The Effect of Betaine and Cisplatin in Combination on Cell Viability, DNA Damage, and Oxidative Stress on the DU-145 Cell Line

BMED 3610: Quantitative Engineering Physiology Lab II, Section A01

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Abstract

Prostate cancer treatment has long been a delicate balance between efficacy and patient safety. This study investigates previously unexplored synergism between cisplatin and betaine in combination. It tests the addition of betaine with cisplatin to measure cytotoxic effects on cell viability, DNA damage and oxidative stress in DU-145 cells. Inhibitory concentration values for betaine and cisplatin determined baselines to run three tests comparing combination results with betaine, cisplatin, and media controls. CCK-8 assays tested DU-145 cells with three triplicate combination variables for cell viability. Flow cytometry was run with DNA damage and oxidative stress kits to compare combination results. Analysis of the resulting luminescence values measured from the CCK-8 kits revealed a noticeable difference in cell viability with higher concentration combinations of cisplatin and betaine. ANOVA statistical testing of the combination treatment for cell viability had a P value of 0.072 for higher concentrations of cisplatin while the lower concentrations value was 0.4. The combination also exhibited higher DNA damage than cisplatin or betaine alone. Interestingly, the oxidative stress of the combination was less than the highly cytotoxic cisplatin and remained at the level of betaine. Despite this, it killed the most cells with the lowest total cell concentration. The combination's ability to keep oxidative stress low while increasing DNA damage shows a unique relationship between natural and chemical treatments. These results demonstrate the combination's potential to reduce prostate cancer's resistance to treatment yet still be effective while taming side effects from high dose cisplatin treatments.

Introduction

Background

This study focuses on a new treatment avenue for prostate cancer as modeled by the DU-145 cell line. One in eight American men will be diagnosed with prostate cancer in their lifetime. Despite its prevalence and large amounts of funding, prostate cancer remains the second leading cause of cancer death for American men.[1] This highlights the need for new and more effective treatment methods for prostate cancer. The DU-145 cell line is an epithelial, androgen independent, and hypotriploid cell line originally isolated from the brain of a 69-year-old male with prostate cancer.[2] These characteristics help ensure the DU-145 cell line is an effective model for studying prostate cancer.

In this study, the DU-145 cell line is treated with a combination of two compounds: betaine and cisplatin. Betaine is a non-toxic, naturally occurring substance that is found in everyday food items such as beets, spinach, quinoa, and wheat and oat bran.[3] Also known as trimethyl glycine, betaine is a glycine molecule with three extra methyl groups. Betaine is a compound that has been shown to have anticancer effects, and these anticancer effects apply to the DU-145 cell line. When the DU-145 cell line was treated with high levels of betaine cell viability decreased and oxidative stress increased. However, at low levels, 0.78 mg/mL to 1.56 mg/mL, there is an increase in cell viability and a decrease in oxidative stress. [3] At these low levels, betaine helps maintain normal cell volume when cells are undergoing osmotic stress and provides protection against protein denaturation. Conversely at high concentration levels, betaine can act as an oxidant and cause cell damage and induce apoptosis.[3]

The second compound, cisplatin, is a platinum-based chemotherapy that is used to treat a wide array of cancers. The platinum in cisplatin binds to and damages the cell's DNA, blocking the cell's ability to self-repair. Cisplatin is activated following its entry into the cell. From that point cisplatin creates cross-linkages in the cell's DNA. This binds the DNA nucleotide pairs together and prevents the cell from synthesizing new DNA and dividing. Additionally, cisplatin increases the oxidative stress within the cell which contributes to the likelihood of cell death. [5] The DNA damage and high oxidative stress levels caused by cisplatin lead to cell cycle arrest and apoptosis. However, due to its high cytotoxic levels and severe side effects (such as hearing loss), it is dose limited.[4] Prostate cancer is often resistant to cisplatin making this chemotherapy a less viable option for this type of cancer. Based on previous literature it was hypothesized that the addition of high levels of betaine affect oxidative stress in the DU-145 cells and make them more susceptible to cisplatin-based treatments. [6]

Hypothesis

The addition of betaine in combination with cisplatin will increase the cytotoxic effect of cisplatin through increasing DNA damage and oxidative stress in the DU-145 cells.

Purpose

When treating cancer patients, it is imperative that the treatment plan is effective in fighting cancer while also minimizing harm to the patient. Cisplatin has the potential to be a highly effective drug in treating prostate cancer, however, prostate cancer's resistance to cisplatin and cisplatin's high toxicity limits its usability. Betaine is a natural compound that decreases cell viability and increases oxidative stress in prostate cancer. Yet still, it is not known if betaine will have a synergistic effect when combined with cisplatin. These experiments help determine if

betaine's effects of increasing oxidative stress results in a larger amount of DNA damage, oxidative stress, and cell death when combined with cisplatin. If betaine and cisplatin do have a synergistic effect, then cisplatin would be less dose limited and produce more positive patient outcomes.

