Enhancement of Anti-Cancer Efficacy through Combination Chemotherapy of Ciprofloxacin with either 5-Fluorouracil or Gemcitabine

By

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Abstract

Pancreatic cancer is one of the most lethal cancers, with most patients dying within the first 5 years after diagnosis. Frequently it arises from cells in the pancreatic ducts (pancreatic ductal adenocarcinoma) and often fails to be diagnosed until it has already metastasized to other organs and tissues in the body, and thus is significantly harder to treat. For these reasons, alternative therapy options should be investigated. Previously, we observed in our hands that ciprofloxacin selectively kills pancreatic ductal adenocarcinoma cells but not non-malignant human cells. 5-fluorouracil (5-FU) is a commonly used chemotherapeutic agent for pancreatic cancer which inhibits DNA replication in the S-phase of the mitotic cell cycle. Another front-line chemotherapeutic is the nucleotide analog gemcitabine (dFdC) which functions in a similar manner to 5-FU. Given that all three of these drugs appear to have both anti-cancer activity *and* act in the same cell cycle phase, we hypothesized ciprofloxacin would augment the cytostatic and/or cytotoxic effect of either 5-FU or dFdC. To test this hypothesis, MIA PaCa-2 human ductal adenocarcinoma cells were cultured and treated singly with 5-FU, or dFdC, or Cipro, or in simultaneous combination (either 5-FU or dFdC with Cipro) for 24 hour periods of time. As a result, we found that Cipro could significantly ($p \leq 0.05$) and in dose-dependent fashion enhance the activity of both 5-FU and dFdC. We conclude that Cipro is a valid candidate as an adjuvant to standard forms of chemotherapy which involve use of 5-FU and/or dFdC. Furthermore, we propose that additional studies be

conducted to further assess the validity of such combinations for human patients in the future.

Acknowledgments

This project is dedicated to my parents for always supporting my pursuit of science and for their continued encouragement, guidance, and love. They have taught me what it means to pursue my goals even though at times it will not be easy.

This project is also dedicated to my dear family friend Edith Mossner who lost her battle with Pancreatic Cancer on June 19, 2015. Her perseverance continues to inspire my work on this project and reminds me of why this work is so important.

I would like to acknowledge many people who have supported my project. I so appreciated the contributions of other students who worked alongside me in the lab on this project, Colton Starcher, Brandy Ploetner, Amanda Khan, and Kennedy Nies. Special thanks go to Dr. Patrick Fueger at Indiana University School of Medicine for allowing use of his equipment. This research would not have been possible without funding from the Strain Honors College at the University of Indianapolis and the Department of Biology. Finally, I would like to thank my Project Advisor Dr. Dean Wiseman for his constant patience, support, and assistance on this project. I have been so blessed to work in his lab over the past two years. I truly appreciate him motivating me when I needed it and being there for me during tough moments. He has taught me that research is about more than just the numbers on a report but about becoming a more independent thinker and a better student of the world.

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Statement of Purpose

Pancreatic cancer is one of the most lethal cancers, and most current chemotherapeutic agents are capable of providing only a slight increase in the life expectancy and/or improving the patient's quality of life during that time (Hildago, et al., 2015). Given our initial observation that ciprofloxacin (Cipro) preferentially kills cancer cells but not non-cancer cells, the purpose of this honors project was to test the effectiveness of a potential (and unexpected) chemotherapeutic agent, the antibiotic ciprofloxacin, independently and in combination with two commonly used chemotherapeutics, 5-fluorouracil (5-FU) and gemcitabine (dFdC). The experiments in this study assessed viability and/or death of pancreatic cancer cells when exposed to either single-agent Cipro, 5-FU, or dFdC with Cipro. Our goal was to ascertain if ciprofloxacin can enhance the known anti-cancer efficacy of 5-FU and/or gemcitabine in a standard *in vitro* model of human pancreatic cancer.

Introduction

This introduction will provide a brief survey of the important concepts presented within this thesis. Such important concepts include a general background of the disease of cancer, some specific aspects of pancreatic cancer, mammalian cell cycle, the drugs ciprofloxacin, 5-fluorouracil, and gemcitabine, adjuvant chemotherapy, and some background information on the condition of pancreatitis. Herein, each concept is explained through a literature review and/or detailed mechanism.

I. Cancer

According to the American Cancer Society (2016), cancer begins when cells grow uncontrollably and push out the healthy cells from a certain area of the body. Cancer can develop in a variety of organs and tissues including the skin, breast, lung, blood, colon, and pancreas. At the time of diagnosis, cancer is typically categorized in one of four possible stages, indicating the severity and spread of the cancer cells within the tissues of the body. Stages 1 and 2 indicate that the cancer has not spread from the specific tissue it arises from (i.e. breast, lung, skin, or other tissue/organ of origin). When a cancer reaches stage 3 and 4 it has spread from tissue of origin, and has proceeded to invade other tissue, or even distant regions of the body (metastasis). Generally speaking, patient prognosis deteriorates as the stage number increases.

II. Pancreatic Cancer

The American Cancer Society (2016) explains that the pancreas is an organ located behind the stomach in the abdomen, with exocrine secretory roles in digestion, as well as endocrine regulatory roles in the bloodstream. The exocrine role involves the production of digestive enzymes to facilitate breakdown of food and nutrients, as well as bicarbonate ion (HCO_3^-) which is critical in neutralizing stomach acid as digestive products enter the small intestine. In terms of endocrine function, specific endocrine cells release hormones, including insulin and glucagon for proper regulation of blood sugar, while other endocrine cells secrete hormones involved in the coordination of digestive activity in other organs, such as the liver, small and large intestines. Epidemiological data indicates that most cancers of the pancreas develop within the cells which line the exocrine ducts; accounting for more than 90% of pancreatic cancer diagnoses (Hidalgo et al., 2015). According to the Mayo Clinic (2016) the vast majority of pancreatic malignancies are silent (asymptomatic), and not usually diagnosed until they have invaded other tissues and organs of the body. Unfortunately, if the stage of disease is 3 (spread to other organs in the same body region) or 4 (metastasized to distant tissues), it is highly likely that surgery is no longer a viable treatment option.

