasopharyngeal carcinoma, and Hodgkin’s disease. Furthermore, studies have shown that expression of some EBV proteins, such as the immediate early gene BZLF1 encoding for the lytic transactivator protein ZEBRA, results in the upregulation of expression of several factors implicated in cancer progression including vascular endothelial growth factor (VEGF). Our laboratory is interested in the mechanism(s) of EBV reactivation and how it impacts EBV-related cancer progression in hopes of finding ways to treat these diseases. AM404, an active metabolite of analgesic acetaminophen, binds to transient receptor potential vanilloid type 1 (TRPV1) receptors. TRPV1 agonists, including AM404, have been shown to induce cell death in gliomas. The effect of AM404 on EBV cell lines is currently unknown. Therefore, using the Daudi Burkitt’s lymphoma cell line as a model, we aimed to test whether AM404 can be to treat EBV-related malignancies. We tested the impact of AM404 on cell viability and mRNA expression after treatment with Dexamethasone to reactivate latent EBV. The impact of AM404 on several aspects of Daudi (an EBV genome positive Burkitt’s lymphoma cell line) cells was examined: * Viability; * Reactivation of latent EBV, we focused on the mRNA expression of the following EBV genes: * BZLF1 immediate early gene that encodes for the lytic transactivator protein ZEBRA; * BLLF3 early gene encoding for DUTPase needed for virus DNA replication; * EBV Latent Membrane Protein LMP1 whose expression has been associated with cancer; * mRNA expression of angiogenic genes; * VEGF and FGFR proteins that have a significant role in angiogenesis during tumor progression; * Angiogenic cytokines, IL-6 and IL-8 that affect the immune and surrounding vascular systems; * Ezrin, a protein kinase substrate in the microvilli which is a membrane cross-linker protein, which has been implicated in malignant progression of EBV positive Nasopharyngeal (NPC) cell lines. Our results show that AM404 decreases the viability of Daudi cells in culture in a dose-dependent manner even when the virus is reactivated. We observed that treatment of Daudi cells with AM404 modulated the expression of BZLF1, BLLF3, LMP-1, IL-6, IL-8, VEGF, FGFR, and Ezrin in a dose-dependent manner. These factors are implicated in the progression of EBV-related malignancies and EBV reactivation. Future work will involve the examination of the impact of AM404 and Dexamethasone on the expression of other EBV genes and on other pro- and anti-tumorigenic factors. Future work will also take the in vitro models to in vivo with immunodeficient mouse models.

Keywords: AM404, Epstein-Barr virus (EBV), Burkitt’s Lymphoma