Materials and Methods

Materials

Hemocytometer, Trans/phase contrast microscope, Pipet, Betaine - 50mM in DMSO and Cisplatin- 20mM in $CHCl_3$ stock solutions, EMEM Growth media, Flask of DU-145 cells, Well Plates (96,12), CCK-8 assay, Infinite 200Pro Multimode Microplate Reader, Cytek Muse Flow cytometer Cell Analyzer, MCH200107 Multi-Color DNA Damage Kit 1.8, Muse Oxidative Stress 1.8 Kit, standard cell Lab equipment

Significant Variables

Control Variables:

Initial cell count: ~5,000 in CCK-8 96 well plate experiments, ~50,000 in Flow Cytometry 12 well plate experiments

Incubation: 24-hour period in 37 C 5% CO_2 atmosphere

Treatment concentrations and types: (5 μM) cisplatin, (500) μM betaine, media

Dependent Variables:

Cell viability, Cell DNA damage, Cell oxidative stress

Experimental Methods

IC values testing cell viability were determined using CCK-8 assay kits. On day one, 5000 cells were prepped and pipetted into 10 triplicate groups in a 96 well plate. The cells were then incubated in 37 C 5% CO_2 for 24 hours. Day two changed the media and treated the cells with the following concentrations of betaine (50 μM, 100 μM, 200 μM, 500 μM, 1000 μM), cisplatin (5 μM, 10 μM, 20 μM, 50 μM), and media for control. The cells were then incubated for 48 hours and then the CCK-8 assay kit procedure was performed to determine IC values from the cell viability results. There was an alternative approach of testing additional concentrations if IC-50 values were not found or if too many cells were killed to gather usable IC values. Cell viability testing of the combinations was performed using the same method with 3 groups of cisplatin+ betaine triplicates concentrations of (1 μM cisplatin+100 μM betaine,1 μM cisplatin +500 μM betaine,1 μM cisplatin +1000 betaine μM) and media control. There was an alternative approach of changing the cisplatin concentration to 5 μM if the results did not significantly differ from the control. DNA damage and oxidative stress testing were determined through flow cytometry. On day one, 4 triplicate groups of 50,000 cells were prepped and pipetted into a 12-well plate. The cells were then incubated in 37 C 5% CO_2 for 24 hours. Day two changed the media and treated the cells with 1 group of 500 μM betaine, 1 group of 1 μM cisplatin, 1 group of 1 μM cisplatin + 500 μM betaine combination, 1 group of 1 μM cisplatin, and a control group of media. The cells were then incubated for 48 hours. The procedure for muse kits, MCH200107 Multi-Color DNA Damage Kit 1.8 and Muse Oxidative Stress 1.8 Kit were performed on the test groups followed by analyzing of the results using a flow cytometer. There was also an alternative approach of starting with a higher number of healthy cells and rerunning the experiment if cell concentrations following treatment incubation period was too low.