Pancreatic cancer was cited as the cause of death in 330,000 patients globally in 2012, and among all major cancer types pancreatic adenocarcinoma has the lowest survival rate. Moreover, fewer than 25% of patients even survive their first year following diagnosis (Jiang et al., 2014). These statistics illustrate that pancreatic cancer is a truly severe condition and should be a focus for further research.

III. Cell Cycle

The cell cycle is the combination of two fundamental processes: replication of the genome and the formation of two new daughter cells (Murray and Kirschner, 1989; Waldman et al., 1996; Wiseman, 2004). There are three primary phases of the cell cycle: the G phases in which cells are relatively inactive, the S phase is when the cell is producing a copy of its genome, and the M phase is when the cell is split into two daughter cells (see Figure 1 below). The cells enter the G_1 phase when they have received a trigger to divide but has not yet fully committed to cell division (Wiseman, 2004).

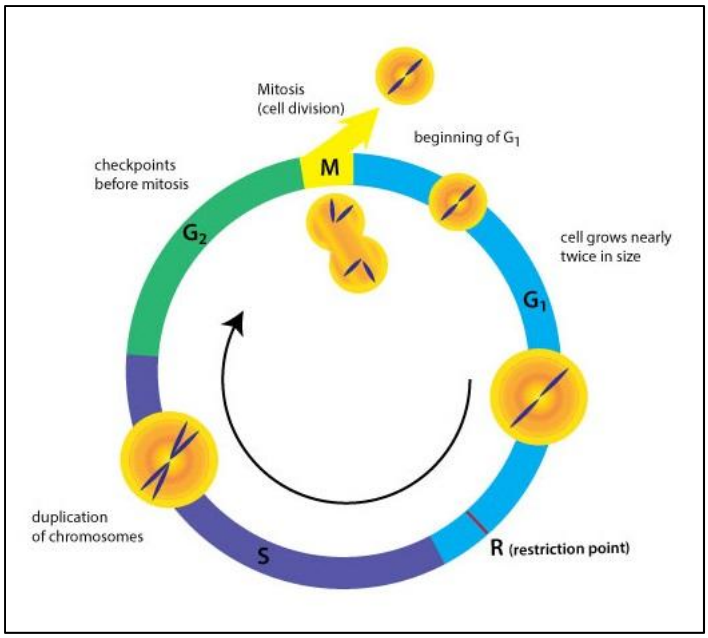

Figure 1. Mammalian Cell Cycle

Research has suggested that ciprofloxacin acts during S-phase and inhibits the duplication of the chromosomes preventing the cancer cells from increasing in number (Yadav et al., 2015).

IV. Ciprofloxacin

Ciprofloxacin is an existing FDA-approved antibiotic which is most often used for severe bacterial infections, and is generally considered to be a safe and effective treatment for acute infectious colitis (bacterial infections of the colon), and many other infections, particularly in hard-to-reach areas of the body. It is a synthetic

4-quinoline derivative and is a broad-spectrum, widely used antibiotic. Based on its chemical structure, ciprofloxacin is classified as a fluoroquinolone, many of which have been used as chemotherapeutics (Yadav et al., 2015).

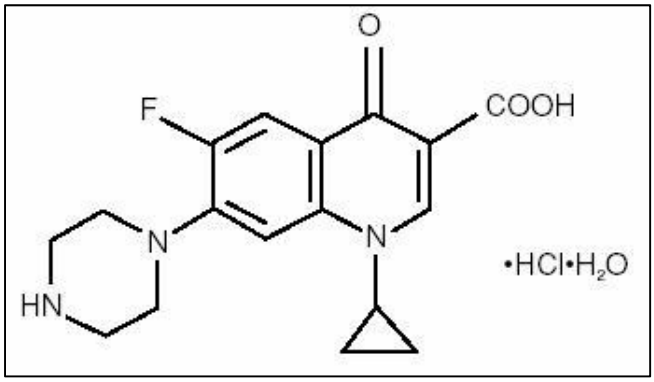

Figure 2. Chemical structure of Ciprofloxacin

However, this drug in non-cancer patients has been known to cause drug-induced pancreatitis (Sung et al., 2014). Ciprofloxacin can also suppress the human immune system by killing off the T-cells, which is also a common characteristic of other chemotherapeutic agents. (Kaminski et al., 2010).

V. 5-Fluorouracil

5-Fluorouracil is a compound that inhibits DNA replication in the S-phase of the cell cycle. It works as an enzyme the blocks the production of thymine, a

nitrogenous base in the DNA molecule. Due to this inhibition the cancer cells are no longer able to divide rapidly.

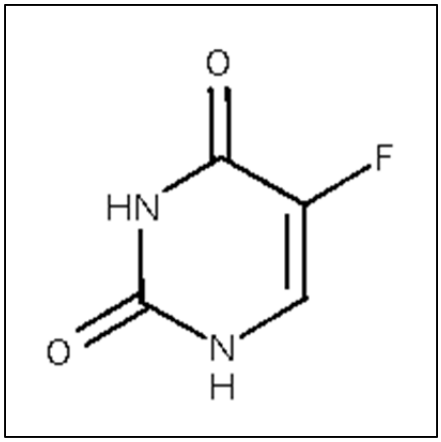

Figure 3. Chemical structure of 5-fluorouracil

5-Fluorouracil has been the most commonly used regimen for esophageal cancer over the past 15 years (Almhanna et al., 2015). 5-Fluorouracil has also been found to be effective in inducing cell death in MIA PaCa-2 cell lines especially when used in combination with traditional radiation treatments (Mohiuddin et al., 2002).

