Analysis of the Effects of Caffeine, Capsaicin, and their Combination on OE33 Cancer Cell Line Proliferation

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Abstract

Caffeine and capsaicin separately have shown to have effects on similar pathways that affect cell death pathways. However, the combination of these two chemicals has not been studied. In this study, we will examine the effects of various concentrations of caffeine and capsaicin alone, and combined treatment groups of caffeine and capsaicin on the OE-33 (esophageal cancer) cell line. There were 6 treatment groups for experiments run with caffeine and capsaicin separately, and 5 treatment groups for the combined concentrations. In addition to the treatment groups, there was a control group for each experiment. Once treated and incubated, a CCK-8 assay measured absorbance on all treatment groups and a MUSE annexin V assay was run to measure apoptosis effects in the 3 treatment types. Results were analyzed via ANOVA and a Tukey's multiple comparisons test. As hypothesized, both caffeine and capsaicin affect OE-33 cell line proliferation at a more significant amount than the chemicals alone. In addition to capsaicin alone affecting cell proliferation, it was also found that caffeine affects proliferation, which was not expected. This data demonstrates that the combination of caffeine and capsaicin can be considered as a possible esophageal cancer treatment.

Introduction

There are certain chemicals with anti-cancer properties that can be found in common foods [7]. Two of these foods that have been researched regarding anti-cancer properties are caffeine and capsaicin[1]. Caffeine is one of the most ingested neuroactive compound that has shown to sensitize certain cell death pathways, such as the p53 pathway [8]. This is achieved by enhancing the cytotoxicity of various chemotherapeutics such as TMZ. Cancer has also been shown to affect the PI3K pathway in cells [8]. Capsaicin is a commonly ingested chemical that is known to

give food their “spicy” sensation [2]. It has been shown in previous literature to induce apoptosis in cell lines and inhibit cell proliferation by targeting pathways such as the PI3K pathway and activates the p53 pathway. Capsaicin affects the same pathways as TMZ. While there have been multiple studies conducted on the effects of caffeine and capsaicin alone on cell proliferation, there is no literature on the combination of the two chemicals.

The OE33 cell line is an esophageal adenocarcinoma cell line in stage IIA that exhibits poor differentiation. This cell line was chosen for these experiments due to the lack of literature on the effects of capsaicin and caffeine. Additionally, when ingested, caffeine and capsaicin can directly interact with esophageal cells. The PI3K pathway can be affected by both caffeine and capsaicin and this pathway is present in the OE33 cell line. The following experiments’ objective is to determine the effects of caffeine, capsaicin, and their combination on OE-33 cell line proliferation. This will be done by running a CCK-8 assay to measure cell viability, and a MUSE Annexin V assay on the treatment groups with differing concentrations and combinations of the two chemicals. We hypothesize that: OE33 proliferation will not decrease significantly when exposed to only caffeine, but will decrease significantly when exposed to capsaicin. When exposed a combination of caffeine and capsaicin, OE33 proliferation will decrease even more significantly than when exposed to capsaicin alone.

Materials and Methods

The OE33 cells were trypsinized and counted using trypan blue in a hemocytometer, and plated in a 96 well plate with RPMI medium. They were incubated for 24 hours at 37ºC, 5% CO_2, and then treated with either caffeine or capsaicin. The treatment groups for caffeine were 10μM,

50μM, 100μM, 250μM, 400μM, and 500μM. The treatment groups for capsaicin were 20μM, 40μM, 60μM, 80μM, 100μM, and 120μM. A control of 0μM was also included. Once treated, they were incubated for 72 additional hours. After incubation, 20% CCK-8 was added after aspirating medium from the wells and incubated 45 minutes before absorbance was read at 450nm in a plate reader. All tests were run in triplicate. Using the control group, the values for the treatment groups were normalized.

Using the IC50 values of caffeine and capsaicin from previous experiments, the above process was repeated with the following concentrations of caffeine and capsaicin combined. The concentration of caffeine in order of treatment group was 430μM, 215μM, 107.5μM, 53.75μM, 26.875μM, 13.44μM, and the concentrations of capsaicin in the combined treatment groups were 120μM, 60μM, 30μM, 15μM, 7.5μM, and 3.75μM. In addition to a CCK-8 assay, a MUSE Annexin assay was performed to measure apoptosis in the differing groups. Once all data were collected, an ANOVA test and Tukey's multiple comparisons test were performed to measure statistical significance and analyze the results.

Results and analysis

The figures below show the effects of capsaicin and caffeine alone on OE33 cell viability. As seen in the graphs, both caffeine (Figure 2) and capsaicin (Figure 1) decrease proliferation when the concentration reaches a certain threshold. In capsaicin, it was found that in concentrations less than 60μM, capsaicin may promote cell proliferation. Only half of the cells survived when the capsaicin concentration reached 120μM, making that the IC50 value for capsaicin. The absorbance results for caffeine treatment groups showed that caffeine affects cell proliferation.