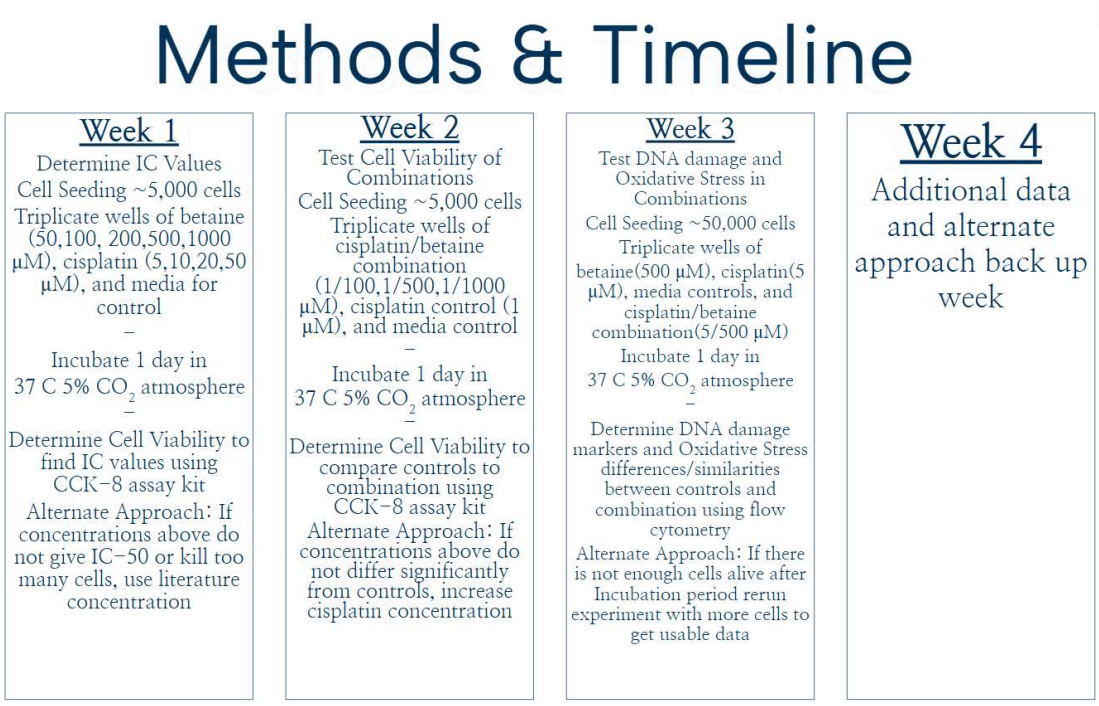


Figure 1: Concise Timeline and Method Description

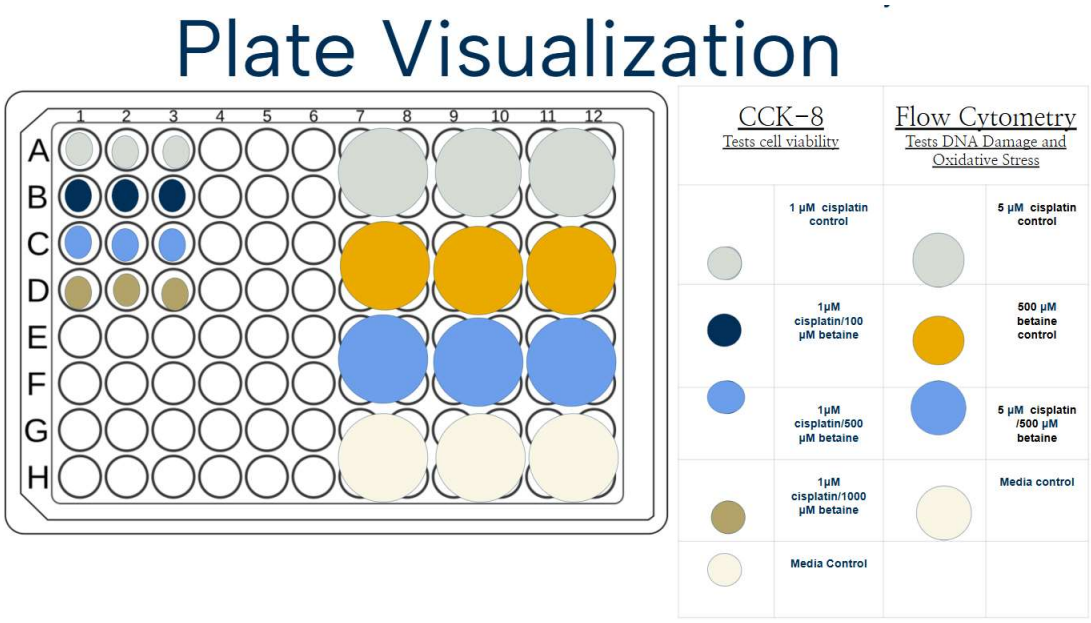


Figure 2: Plate Visualization The left side of the plate represents the different treatments and example size of a 96 well plate that was used for CCK-8 assays. The right side of the plate represents the different treatments and example size of a 12 well plate that was used for flow cytometry which tested for DNA damage and oxidative stress.

Statistical Methods

CCK-8 data from the plate reader can be interpreted with the assistance of the software GraphPad Prism, which has capabilities of normalizing the data, and placing it into a graph with cell viability on the y axis and drug concentrations on the x axis. From this data, one can identify the IC 10, IC 25, and IC 50 concentrations from their correlative cell viability. Linear regression is performed using GraphPad Prism to give statistical results. These values are applicable to the second and third portions of the experiment and are used as the drug combination independent variables. For the second portion of the experiment, cell viability and the capabilities of the drug combinations in inducing cell death is observable with the same type of graph in GraphPad Prism. ANOVA analysis is then used to determine the statistical significance between differences between both the controls and combinations, and between the varying concentrations for cell viability testing.

Flow cytometry data outputs histograms of fluorescent signals, and for DNA damage assessment, the sample's quadrant association (double positive, singular positive, or double negatives for each antibody fluorescent signal) is indicative of their level of DNA damage. The higher the fluorescent level for each antibody, the higher levels of DNA damage present in the sample. [8] The Muse Oxidative Stress Kit detects reactive oxygen species (ROS) levels with fluorescence, high levels of fluorescence are indicative of high levels of oxidative stress within the sample. [9] In summary, both assay kits have data output from the flow cytometer in the form of a histogram, with direct correlation between fluorescence levels and their targeted observations. The positive results for DNA damage and oxidative stress produced by the Muse analysis are compared

between the controls and combination to determine an absolute % difference for statistical reference.