VI. Gemcitabine

Gemcitabine is another nucleoside analog that inhibits DNA replication and has been commonly used as a chemotherapeutic against pancreatic cancer (Heinemann et al., 1988).

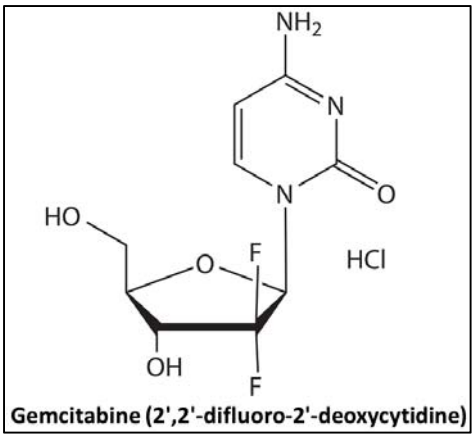

Figure 4. Chemical structure of gemcitabine

Once Gemcitabine enters the cells it is phosphorylated and then inhibits DNA replication, in an interesting mechanism described as "masked chain termination." Gemcitabine inserts itself into the DNA sequence and then two more nucleotides are added to "mask" it and the drug becomes stuck in the DNA (Plunkett et al., 1995).

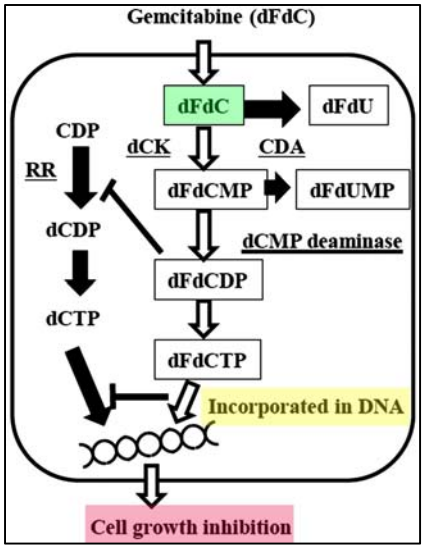

Figure 5. Metabolism of gemcitabine in mammalian cells (Kamada et al., 2014)

One key study which demonstrated the clinical effectiveness of gemcitabine showed that 23.8% of patients experienced what was termed a "clinical benefit" of gemcitabine therapy, where patients lived for longer periods of time, with less discomfort and less pain than with other front-line drug therapies (Burris et al., 1997). Gemcitabine has limited toxicity and therefore is a focus of investigation in combination chemotherapy (Araneo et al., 2003; Wiseman, 2004).

VII. Adjuvant Chemotherapy

Adjuvant chemotherapy is the term used to describe a combination of multiple drugs for chemotherapy, where one drug is *enhancing* the effect of another, or allowing a particularly toxic drug to be used effectively at lower doses. Adjuvant therapy has been shown to be effective in gastrointestinal, pancreatic, and breast cancers (Schwentner et al., 2014). It was recently shown that moxafloxacin and ciprofloxacin, when combined, had a synergistic effect on MIA PaCa-2 cells, meaning the two-drug combination was much more potent than a "one plus one" dose-to-effect prediction. Specifically, this treatment led to mitochondrial apoptosis and arrest in the S-phase of the cell cycle (Yadav et al., 2015). Currently approved forms of adjuvant chemotherapy increase the life expectancy of pancreatic cancer patients in clinical settings. However, it is typically only used for younger patients with more advanced stage cancers (Nagrial et al., 2014). *In vivo*, combination

chemotherapy significantly reduced pancreatic adenocarcinoma tumor size in mouse models (Conger, 2015).

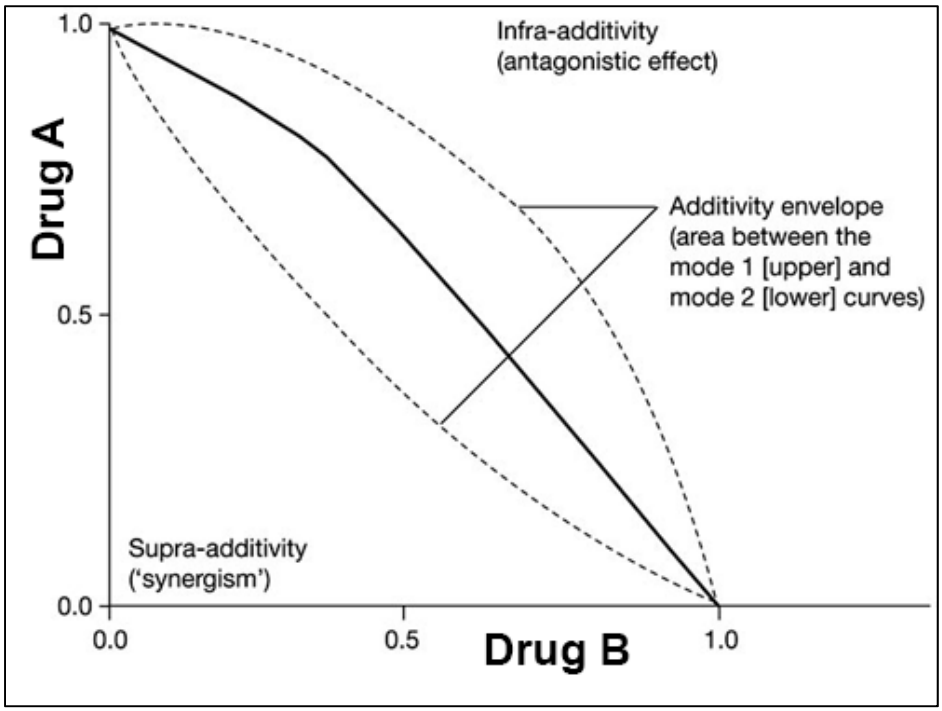

Figure 6. Example of a normalized isobologram plot for drug combinations (Seiwert et al., 2007)

One graphical method of assessing the effective quality of drug combinations is termed a "normalized isobologram" (shown above, Figure 6). By plotting the effect of dose combinations in this manner, we get a rapid visual representation of the relative effectiveness of a drug combination versus known effect with each drug individually (Chou et al., 2005).