Previous literature has not been clear on the effects of caffeine, but these experiments support caffeine as an anti-cancer treatment. At a concentration of 500µM, only 24% of the cells survived. Using interpolation, the IC50 for caffeine was determined to be 430µM.

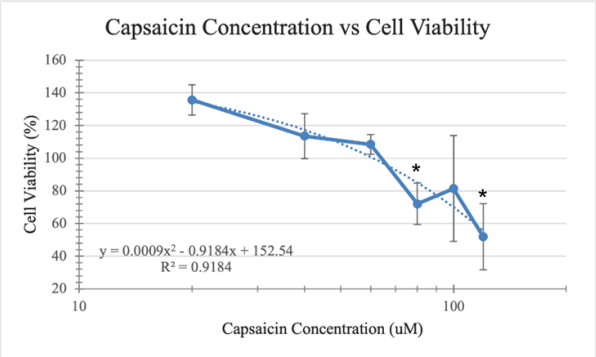

*Figure 1: Effect of capsaicin on OE33 cell line viability. IC50 concentration was 120 µM of capsaicin. * p < 0.05 indicate significant differences between the treated and control cells by two-way ANOVA and Tukey's multiple comparisons test*

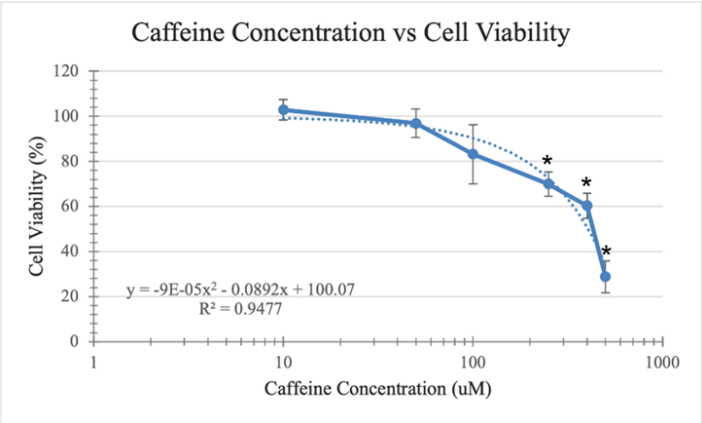

*Figure 2: Effect of caffeine on OE33 cell line viability. IC50 concentration was 430µM of caffeine. * p < 0.05 indicate significant differences between the treated and control cells by two-way ANOVA and Tukey's multiple comparisons test*

The figure below shows the effect of combined caffeine and capsaicin on cell viability. As predicted, the combination treatment has a greater effect on cell viability than the chemicals alone. At 100% IC-50 for both chemicals, cell viability dropped to 30%. For the rest of the percentages, combined treatment groups outperform the individual treatments by at least 10 *pp.*

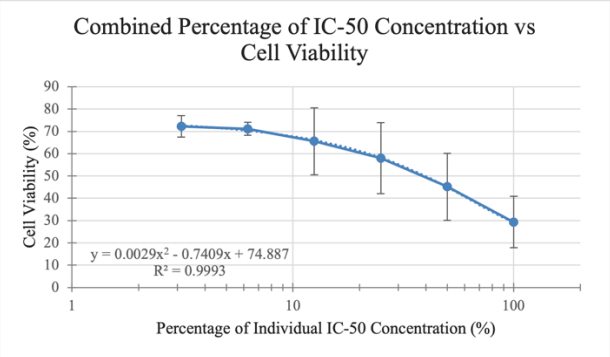

Figure 3: Effect of both caffeine and capsaicin on OE33 cell line viability. Starting concentrations were the IC50's determined above and were reduced by a factor of two for subsequent tests.

The MUSE Annexin V assay results also support what was determined from the CCK-8 assays. The combined treatment of 100% IC-50 concentrations showed 94.76% of cells in the late apoptotic stage, or dead. In comparison to the only caffeine, capsaicin, and control groups, which had 64.65%, 64.65%, and 79.95% of cells still alive, respectively.