Results

IC Testing

IC testing yielded variable results for the betaine concentration range chosen. Higher concentrations had slightly lower viability, but it was not statistically significant. There was a slight correlation between cell viability and cisplatin concentration. The cell viability following cisplatin treatments was significantly impacted to the point of diminishing returns with higher concentrations. Lower cisplatin concentrations were chosen to test combinations with betaine as the IC testing revealed the highly cytotoxic nature of concentrated cisplatin.

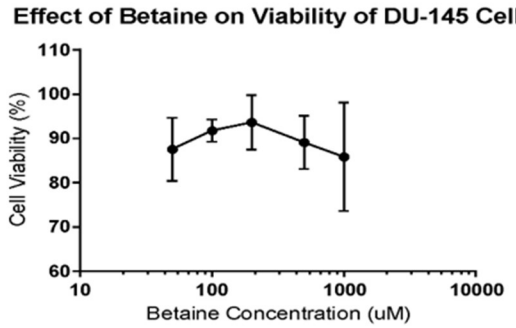


Figure 3

Figure 3: Effect of Betaine on Viability of DU-145 Cells displays DU-145 cell viability following 48-hour treatment for betaine. There was no significant decrease in cell viability as betaine concentration increases. Statistical analysis performed through linear regression displayed on figure.

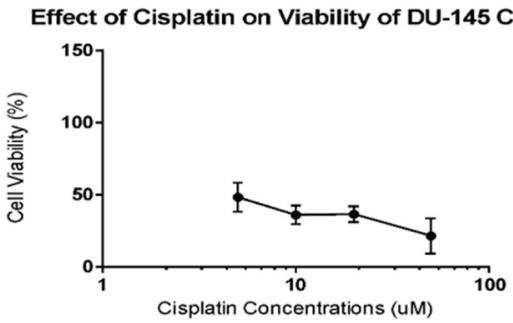


Figure 4

Figure 4: Effect of Cisplatin on Viability of DU-145 Cells displays DU-145 cell viability following 48-hour treatment of cisplatin. There was a consistent decrease in cell viability as cisplatin concentration increases. Statistical analysis performed through linear regression displayed on figure.

Cell Viability

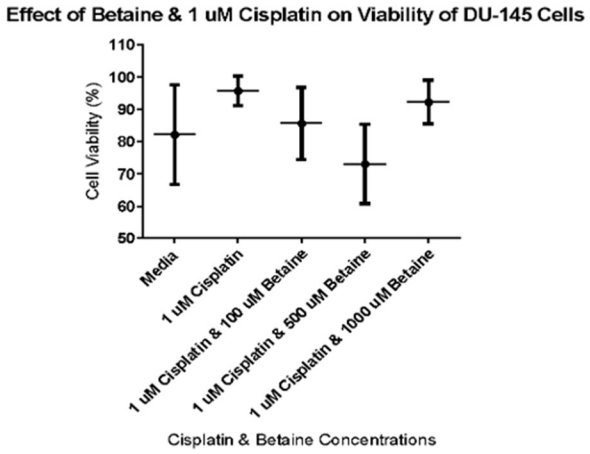


Figure 5: Effect of Betaine & 1 µM Cisplatin on Viability of DU-145 Cells displays DU-145 cell viability following 48-hour varying betaine combination treatments with 1 µM cisplatin. There was no significant decrease in cell viability with low betaine concentrations. There was a consistent decrease in cell viability with higher betaine concentrations particularly the 500 µM betaine combination with 1 µM cisplatin. Statistical analysis performed using ANOVA had a P- value of 0.399 for this combination.

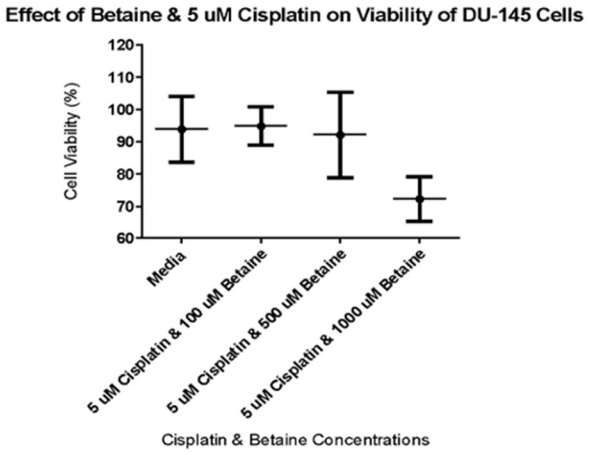


Figure 6: **Effect of Betaine & 5 µM Cisplatin on Viability of DU-145 Cells** displays DU-145 cell viability following 48-hour varying betaine combination treatments with 5 µM cisplatin. There was no significant decrease in cell viability with low betaine concentrations. There was a consistent decrease in cell viability with higher betaine concentrations particularly the 500 µM and 1000 µM betaine combinations with cisplatin. Statistical analysis performed using ANOVA had a P- value of 0.072 for 5 µM cisplatin in combination with 500 µM betaine.