As a final note, combination chemotherapy has yielded extraordinarily beneficial outcomes in certain highly metastatic and previously extremely lethal forms of

cancer. In particular, triple-adjuvant chemotherapy was used in Lance Armstrong's treatment for metastatic testicular cancer. Over time, this triple-adjuvant regimen has reversed what was originally a disease with a 90% mortality into one which is now 90% curable (Washington Post, 2002).

VIII. Pancreatitis

Pancreatitis is either acute or chronic inflammation of the pancreas, and can be caused by a number of different factors. Causes of pancreatitis may include, infection coming up through the pancreatic duct from the intestine, or microorganisms traveling through the lymph nodes into the pancreas, be the result of physical occlusion of pancreatic ducts, or in some cases an adverse reaction to certain drugs or pharmaceuticals (Ignatavicius et al., 2012). According to the National Institutes of Health, pancreatitis leads to a hospitalization in 1,000 in every 50,000 people. Ciprofloxacin has been observed to induce a form of acute pancreatitis (Sung et al., 2014), and we consider this side effect to be perhaps a harbinger of its unusual anti-cancer effect on pancreatic ductal adenocarcinoma cells.

Methods/Procedure

I. Cell Culture

The human pancreatic ductal adenocarcinoma cell line MIA PaCa-2 (CRL-1420) was purchased from the American Type Culture Collection (Manassas, VA). Cells were maintained in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine (Invitrogen, Carlsbad, CA) at 37°C in humidified air and 5% CO_2. Cells used in experimentation were between passages 5 (post-American Type Culture Collection) and 20. Four days before the MTS assay MIA PaCa-2 cells were seeded in 96-well culture plates at a density of 1.0×10^5 cells per mL.

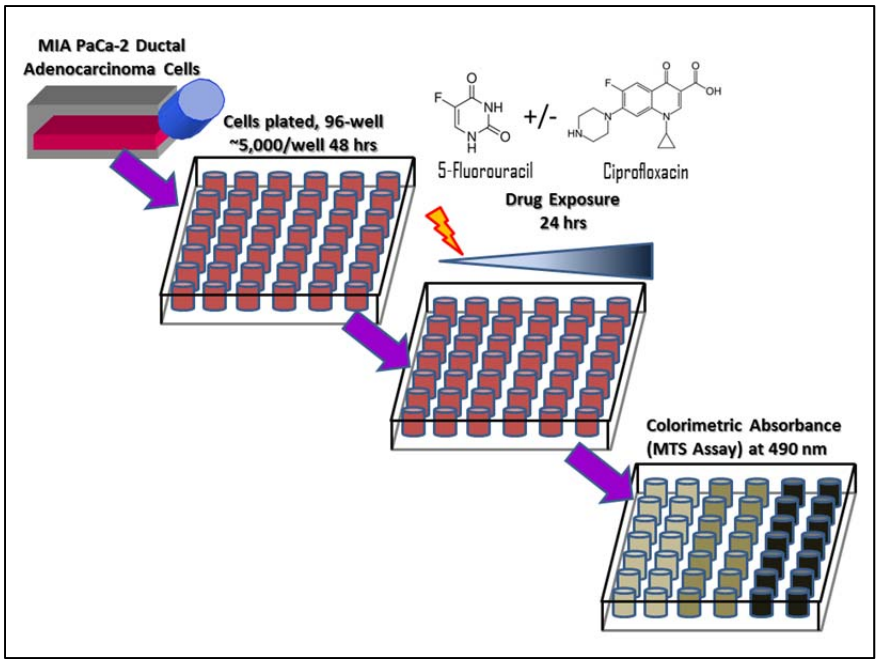

Figure 7. General workflow of cell viability assays

II. Drug Treatments

24 hours before MTS assay, MIA PaCa-2 cells were treated with 5-fluorouracil (5-FU), gemcitabine (dFdC), or ciprofloxacin (Cipro) as single agents, or in combinations of either 5-FU or dFdC with Cipro. For single-drug exposures drug concentrations of 5-FU dissolved in DMEM ranged from 0.625-100 μM and drug combinations of dFdC dissolved in DMEM ranged from 0-50 μM, while concentrations of Cipro ranged from 25-800 μM. In combination regimes, 5-FU concentrations varied as before (0.625-100 μM) with either 200 or 400 μM Cipro. dFdC concentrations varied from 0-50 μM with 400 μM Cipro.

III. MTS Assay

In this assay, we optically measure the creation of a dark-colored formazan product (which is opaque to 490 nm wavelength light) created by mitochondrial enzymes within viable cells. Formazan is a dye which is converted by NAD(P)H-dependent dehydrogenase enzymes, which function in mitochondrial metabolism (Cory et al., 1991). This mitochondrial enzymatic activity is directly proportional to the number of living cells present in a given culture of cells. According to the manufacturer's protocol (BioVision, 2015), cells were incubated in MTS solution and phosphate buffered saline (PBS) for 1-4 hours then the absorbance is measured at 490 nm using the Molecular Devices SpectraMax M2 microplate reader with the Softmax Pro 6 software.

IV. Data Analysis

Each individual experiment was conducted with an independent sample number, n=16. Statistical analysis was conducted using Student's *t*-Test comparing the average absorbance of formazan product (A_{490}), along with standard error of the mean (standard deviation divided by the square root of the number of samples) was calculated. For statistical significance, we considered a value of $p \leq 0.05$ between experimental groups to be significant.

