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Cannabidiol Alters Chemotherapy Drug Effectiveness in A Cell Culture Model

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ABSTRACT

Chronic myelogenous leukemia (CML) accounts for 15-20% of all leukemia cases across the globe, with an incidence of 1-2 people in every 100,000. Doxorubicin (DOX) is a popular anti-cancer chemotherapy drug used to treat CML and has an IC50 of 0.412μM in the immortal K-562 cell line. Although DOX is effective in destroying cancer cells, DOX affects other tissues as well. Unfortunately, DOX is often associated with adverse physiological and psychological side effects, leading to decreased quality of life and survival. Cannabidiol (CBD), a compound found in *Cannabis Sativa,* is a non-toxic exogenous agent with the potential to reduce chemotherapy side effects including anxiety, inflammatory conditions, nausea, and inhibits human breast cancer cell proliferation and tissue invasion. Given this therapeutic potential, the purpose of the present investigation was to explore whether CBD would alter the ability of DOX to induce cell death in K-562 cells. Briefly, 3 groups of $7.5x10^5$ K-562 cells were plated, cultured and incubated for 24h and 48h and treated with both CBD and DOX in the following concentrations (CBD 1 and 5uM; DOX 1 and 5uM; CBD 1μM: DOX 5μM; CBD 5μM: DOX 1μM; CBD 1μM: DOX 1μM). Flow-cytometry was used following the 24h and 48h incubation to determine cell viability using Calcenin-AM cell viability dye. Preliminary results revealed that median fluorescence intensity of Calcenin-AM was lower than control at 24h (47710 MFI) in CBD 1μM:46909 and DOX 5μM:39740 MFI; all other groups MFI increased above control at 24h. Following 48h only DOX 5uM MFI decreased below control. Both concentrations of DOX in combination with CBD resulted in higher MFI than control and DOX 5uM. This suggests that higher concentrations of CBD alone and CBD in combination with DOX, may increase K562 cancer cell proliferation in addition to interfere with K562 cancer cell death upon DOX treatment. Although these results are preliminary; they are novel. This study provides a foundation for further exploration related to the use of CBD in tandem with DOX.

INTRODUCTION

Chronic myelogenous leukemia (CML) accounts for 15-20% of all leukemia cases across the globe, with an incidence of 1-2 people in every 100,000. One of the more common anti-cancer chemotherapy drugs used to treat CML is doxorubicin (DOX). Despite the ability of DOX to effectively kill leukemia cells, DOX also damages non cancer cells leading to adverse, potentially life threatening side effects. Cannabidiol (CBD) is a compound found in cannabis and is most well-known for its potential to reduce the negative side effects of chemotherapy. Although CBD inhibits human breast cancer cell proliferation and tissue invasion, relatively little is known about how CBD may change the action of DOX on leukemia cells.

The purpose of this novel investigation was to explore whether CBD would alter the ability of DOX to induce cell death in leukemia (K-562) cells. We hypothesized that the co-administration of CBD and DOX would increase the ability of DOX to induce cell death in K562 cells.

METHODS

Cell Culture: K562 cells were cultured in RPMI-1640 medium supplemented with 10% Fetal Bovine Serum and 1% Penicillin Streptomycin at 37˚ C in a humidified atmosphere of 5% CO_2.

Cell Viability Assay: Cell viability was determined using calcein AM, which is a a cell-permeant dye used to determine cell viability. When cells are alive, the nonfluorescent calcein AM is converted to a green fluorescent calcein through cleavage of the acetoxymethyl portion by cellular esterase. This fluorescence can be read on a flow cytometer.

Briefly, K562 cells (ATCC, Manassas, VA) were seeded in a 6-well plate at a density of $7.5x10^5$ and treated with either DOX and/or CBD (1μM CBD; 5μM CBD; 1μM DOX; 5μM DOX;1μM CBD+5μM DOX; 5μM CBD+1μM DOX;1μM CBD+1μM DOX). Cultures were then incubated for 24 or 48h in the atmosphere stated above. Calcein AM was thawed and resuspended in Dimethyl sulfoxide and was incubated with cultures in the dark on ice for 20 minutes. Then, cells were washed and resuspended in Dulbecco's phosphate buffered saline. Cell cultures were then incubated with CD-71 on ice in the dark for 20 minutes, washed, resuspended, and read on the Attune™ NxT Flow Cytometer (Thermo Fisher Scientific).