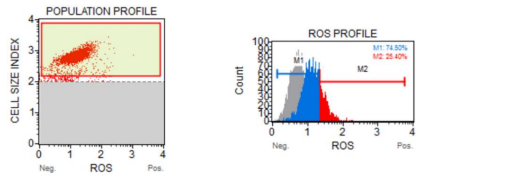
There was no noticeable difference in cell viability for lower values of betaine in the first round of combinations using 1 µM of cisplatin. Cisplatin's driving force was lowered by 5 times in this first round and appeared to be too much for synergy to overcome. A small synergistic effect was observed in high betaine concentration concentrations like 500 µM, where it averaged 25% more cell death.

There was more of a noticeable difference in cell viability when using the higher concentration of cisplatin in the second round of combinations using 5 μM of cisplatin. The media had the highest cell viability, and the combination with 1000 μM betaine was the lowest as expected. The lesser concentrations had a slight drop off from the media, but their significance was limited. It can be determined to see more synergistic effects of betaine in cell viability, higher treatment doses such as 5 μM cisplatin + 1000 μM betaine are needed.

Flow Cytometry

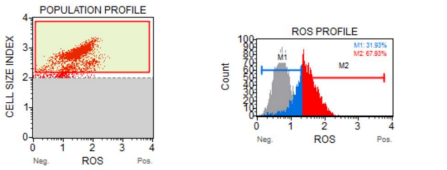
The results from the first run of this experiment had such low cell counts, that the data is unreliable, so the results shown are from the second attempt of the experiment. Four variables were tested, media control, Cisplatin 5 μM baseline control, Betain 500 μM baseline control, and the optimal combination for experimental purposes of 5 μM cisplatin + 500 μM Betain. This combination would leave enough cells viable for testing but was enough to reach a significant threshold of cell death. Flow cytometry was used to determine both oxidative stress and DNA damage in the cells. Both assay kits had significantly better results in the second run. This is due to them having much higher cell concentrations per volume.

Oxidative Stress



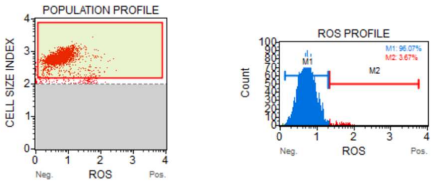
Oxidative Stress	% Gated	Cell Conc. (Cells/mL)
ROS(-) Cells (M1)	74.50	2.31E+05
ROS(+) Cells (M2)	25.40	7.89E+04
Total Cell Concentration	3.11E+05 Cells/mL	

Figure 7: Oxidative Stress Profile of 500 µM Betaine displays DU-145 cells oxidative stress positive markers determined through flow cytometry of Muse Oxidative Stress 1.8 kit. Following 48-hour treatment of betaine, this was used as control to compare with combination treatment results and had an absolute % difference of +0.57



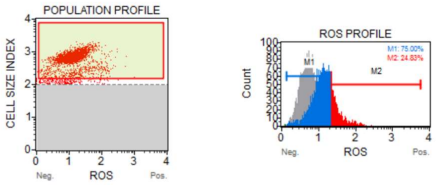
Oxidative Stress	% Gated	Cell Conc. (Cells/mL)
ROS(-) Cells (M1)	31.93	8.79E+04
ROS(+) Cells (M2)	67.93	1.87E+05
Total Cell Concentration	2.75E+05 Cells/mL	

Figure 8: Oxidative Stress Profile of 5 µM Cisplatin displays DU-145 cells oxidative stress positive markers determined through flow cytometry of Muse Oxidative Stress 1.8 kit. Following 48-hour treatment of cisplatin, this was used as control to compare with combination treatment results and had an absolute % difference of +43.1



Oxidative Stress	% Gated	Cell Conc. (Cells/mL)
ROS(-) Cells (M1)	96.07	1.25E+06
ROS(+) Cells (M2)	3.67	4.77E+04
Total Cell Concentration	1.30E+06 Cells/mL	

Figure 9: Oxidative Stress Profile of Media Control displays DU-145 cells oxidative stress positive markers determined through flow cytometry of Muse Oxidative Stress 1.8 kit. After a 48-hour incubation period, this control treatment of media was used as the baseline for other oxidative stress results.