V. Graphs and Isobolograms

All graphs were generated using Prism™ software by Graphpad.

Isobolograms were constructed using CompuSyn software by Ting-Chao Chou and collegues (Chou et al., 2005).

Results

I. Ciprofloxacin-mediated inhibition of pancreatic cancer cells.

This first experiment involved single-agent Cipro in order to establish the relevant range of anti-cancer activity in our PaCa-2 cells. Using the MTS assay to quantify mitochondrial activity, we found that Cipro significantly inhibits PaCa-2 cell growth in a dose-dependent manner over a 24 hour period with concentrations ranging from 50 to 800 μM (Figure 8, below). The approximate IC_{50} was determined to be 400 μM.

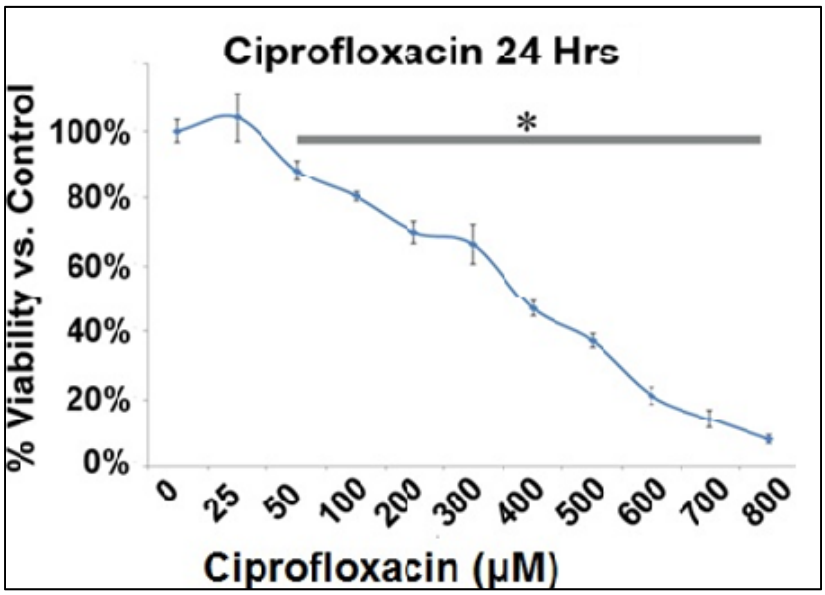

Figure 8. Dose-dependent inhibition of PaCa-2 cell viability by ciprofloxacin. MIA PaCa-2 human pancreatic adenocarcinoma cells were incubated under standard conditions for 24 hrs with 0-800 μM ciprofloxacin. Following incubation, MTS reagent was added to the media, and the cells were incubated for an addition 1-4 hr, and colorimetric analysis of the mitochondrial conversion of MTS was performed. Graph represents the average A_{492} absorbance of samples (n=16), and error bars represent the standard error of the mean (SEM). * $p \leq 0.05$ vs. untreated cells.

II. Anti-cancer effect of single-agent 5-FU.

5-Fluorouracil (5-FU) is classified as a fluoroquinoline antibiotic. It has been shown to inhibit DNA replication during the S-phase of the cell cycle, and several other less well-characterized mechanisms. In our hands, we found 5-FU causes a significant dose dependent decrease in PaCa-2 cell viability after 24 hours in the range of 1.25-100 μM (Figure 9, below).

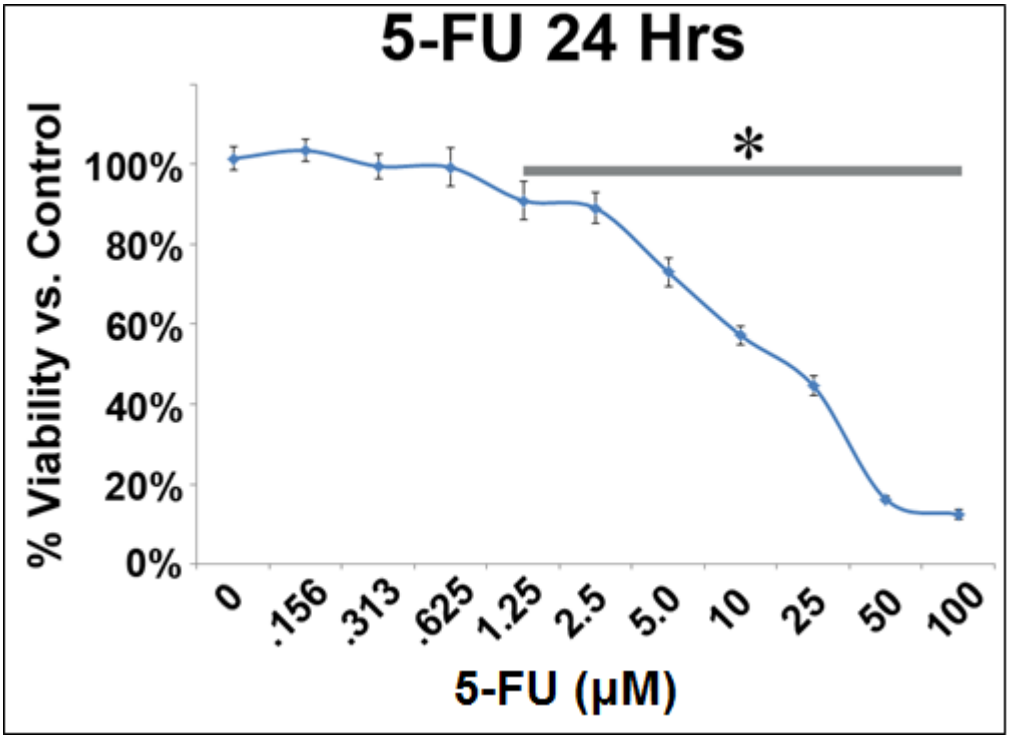

Figure 9: 5-Fluorouracil Inhibits Pancreatic Ductal Adenocarcinoma Cell Growth
MIA PaCa-2 cell viability is presented as a function of 5-FU concentration over a 24 hour period. Data represent the mean ± SEM. * $p \leq 0.05$ vs. untreated cells.