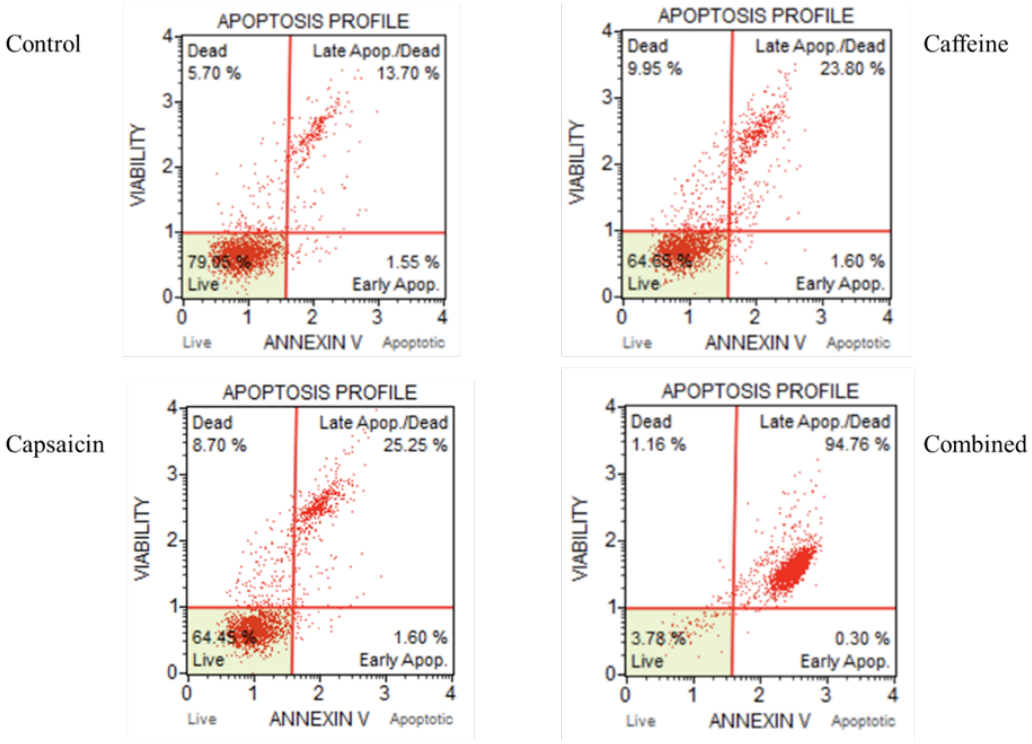

Figure 4: MUSE assay of control, caffeine, capsaicin, and combined treatment groups *(from left to right, top to bottom). Apoptosis profile shows that combination treatment has greater effect.*

The ANOVA table for the treatment groups are below. Values that are statistically significant with the control group are marked with an asterisk (*).

Anova Table for Treatment Groups (concentration decreases as treatment group number increases)	Caffeine	Capsaicin	Combined
1	.000487*	.013018*	.028759*
2	.005858*	.341025	.06616
3	.017426*	.041748*	.129634
4	.16558	.373725	.190234
5	.74036	.254569	.225223
6	.750225	.01235	.242936

Figure 4: ANOVA test shows that higher concentration treatment groups of each chemical are statistically significant compared to the control group

Discussion

Our results demonstrated that, as hypothesized, the combined treatment of caffeine and capsaicin decreases cell proliferation more than the two chemicals separately. Additionally, which was not expected, caffeine does have anti-cancer properties as cell viability significantly decreased when exposed to an increasing concentration of caffeine. Previous literature has been ambiguous about the caffeine's effects on cancer cells. However, these experimental results support caffeine as an anti-cancer treatment.

As for capsaicin, it was found that the OE33 cancer cell line actually increased proliferation when the concentration was ≤ 60μM. This may have been due to an error, because the cell viability values were normalized against the control group. If the control group was underpopulated at the start, or was compromised, the absorption values read from the CCK-8 assay would be much lower than what the other wells would have, representing an increase in proliferation. Once concentrations passed 60μM, the substance demonstrated anti-cancer effects

that previous literature suggests. When capsaicin concentration was 120μM, only 50% of the cells survived.

The MUSE Annexin V assay reinforced that combining caffeine and capsaicin decreases cell viability more than that of the individual concentrations. At 100% IC-50 concentrations for both caffeine and capsaicin, the cell viability dropped to 30%, and almost the entire cell population was in a late apoptosis stage or dead. In the other treatment groups, half of the cells were still alive. However, this difference could be due to an error, because the treatment concentrations for caffeine and capsaicin groups were not from our determined IC50 values. The concentration of caffeine in the MUSE Annexin V assay was 500μM instead of the 430μM that we determined, and the concentration of capsaicin used was 80μM rather than our determined 120μM.

Conclusions

It has been concluded that both caffeine and capsaicin affect proliferation of the OE33 cell line when exposed past a threshold concentration. This is different than the expected results, because previously it was not expected for caffeine to have an effect on cell viability. These findings are important because they demonstrate the effects of the OE33 cell line when exposed to such chemicals and can further be researched for use in anti-cancer treatments. These chemicals can be used as a low risk form of treatment for esophageal adenocarcinoma as these found in common foods.

However, there are still limitations, as the effectiveness of the combined treatment is only statistically significant at the 100% IC-50 concentrations (430μM caffeine and 120μM

capsaicin). 500μM of caffeine is equivalent to consuming 25 cups of coffee. Even just 12-15 cups of coffee can lead to toxic effects due to the overconsumption of caffeine. While the effects of caffeine and capsaicin need to be further researched before using as an anti-cancer treatment, this is a step in the correct direction towards developing new treatments.

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