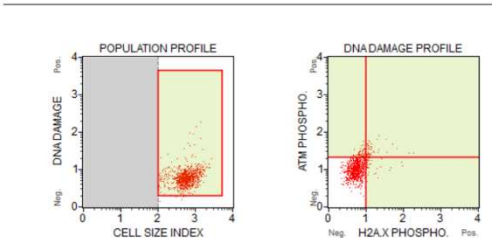


Oxidative Stress	% Gated	Cell Conc. (Cells/mL)
ROS(-) Cells (M1)	75.00	1.98E+05
ROS(+) Cells (M2)	24.83	6.55E+04
Total Cell Concentration	2.64E+05 Cells/mL	

Figure 10: Oxidative Stress Profile of 500 µM Betaine + 5 µM Cisplatin Combination displays DU-145 cells oxidative stress positive markers determined through flow cytometry of Muse Oxidative Stress 1.8 kit. Following a 48-hour incubation of betaine + cisplatin combination, this was compared against controls to determine the treatments effect on oxidative stress.

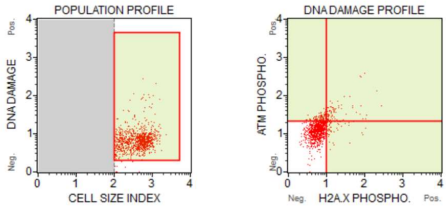
The cisplatin baseline had the highest oxidative stress with ~68% of cells testing positive. This was expected as cisplatin is known to induce oxidative stress which leads it to be very cytotoxic. The betaine baseline and combination tests had the second highest oxidative stress at ~25% of cells testing positive. Unexpectedly, the combination had less total cell concentration, yet it kept the oxidative stress at levels similar to betaine. The media control results were expected with a very low oxidative stress at ~3.5% and the highest total cell concentration.

DNA Damage



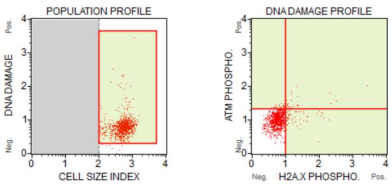
Total Events	% Total	Cell Conc. (Cells / µL)
Negative (LL) :	85.07 %	437.62
pATM (Single Pos) (UL) :	5.75 %	29.59
DNA double-strand breaks (UR) :	4.74 %	24.40
pH2A.X (Single Pos) (LR) :	4.44 %	22.84
Total DNA Damage (UL+UR+LR) :	14.93 %	76.83

Figure 11: DNA Damage Profile of 500 µM Betaine displays DU-145 cells DNA damage positive markers determined through flow cytometry of MCH200107 Multi-Color DNA Damage Kit 1.8. Following 48-hour treatment of betaine, this was used as control to compare with combination treatment results and had an absolute % difference of -13.8



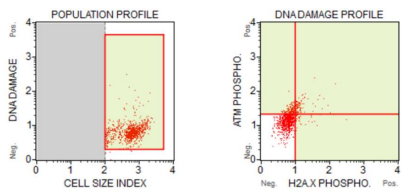
Total Events	% Total	Cell Conc. (Cells / µL)
Negative (LL) :	73.84 %	138.45
pATM (Single Pos) (UL) :	11.01 %	20.64
DNA double-strand breaks (UR) :	8.28 %	15.53
pH2A.X (Single Pos) (LR) :	6.87 %	12.88
Total DNA Damage (UL+UR+LR) :	26.16 %	49.05

Figure 12: DNA Damage Profile of 5 µM Cisplatin displays DU-145 cells DNA damage positive markers determined through flow cytometry of MCH200107 Multi-Color DNA Damage Kit 1.8. Following 48-hour treatment of cisplatin, this was used as control to compare with combination treatment results and had an absolute % difference of -2.57



Total Events	% Total	Cell Conc. (Cells / µL)
Negative (LL) :	88.00 %	344.03
pATM (Single Pos) (UL) :	3.02 %	11.82
DNA double-strand breaks (UR) :	2.72 %	10.64
pH2A.X (Single Pos) (LR) :	6.25 %	24.43
Total DNA Damage (UL+UR+LR) :	12.00 %	46.89

Figure 13: DNA Damage Profile of Media Control displays DU-145 cells DNA damage positive markers determined through flow cytometry of MCH200107 Multi-Color DNA Damage Kit 1.8. After a 48-hour incubation period, this control treatment of media was used as the baseline for other DNA damage results.



Total Events	% Total	Cell Conc. (Cells / µL)
Negative (LL) :	71.27 %	215.29
pATM (Single Pos) (UL) :	14.62 %	44.15
DNA double-strand breaks (UR) :	9.98 %	30.15
pH2A.X (Single Pos) (LR) :	4.13 %	12.49
Total DNA Damage (UL+UR+LR) :	28.73 %	86.79

Figure 14: DNA Damage Profile of 500 µM Betaine + 5 µM Cisplatin Combination displays DU-145 cells DNA damage positive markers determined through flow cytometry of MCH200107 Multi-Color DNA Damage Kit 1.8. Following a 48-hour incubation of betaine + cisplatin combination, this was compared against controls to determine the treatments effect on DNA damage

The combination had the highest DNA damage with ~29% of cells displaying DNA damage. This is promising results as it exceeds the known DNA damage inducer tested, cisplatin, which damaged ~26% of cells. This is important as cisplatin is a treatment for cancer because it damages DNA which induces cell death. The betaine also dealt DNA damage on ~15% of cells, but it was not much more than the baseline damage done by the media control being 12%. It should be noted that the highest type of DNA damage identifiers was pATM (Single Pos) for all three treatments, while the media's highest marker was pH2A.X (Single Pos) (LR) which was the lowest for the treatments.