III. Simultaenous administration of ciprofloxacin and 5-FU have a superadditive anti-cancer effect on PaCa-2 cells.

Given that both agents demonstrate reasonable effectiveness on our PaCa-2 cells, and that both agents appear to work primarily during the same phase of the cell cycle, we hypothesized that both agents would function with increased efficacy in a tandem combination. Using isobologram analysis (Figure 12) We found that, when used together, 5-FU and Cipro have a greater dose-dependent effect than either 5-FU or Cipro alone as quantified by the MTS assay after 24 hours.

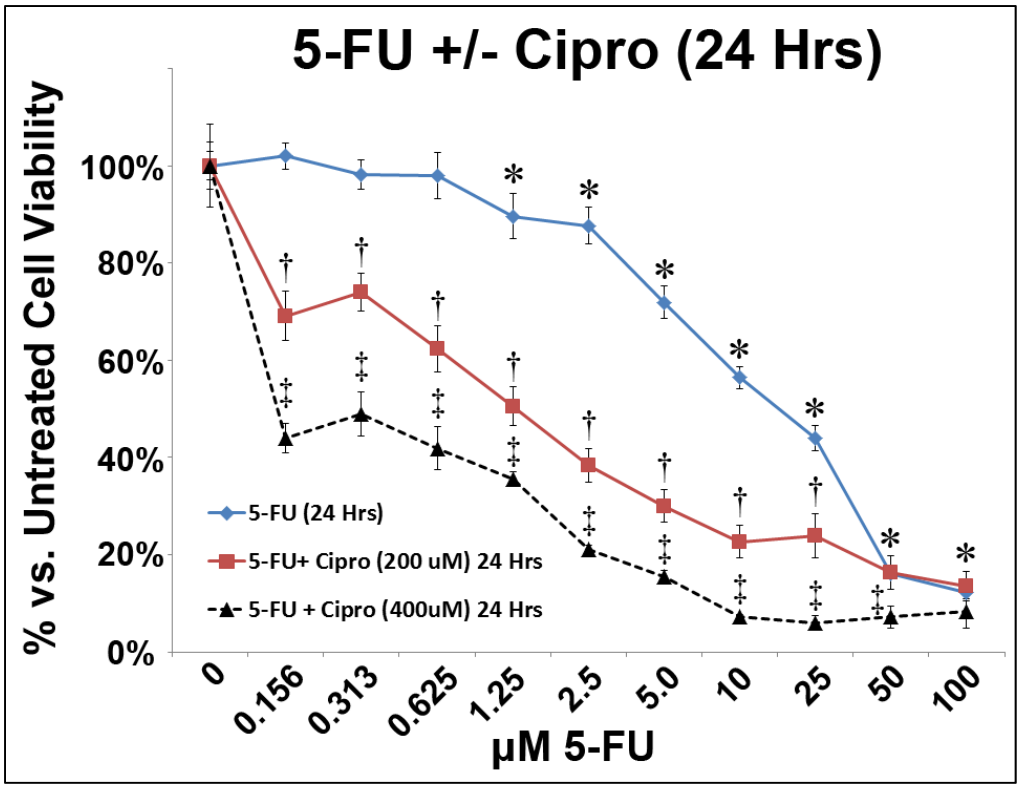

Figure 10: The anti-cancer effect of 5-FU is enhanced when combined with ciprofloxacin. MIA PaCa-2 cell viability is decreased when both drugs are used together and the combinatorial anti-cancer effect is dose-dependent at all but the very highest doses of 5-FU. Data represent the mean (n=16) ± SEM. * $p \leq 0.05$ vs untreated cells; † $p \leq 0.05$ vs single-agent 5-FU; ‡ $p \leq$ vs. 5-FU+200 µM combination.

IV. Simultaenous administration of ciprofloxacin and gemcitabine (dFdC) also has a superadditive anti-cancer effect on PaCa-2 cells.

Given that we found enhanced anti-cancer effect with a known DNA synthesis inhibitor in 5-FU, we elected to see if there is any additional benefit in using another known DNA synthesis antagonist. We found that this combination

was superior to single-agent dFdC administration in our cells. Using isobologram analysis (Figures 12 and 13) We found that, when used together, 5-FU and Cipro have a greater dose dependent effect than either 5-FU or Cipro alone as quantified by the MTS assay after 24 hours.