RESULTS

Preliminary results revealed that the median fluorescence intensity of calcenin AM was lower than control at 24h (47710 MFI) in CBD 1μM and DOX 5μM. However, CBD 5μM and DOX 1μM had a higher MFI following 24h incubation (Figure 1. and Table 1). Following 48h incubation only DOX 5μM MFI decreased below control values, while CBD 5μM increased above control values. All concentrations of DOX in combination with CBD resulted in higher MFI than control (Figure 2). Cannabidiol at lower concentrations had no effect on cancer cell viability; however, CBD at higher doses after 2 days of exposure, increased cancer cell viability. A representative figure of the data obtained with the flow cytometer is presented in Figure 3.

Figure 1. K562 Cell Viability

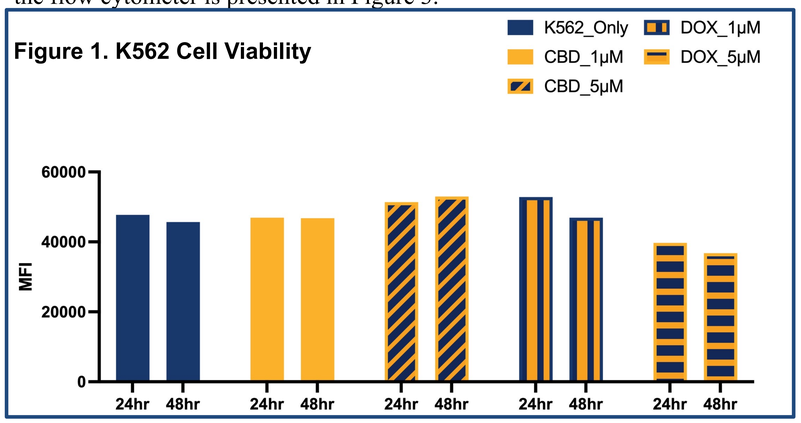


Table 1. Mean Fluorescence Intensity

	K562 ONLY	CBD 1μM	CBD 5μM	DOX 1μM	DOX 5μM	CBD 1μM DOX 5μM	CBD 5μM DOX 1μM	CBD 1μM DOX 1μM
24HR (MFI)	47710	46909	51398	52808	39740	51051	63613	48185
48HR (MFI)	45656	46751	52987	46909	36764			

RESULTS

Figure 2. CBD+DOX

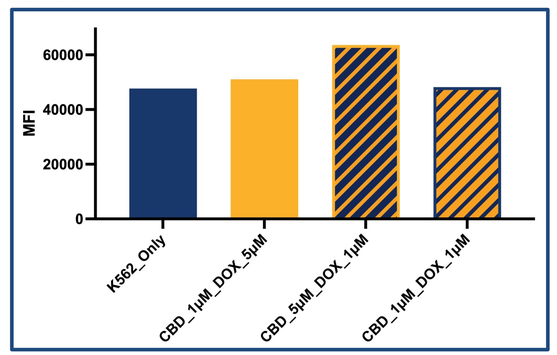
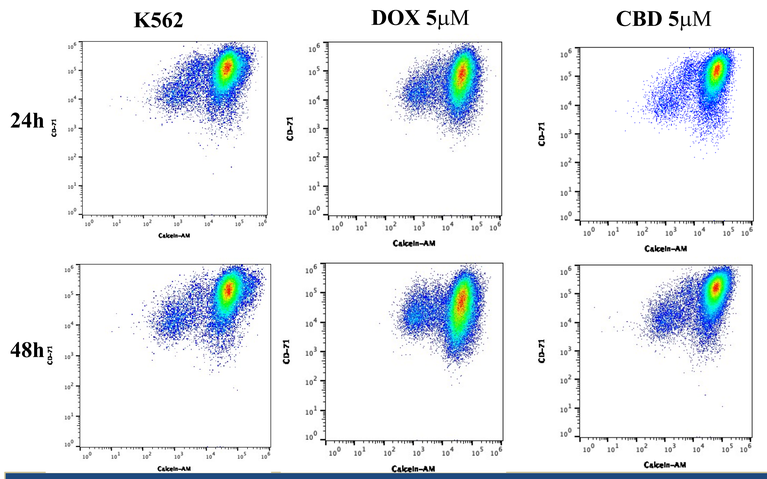


Figure 3. Flow Cytometry Cell Viability



CONCLUSION

When cancer cells are treated with CBD alone or combination with DOX, cancer cell viability increased. Therefore, we conclude that CBD, at 5μM concentration for 2 days, may interfere with DOX-induced cancer cell death. Given that some cancer survivors use CBD to help with their pain, inflammation and anxiety management, these individuals deserve to know whether CBD has the potential to interfere with their chemotherapy. **Although these results are preliminary in nature, they are striking and suggest that future efforts should focus on whether CBD may actually hinder that actions of DOX on cancer cells.**

ACKNOWLEDGEMENTS

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