Discussion

The first round of cell viability testing with the CCK-8 assay used a smaller dose of cisplatin, 1 μM. This was chosen to determine the strength of the synergism between cisplatin and betaine as it was unknown and never tested before. The results did not display a large effect and in this case had a P value of 0.4 using the ANOVA statistical methodology. These initial results did not confirm the hypothesis. This was most likely due to the chosen lower concentration of cisplatin in the hope for a prominent synergistic effect. But since cisplatin was reduced by 5 times, taking away much of the driving factor was too much for the synergy to overcome. The cell viability results were consistent between the three combination treatments, 1 μM cisplatin, and media control. The alternate approach of increasing the cisplatin concentration back to 5 μM for a second round of cell viability was decided upon. In the second round of cell viability testing, the less potent combination treatment of 100 μM betaine + 5 μM cisplatin and had a slight drop off from the media, but it was not significant. Despite this, the stronger dosage of 1000 μM betaine + 5 μM cisplatin and 500 μM betaine + 5 μM cisplatin did show a more significant reduction in cell viability compared to the control. These results had a more significant P value of 0.072 using the ANOVA methodology. This tentatively confirms the hypothesis that cell viability would be decreased with the combination treatment. It also shows that the combination follows previous research on betaine, that only at higher concentrations will betaine reduce cell viability. A microscope also confirmed these results as visual images displayed changes in living cell density correlating with increased dosages.

The first round of DNA damage and oxidative stress had inconclusive results due to low cell count and did not confirm or reject the hypothesis. Further discussions of this first round are in

the errors and limitations sections. The alternate approach was then taken with a second round of flow cytometry testing for DNA damage and oxidative stress. This round used closer to 100,000 cells per well and had enough living cells to be analyzed post treatment. It confirmed the hypothesis with the combination displaying a high cytotoxic effect for both DNA damage and oxidative stress. Testing results of the combination were compared to baselines for cisplatin and betaine along with media for control. The combination treatment had the highest DNA damage by roughly 2.5%. The combination performing just as good or better than the known DNA damage inducer cisplatin, helps to confirm the first part of the hypothesis. Since it was not a significant difference between the combination and cisplatin treatment, full synergism cannot be claimed, but it could be in part due to this or additive effects of the treatments. This is important as cisplatin is a treatment for cancer because it damages DNA which induces cell death. The highest damage identifier was pATM (Single Pos) for all three treatments, while the media's highest marker was pH2A.X (Single Pos) (LR) which was the lowest for the treatments. Both types are used to repair DNA, but pATM (Single Pos) is more of a broad DNA damage response in cell cycle regulation and it is more responsive to oxidative stress. [11] This is further proof that one of the mechanisms that the treatments cause is increased oxidative stress. The combination also has the second highest oxidative stress and lowest total cell concentration. Keeping its oxidative stress at the level of betaine is an advantage of the combination over the highly cytotoxic cisplatin. This allowed it to achieve the lowest total cell concentration, killing the most cells, post treatment. This partly confirms the second part of the hypothesis as the oxidative stress results were affected but not increased. Discussed in previous studies, cells develop a strong resistance to high oxidative stress. [4] The combination's ability to keep the oxidative stress low while increasing DNA damage shows the potential for synergism. The combination's

oxidative stress being lower than cisplatin's baseline limits the cell's ability to develop resistance to the treatment. This will yield long term benefits in killing the cancer cells as well as keeping the normal cells in a healthier environment.

Errors Encountered

The first round of cell viability testing using CCK-8 assay had inconclusive results. More time was waited, and the cells were tested again yet the results still had little cell viability difference between the media control and treatments. This could have occurred for a couple of reasons. The first was that there may have been some contamination in the wells as this was the first experiment, which could cause triplicates to vary more than expected and no noticeable difference between cell viability for some treatments. The second more likely reason would be the errors were caused by the initial concentration of 1 μM cisplatin being too low. Since cisplatin is the driving force of the treatment, if its concentration is too low the effectiveness of the synergism between betaine and cisplatin will also be too low to yield significance. When the cisplatin concentration was increased for the second round of cell viability testing to 5 μM decreased cell viability was observed.

