Assessment of the Effects of Caffeine, Gallic Acid, and Epigallocatechin-3-gallate on Cell Inhibition, PIM-3 and E. cadherin Protein Levels in Two Lines of Pancreatic Cancer Cells

Lena Haddad  
Chapman University, hadda106@mail.chapman.edu

Melissa Rowland-Goldsmith  
Chapman University, rowlandg@chapman.edu

Follow this and additional works at: http://digitalcommons.chapman.edu/cusrd_abstracts

Part of the Cancer Biology Commons, Chemicals and Drugs Commons, Dietetics and Clinical Nutrition Commons, Food Chemistry Commons, and the Other Analytical, Diagnostic and Therapeutic Techniques and Equipment Commons

Recommended Citation
http://digitalcommons.chapman.edu/cusrd_abstracts/76

This Poster is brought to you for free and open access by the Office of Undergraduate Research and Creative Activity at Chapman University Digital Commons. It has been accepted for inclusion in Student Research Day Abstracts and Posters by an authorized administrator of Chapman University Digital Commons. For more information, please contact laughtin@chapman.edu.
**Introduction**

According to the American Cancer Society, pancreatic cancer is currently the fourth leading cause of cancer related deaths in the United States. In addition to being an exceptionally aggressive form of cancer, it is particularly difficult to treat because it is usually diagnosed in late stages after the onset of metastasis (1). Consequently, the current treatments used, including chemotherapy and radiation, have been found to be ineffective (2). As a result, efforts have been placed on using dietary alternatives which are known to possess chemopreventive properties (3). Previous studies have indicated that Gallic acid (an important phytochemical in pomegranates) and Epigallocatechin gallate (the primary catechin in Green Tea) have inhibited cancer cell growth in a variety of cancer cell types (4-9). Similarly, studies have shown that caffeine, the primary compound found in coffee, when combined with coffee, has been successful in stimulating apoptosis in endometrial, liver, skin, breast, colorectal, oral, and bone cancers (10-20). Although these compounds have individually found in coffee beans, has been successful in stimulating apoptosis, it is not clear that the combination of both caffeine and EGCG is a potential viable treatment for inhibiting cancer cell growth. In vivo studies would be required to validate that these compounds would be effective in inhibiting pancreatic cancer cell growth in humans. Although the combination of Epigallocatechin-3-gallate and caffeine produced the greatest decrease in the expression of the PIM-3 protein in the CLO-357 line of pancreatic cancer cells, the difference relative to the non-treated cells was not statistically significant (Figure 4A-4B). So, while EGCG is able to inhibit cancer cell growth, it is predominantly not through the apoptotic PIM-3 pathway. The combination of EGCG and caffeine did increase the level of E. cadherin to a great degree, although it was still not statistically significant (Figure 4C-D). These compounds in combination are making cells “stickier,” potentially helping prevent migration (or metastasis of these cells).

**Hypothesis**

We hypothesized, due to results from previous experimentation, that caffeine and Gallic acid will be the most powerful cancer fighting agents. Moreover, the combination of these two compounds will cause greater increase in cell inhibition, a greater increase in PIM-3 protein levels, and a greater increase in E. cadherin protein levels than either of the compounds individually in human pancreatic cancer cells.

**Experimental Methods**

**Cell Maintenance**

Human pancreatic cancer cells (CDO-357) were grown in Dulbecco's Modified Eagle's (DME) complete media. The following components were added to the media: 10% fetal bovine serum, 100 μg/ml Penicillin, 100 μg/ml Streptomycin, and 1x ITS before being treated. Cells were grown for 48 hours in DME SFM containing varying concentrations of EGCG (0.046 mg/ml, 0.18 mg/ml, or 0.32 mg/ml). The control cells were treated with DME SFM. Post-treatment cell counts were determined using a hemocytometer. The cell concentrations measured were compared to the pre-treatment data and was analyzed using analysis of variance (ANOVA).