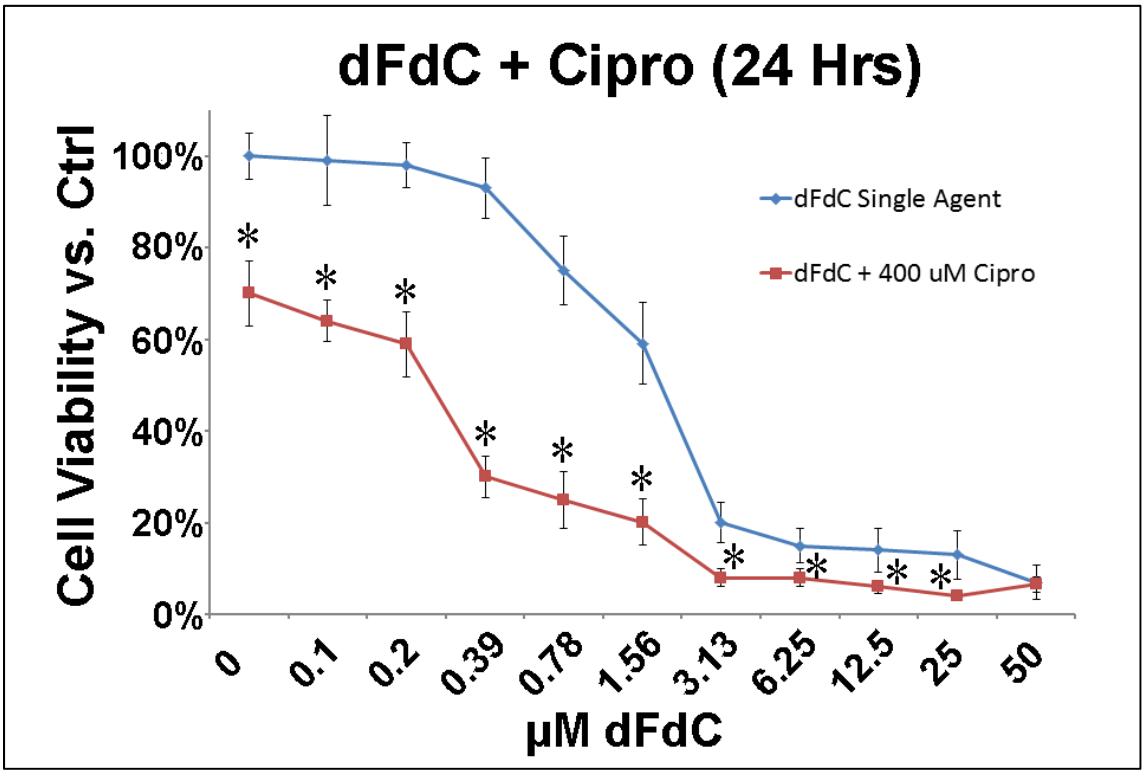

Figure 11: The anti-cancer effect of dFdC is enhanced when combined with ciprofloxacin. MIA PaCa-2 cell viability is decreased when both drugs are used together and the combinatorial anti-cancer effect is dose-dependent at all but the very highest doses of 5-FU. Data represent the mean (n=9) ± SEM. * $p \leq p \leq 0.05$ vs single-agent dFdC.

Analysis/Conclusion

I. Single Chemotherapeutic Agent Effectiveness

It was important that we first treat pancreatic ductal adenocarcinoma cells with each agent independently in order to confirm that they actually decrease viability of cancer cells as well as establish a baseline reference for anti-cancer activity in our cellular model system. We found that Cipro effectively reduced pancreatic cancer cell viability in our 24-hour experiments, as evidenced by MTS colorimetric assay. Our data indicate that cell viability and mitochondrial metabolism are decreased in response to Cipro in a significant and concentration-dependent manner (Figure 8). In retrospect, while it was a surprising finding to us at the time, it turns out that Cipro has been recently shown to have therapeutic promise in treating models of human colorectal cancer. Importantly, a recent study demonstrated anti-cancer effects of combinations of fluoroquinolone drugs – including ciprofloxacin—in human pancreatic adenocarcinoma cell lines (Yadav, et al., 2015), strongly indicating that the drug does have a specific effect on cells of the pancreas.

The chemotherapeutic agents 5-fluorouracil (5-FU) and gemcitabine (dFdC) also demonstrated effective suppression of viability in our 24 hour experiments (Figures 9 and 11). The colorimetric reaction in the MTS assay suggests that the mitochondrial metabolism is significantly decreased by increased doses of 5-FU and dFdC.

II. Effectiveness of Combination Therapy on Cancer Cell Viability

Given that Cipro, 5-FU, and dFdC all appear to act primarily during DNA replication in the S-phase of the cell cycle (Yadav et al. 2015) with resultant reduction in cell viability, our MTS viability data supports our hypothesis that Cipro would enhance the anti-cancer effect of 5-fluorouracil. This suggests that the two drugs would be most effective in a simultaneous combination, though this is merely speculative, as we did not assess an alternative modality of sequential drug administration, which would be an important follow-up experiment to this work.

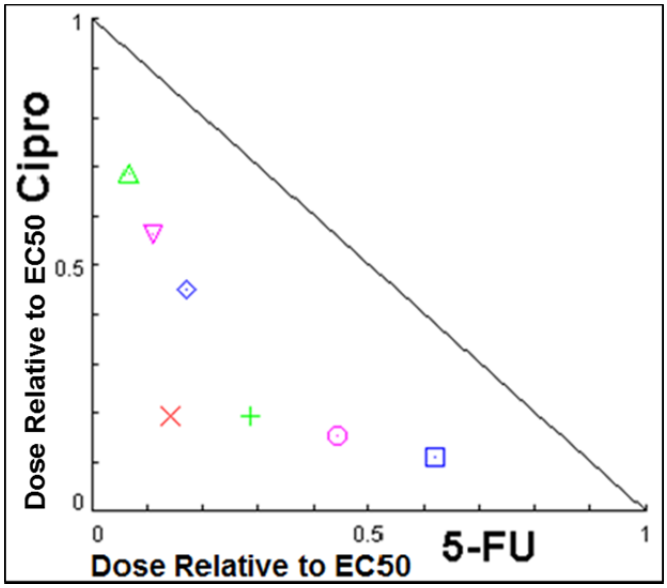

Figure 12: Normalized isobologram demonstrates the enhancement of the anti-cancer effect of 5-FU by ciprofloxacin (400 µM). Single-agent and combination dose vs. effect relationships were graphically analyzed as described previously. This graph represents of 5-FU (0-100 µM) with a constant dose (400 µM) Cipro for 24 hrs. Drug combinations are plotted versus what the predicted effect would be if a perfect additive relationship was present (i.e. EC50 Drug A + EC50 Drug B = EC100 Drug A or B). All combinations tested yielded improved effect over single-agent experimental effect.