The first round of flow cytometry experiments had a low cell count which greatly affected the results. This could have been caused by starting out with too little cells, or unhealthy cells. This experiment was delayed at first due to not having enough cells in good condition. It is possible that even after waiting the cells were still not healthy enough to survive treatment. With the low cell count big disturbances in the data occur depending on which way individual cells are classified. Having more cells dramatically helped the results as seen in the second round of flow cytometry testing.

Limitations

Betaine risks precipitation in higher concentrations. During testing no precipitate was observed, but this was a consideration in limiting our betaine concentration to 1000 μM.

Testing higher concentrations of treatments is a limitation of these experimental methods. For flow cytometry to work properly, there must be enough cells alive to test. This makes it difficult to test higher treatments as they kill too many cells to get reliable data. Otherwise results like round 1 of the flow cytometry are observed.

This study was limited to invitro testing, so there is still a question how the combination treatment would act in a real life treatment method. This study used less concentrated doses than current cisplatin cancer treatments in the medical field. Despite this, some significant alterations in cell viability, DNA damage, and oxidative stress were observed. These results give promise for this treatment to be as effective with less harmful side effects than standard procedures.

Further Research

Future work can be done to determine optimal concentrations of both betaine and cisplatin in combination for in vivo treatments. Tests can also be done to extended treatment periods and observe effects on both healthy and cancer cells. Future studies can also be performed by putting betaine in combination with other known chemo treatments such as carboplatin. This study showed a slight synergistic relationship between cisplatin and betaine when placed in

combination treatments. Longer-term treatments and cellular resistance to this combination would need to be tested to further confirm the relationship.

Conclusion

The relationship between cisplatin and betaine in combination displayed throughout this study has promising implications for future cancer treatment. When treating cancer patients, it is imperative that the treatment plan is effective in fighting cancer while also minimizing harm to the patient. The combination's ability to keep the oxidative stress low while increasing DNA damage shows the possibility of synergism. These results demonstrate the combination's potential to reduce prostate cancer cell's ability to develop resistance and limit side effects from high dose cisplatin treatments. Future studies can build off the framework of these experiments to eventually create effective techniques for fighting patient cancer.

References

1. Key statistics for prostate cancer: Prostate cancer facts. Prostate Cancer Facts | American Cancer Society. Accessed February 17, 2025. https://www.cancer.org/cancer/types/prostate-cancer/about/key-statistics.html.
2. DU-145 cells. Cytion. Accessed March 8, 2025. https://www.cytion.com/us/DU-145-Cells/300168.

3. Kar F, Hacioglu C, Kacar S, Sahinturk V, Kanbak G. Betaine suppresses cell proliferation by increasing oxidative stress-mediated apoptosis and inflammation in DU-145 human prostate cancer cell line. Cell stress & chaperones. September 2019. Accessed March 8, 2025. https://pmc.ncbi.nlm.nih.gov/articles/PMC6717232/.
4. Cisplatin. Cisplatin - an overview | ScienceDirect Topics. Accessed March 8, 2025. https://www.sciencedirect.com/topics/neuroscience/cisplatin.
5. Dasari S, Tchounwou PB. Cisplatin in cancer therapy: Molecular mechanisms of action. European journal of pharmacology. October 5, 2014. Accessed March 8, 2025. https://pmc.ncbi.nlm.nih.gov/articles/PMC4146684/.
6. Liu YQ;Zhang GA;Zhang BC;Wang Y;Liu Z;Jiao YL;Liu N;Zhao YR; Short low concentration cisplatin treatment leads to an epithelial mesenchymal transition-like response in DU145 prostate cancer cells. Asian Pacific journal of cancer prevention : APJCP. Accessed March 8, 2025. https://pubmed.ncbi.nlm.nih.gov/25735324/.
7. Lin K. What is the CCK-8 assay? Bosterbio. March 7, 2024. Accessed March 8, 2025. https://www.bosterbio.com/blog/post/what-is-the-cck-8-assay.
8. MUSE® multi-color DNA damage kit user's guide. Accessed March 9, 2025. https://welcome.cytekbio.com/hubfs/Amnis-and-Guava-Products/muse-multi-color-dna-damage-kit-rsp.pdf.
9. Muse oxidative stress kit. Accessed March 9, 2025. https://welcome.cytekbio.com/hubfs/Amnis-and-Guava-Products/Muse-Oxidative-Stress-Kit-RSP.pdf.

10. Arumugam MK, Paal MC, Donohue TM Jr., Ganesan M, Osna NA, Kharbanda KK. Beneficial Effects of Betaine: A Comprehensive Review. Biology. 2021; 10(6):456. https://doi.org/10.3390/biology10060456

11. Okuno, Y., Nakamura-Ishizu, A., Otsu, K., Suda, T., & Kubota, Y. (2012). Pathological neoangiogenesis depends on oxidative stress regulation by ATM. *Nature Medicine*, *18*(8), 1208–1216. https://doi.org/10.1038/nm.2846