**Experimental Methods (continued)**

**Treatment of Cells (Epigallocatechin-3-Gallate, Caffeine, and the Combination thereof)**

As in the previous tests, 8 x 10^5 CDO-357 cells (4 x 10^5 PANC-1 cells for PANC-3 experiments) were added to each of 6 wells in the cell-culture 6-well plates. The cells were grown overnight in DME Complete media, then overnight again in DME Serum Free Media (SFM). Cells were treated for 48 hours in DME SFM containing either 0.32 mg/ml EGCG, 0.1269 g/mL caffeine, or the combination of the two. The control cells were treated with DME SFM. Post-treatment cell counts were determined using a hemocytometer. Data analyzed using analysis of variance (ANOVA).

**Western Blot Analysis**

Whole cell lysates were generated using Pierce M-PER Mammalian Protein Extraction Reagent with Protease Inhibitor Cocktail. Proteins were quantified with a Pierce BCA protein assay kit. Equal amounts of protein were added to an SDS-polyacrylamide gel and transferred to a PVDF membrane. The membranes were blocked with 5% non-fat milk in TBS and then probed with indicated primary antibodies with gentle shaking overnight. Primary antibodies against PIM-3 (1:100), E-cadherin (1:100), and the ERK-2 (1:6000) loading control were used in this study. After washing the membranes three times, the immunoblots were incubated with the appropriate secondary antibodies for 1 hour. Antibody-bound proteins were detected using Immobilon Western Chemiluminescent HRP Substrate, and images taken utilizing Bio-Rad Image Lab Software.

**Results**

**Cell Inhibition**

A volume of 8 x 10^5 CDO-357 cells (4 x 10^5 PANC-1 cells for PANC-3 experiments) were added to each of 6 wells in the cell-culture 6-well plates. The cells were grown overnight in DME complete media, then overnight again in DME serum free media (SFM) with 100 μg/ml Penicillin, 100 μg/ml Streptomycin, and 1x ITS before being treated. Cells were grown for 48 hours in DME SFM containing varying concentrations of EGCG (0.046 mg/ml, 0.18 mg/ml, or 0.32 mg/ml). The control cells were treated with DME SFM. Post-treatment cell counts were determined using a hemocytometer. The cell concentrations measured were compared to the pre-treatment data and was analyzed using analysis of variance (ANOVA).

**Conclusion**

Based on the results from the cell inhibition studies using Epigallocatechin-3-gallate, it was determined that an increasing dosage of EGCG resulted in a greater decrease in cell proliferation (Figure 1). The results from the EGCG, caffeine, and combination cell inhibition studies illustrated that the combination treatment of EGCG and caffeine together resulted in a greater degree of cell inhibition than EGCG or caffeine alone (Figure 5-6). Based on these preliminary results, it is clear that the combination of both caffeine and EGCG is a potential viable treatment for inhibiting cancer cell growth. In vivo studies would be required to validate that these compounds would be effective in inhibiting pancreatic cancer cell growth in humans. Although the combination of Epigallocatechin-3-gallate and caffeine produced the greatest decrease in the expression of the PIM-3 protein in the CLO-357 line of pancreatic cancer cells, the difference relative to the non-treated cells was not statistically significant (Figure 4A-4B). So, while EGCG is able to inhibit cancer cell growth, it is predominantly not through the apoptotic PIM-3 pathway. The combination of EGCG and caffeine did increase the level of E. cadherin to a great degree, although it was still not statistically significant (Figure 4C-D). These compounds in combination are making cells “stickier,” potentially helping prevent migration (or metastasis of these cells).

**Future Research**

Future research project will focus testing the effects of caffeine and Gallic acid in combination to observe their effects on cell inhibition and apoptosis. Future work would continue to look at other mechanisms of action by which these compounds are able to inhibit cancer cell growth.

**Acknowledgments**

I would like to thank the Office of Undergraduate Research for allowing me to participate in this SURF program. Many thanks to Dr. Murray Korc (Indiana University) for supplying COLO-357 and PANC-1 cells, Dr. Marco Biasotti, and the Chapman University Faculty Scholarly Research Grant, for without them this research would not have been possible.

**References**

5. Haddad, L., Rowland-Goldsmith, M. "Assessment of the Effects of Caffeine, Gallic Acid, and Epigallocatechin-3-gallate on Cell Inhibition, PIM-3 and E. cadherin Protein Levels in Two Lines of Pancreatic Cancer Cells." Department of Biological Sciences, Chapman University, Orange, CA.