Our data corroborates the work of others who have established a preliminary mechanism of action for Cipro, as well as effectiveness of Cipro and 5-FU drug combinations in the MIA PaCa-2 cell line and other malignant pancreatic cell lines *in vitro* (Yadav et al., 2015). In our hands, increased Cipro (from 200 μM to 400 μM) further decreased cancer cell viability, indicating a dose-dependent combinatorial effect (Figure 10), which another standard of evaluation of drug combinations. In summary, the drugs were enhancing each other in a synergistic fashion, rather than acting independently of one another or acting as antagonists to one another.

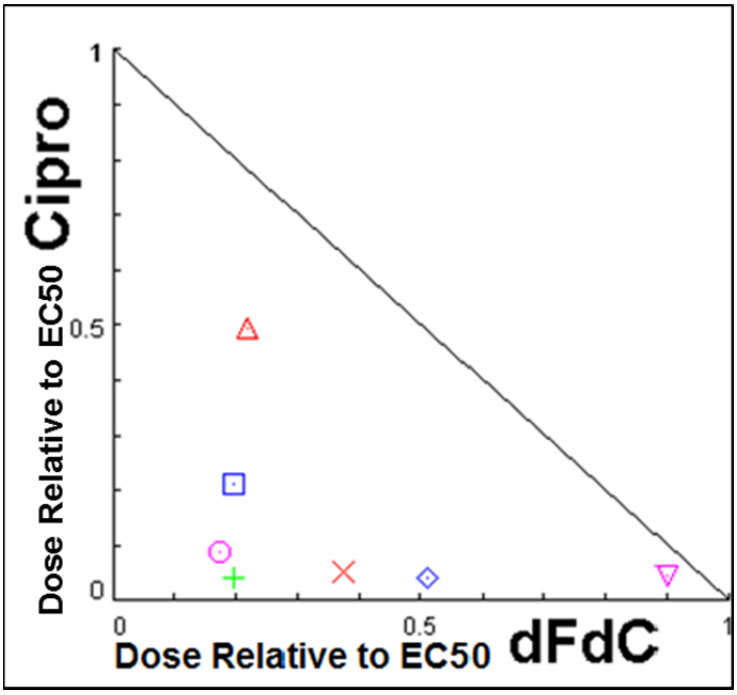

Figure 13: Normalized isobologram demonstrates the enhancement of the anti-cancer effect of dFdC by ciprofloxacin (400 µM). Single-agent and combination dose vs. effect relationships were graphically analyzed as described previously. This graph represents of dFdC (0-50 µM) with a constant dose (400 µM) Cipro for 24 hrs. Drug combinations are plotted versus what the predicted effect would be if a perfect additive relationship was present (i.e. EC50 Drug A + EC50 Drug B = EC100 Drug A or B). All combinations tested yielded improved effect over single-agent experimental effect.

We also tested the combination of Cipro with dFdC, another nucleotide analog which is more potent than 5-FU, and surpassing 5-FU as the frontline drug of choice. Like 5-FU, the combination was more effective in diminishing cell viability than the individual chemotherapeutic agents. Normalized isobolgram analysis further suggests that these drugs act in a synergistic manner (Figure 13), and indicates a strong opportunity for future combinatorial study in advanced models of the disease.

III. Conclusions

We find that either 5-FU or dFdC administered in combination with Cipro leads to a significantly greater dose-dependent response in our pancreatic ductal adenocarcinoma cells. Both 5-FU and dFdC appear to act in a synergistic manner with Cipro, and we hypothesize that this is due to their primary mechanism of action occurring during the same phase of the cell cycle, though this also has yet to be fully examined. This research provides strong evidence for a novel treatment therapy for pancreatic cancer using *currently approved* FDA drugs and further investigation is strongly merited.

Reflection

First of all, we determined ciprofloxacin (Cipro) and 5-fluorouracil (5-FU) do act synergistically in terms of their effect in comparison to each of the drugs independently (as illustrated in Figures 8 and 9). Hye et al. (2014) suggested that ciprofloxacin has been critical in the treatment of gastrointestinal cancers. Further, Almhanna et al. (2015) suggest that 5-FU and radiation work well in inhibiting cancers of the esophagus. Since, the pancreas functions within the digestive system it correlates that these drugs would be effective in treating cancers of the pancreatic duct.

Conger's (2015) work argues that use of drug combinations such as Cipro and 5-FU could be the key to treating pancreatic cancer. We corroborated previous data in Yadav et al. (2015) in Figure 12 where both drugs together were had a greater anti-cancer effect than either drug independently. Our data, as a whole, we conclude supports the contention that these compounds could be used in drug combination strategies.

Through the completion of this project, I was able to use the scientific method to investigate why it is that ciprofloxacin kills cancer cells but does not kill healthy cells. I was also able to corroborate the published work of Yadav, et al. (2015) and was able to better understand the physiological function of both 5-fluorouracil (5-FU), gemcitabine (dFdC), ciprofloxacin (Cipro) as inhibitors of DNA replication. As a result of this

inhibition, the pancreatic ductal adenocarcinoma cells have decreased viability as shown by the MTS assay for mitochondrial function. While the viability is decreased we are as of yet unsure of the specific mechanism of cell cycle inhibition.

Further studies should be conducted to explain the mechanism of action of Cipro and 5-FU in combination and how it impacts mitochondrial metabolism as illustrated in the MTS assay. Another study could be conducted to better understand the most optimal concentrations within the combination.

In addition to the knowledge that I gained in the field of cancer pharmacology, I was able to apply the skills I have gained in aseptic tissue culture and other basic laboratory techniques that I will be able to use in my future research endeavors. I feel that the opportunities that I had to mentor other students was an important part of this honors project. Ultimately, sharing skills with a laboratory colleague helps them develop their skillset and also allows the mentor to gain a better understanding and mastery of the skill by teaching it.

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