4-28-2020

Synthetic Lethality in Pediatric Brain Cancer Cells by Optimized PLGA Nanoparticles and Drug Combinations

Megan Ruckman
University of Nebraska-Lincoln, megan.ruckman@huskers.unl.edu

Megan Otte
University of Nebraska-Lincoln, motte10@huskers.unl.edu

Forrest Kievit
University of Nebraska-Lincoln, fkievit2@unl.edu

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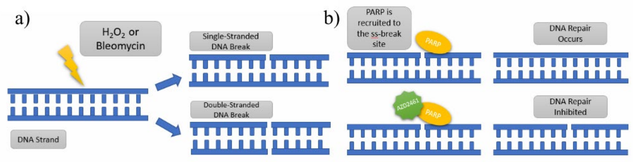
Megan Ruckman, Megan Otte, Dr. Forrest Kievit
Department of Biological Systems Engineering- University of Nebraska Lincoln

Synthetic Lethality and Clonogenic Assay

On the cellular level, cancer cells are able to divide by repair DNA damage through DNA repair pathways. In order to survive, cancer cells utilize a certain repair pathway and rely on it to replicate. The idea of synthetic lethality is that the repair pathway can be inhibited, therefore causing cancer cell death[3]. The objective of this project is to sensitize cells, specifically cancer cells, to radiation by utilizing the concept of synthetic lethality.

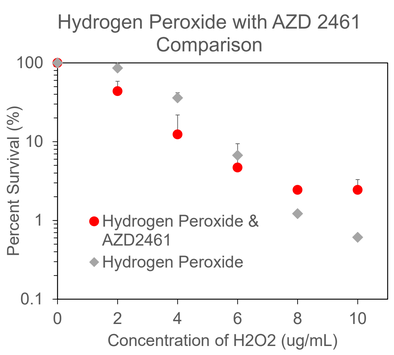
Figure 1: a) Hydrogen peroxide and Bleomycin treatments generate single-strand and double-strand breaks which mimic the DNA damage caused by ionizing radiation. b) AZD2461 inhibits the repair of single-stranded breaks by binding to and inhibiting the nuclear enzyme, poly(ADP-ribose) polymerase (PARP). This protein plays a significant role in DNA repair[2].



The clonogenic assay detects all cells that have the ability to form a significantly sized colony. This is a standard assay for testing replicative cell death because it is able to give a precise cell kill measurement. This assay confirms that DNA damage has occurred and allows for the drug to be assessed in terms of success of blocking the repair pathway. After treatment with the drug and the DNA damage agent, the cells are allowed to grow for 10 days. The growth time allows the replicative ability of the cells to be measured. The cells are dyed with methylene blue (Fig. 2) and the colonies over 50 cells are counted.

Figure 2: Pictured is a set of Methylene Blue stained plates which were utilized to determine plating efficiency and the ideal concentration of cells to be plated for the clonogenic assay. The well concentration of U118 cells per well is labeled in the image.

Results: Drugs



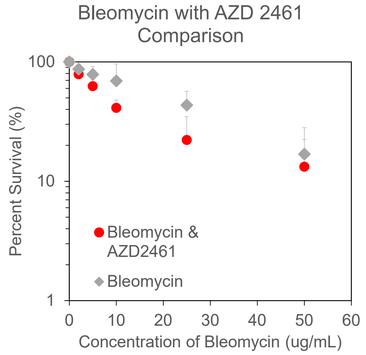


Figure 3: To mimic the damaging effects of radiation, cells were subjected to Hydrogen Peroxide and Bleomycin. DNA damage methods can be compared in the figures to the left. On the y-axis is the log of percent survival, or the cells ability to replicate and create sizable colonies after DNA damage. Each graph shows a variety of concentrations of the chemical that provides DNA damage. The curves compare one set of cells that was first treated with 10 uM of the DNA repair pathway drug AZD 2461.

Nanoparticles and Optimization

To accomplish the delivery of DNA repair pathway inhibiting drugs, we use nanoparticles (NPs). This serves to transport the drugs to cancer cells without damaging healthy tissue so that destroying the tumors with radiation will be more effective. We used poly(lactic-co-glycolic acid), or PLGA, nanoparticles due to their safety and simple preparation methods. We must optimize the NPs for size and drug loading to increase effectiveness. The NPs must be less than 200nm in diameter so they will not be filtered out by the body, and increasing the drug loading makes them more effective and less expensive. We used Doxorubicin, an established cancer drug, as a model drug due to its fluorescent properties. Optimization of the NPs included variation of sonication/stirring, organic and aqueous phases, amount of PLGA, and amount of Doxorubicin. A single emulsion preparation method was used due to more success in our previous experiments than a double emulsion. This method uses differences in polarity between the phases to form layers and force the drug into NPs through sonication (Fig. 4).

Results: Nanoparticles

The maximum yield of Doxorubicin achieved in the NPs was about 10uM. This is significantly higher than in our previous research, and did not produce nanoparticles that were as large either. Fig. 5 and Fig. 6 show the effects of two different conditions that were varied, concentration of poly(vinyl alcohol), or PVA, and amount of PLGA. Lower concentrations of PVA produced more favorable NPs (Fig. 5). Fig. 6 shows 50mg of PLGA producing much less favorable NPs than other amounts, which does not make sense, so it would be worth running this trial again to determine if it can produce a balance of high drug loading and small enough diameter. We believe using more PLGA produces more drug loading in the NPs, but compromises the size.

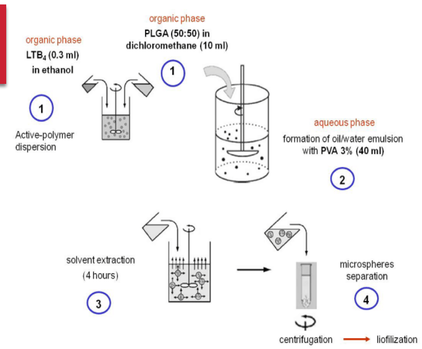


Figure 4: An example of an oil in water emulsion technique similar to what we used our experiments. https://www.omicsonline.org/articles-images/2157-7013-S5-001-g001.gif

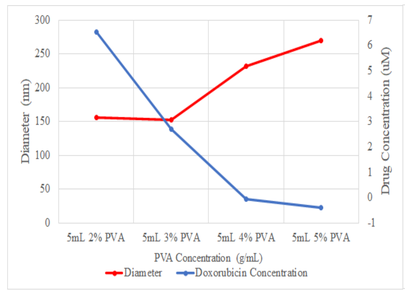


Figure 5: PLGA NPs demonstrating effects of varying w phase PVA concentration on diameter and drug loading under the conditions of sonication for 3.5min at 75% amplitude, stirring for 1.25hr at 900rpm, and o phase containing 100µL of 100µM Doxorubicin, 30mg PLGA, 2.5mL dichloromethane, and 0.5mL methanol.

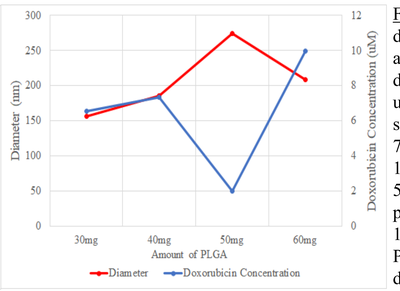


Figure 6: PLGA NPs depicting effects of varying amounts of PLGA on diameter and drug loading under the conditions of sonication for 3.5min at 75% amplitude, stirring for 1.25hr at 900rpm, w phase 5mL of 2% PVA, and o phase containing 100µL of 100µM Doxorubicin, 30mg PLGA, 2.5mL dichloromethane, and 0.5mL methanol.

Discussion

The drug AZD2461 works by blocking the cancer's cells repair pathway. When a single strand break occurs in the DNA, PARP is recruited to repair the DNA break. When AZD2461 is present, PARP is blocked and the DNA cannot be repaired. The data shows that the blocking of this repair pathway makes the cells easier to kill with radiation. The presence of a drug will ideally make less radiation more effective. The combination of AZD2461 and radiation treatment caused a decrease in cell survival at low concentrations of hydrogen peroxide and all concentrations of Bleomycin. The next step in this research is to incorporate drug combinations to determine what treatment best sensitizes cells to radiation treatment. Having produced NPs with significant drug loading, the next step will be to test the Doxorubicin-loaded NPs on glioblastoma cells, as well as incorporate DNA repair pathway inhibiting drugs into NPs.

ACKNOWLEDGEMENTS
Special Thanks to the UCARE program, as well as the Nebraska Research Initiative and the Buffett Cancer Center and Pediatric Cancer Research Group at UNMC for help funding this work.

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3. https://www.omicsonline.org/articles-images/2157-7013-S5-001-g001.gif