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4-29-2021

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Temporal dynamics of caspase activation in PPI-treated cancer cells

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Abstract

Cancer cells rely on glycolysis even under normoxic conditions. The use of this pathway results in measurable intracellular acidification, which is characterized as an early event in the apoptosis program. The pH is restored by activation of voltage-gated proton pumps, preventing acidification. Proton pump inhibitors (PPIs), such as omeprazole, inhibit the H+/K+-ATPase system found at the secretory surface of gastric parietal cells. Research has shown that omeprazole is also capable of inducing caspase-dependent apoptosis in Jurkat T-lymphocytes. However, the effects of PPIs on caspase activity remain largely unknown. The goal of this study was to determine the temporal dynamics of caspase activity in Jurkat cells treated with omeprazole, dexlansoprazole, or esomeprazole for six hours. After the incubation period, cells were held in place by anti-CD71 antibodies on the device's affinity surface and fluorescence microcopy was used to monitor caspase activity in real time. Caspase activation was observed over a six-hour period with the fluorogenic caspase probe, L-bisaspartic acid rhodamine 110 (D_2R). Elucidation of the intensity and timing of caspase activation will be beneficial for evaluating PPIs as potential cancer therapeutics.

Introduction

During mitochondrial apoptosis, caspases become active in a signaling cascade (Fig. 1) (Susin et al., 2000). Cancer cells upregulate proton pumps and hydrogen ion transporters (Huber et al., 2010) to maintain alkalinity and avoid apoptosis. When these methods of maintaining alkalinity are inhibited, the intracellular space becomes too acidic and triggers apoptosis, a form of programmed cell death.

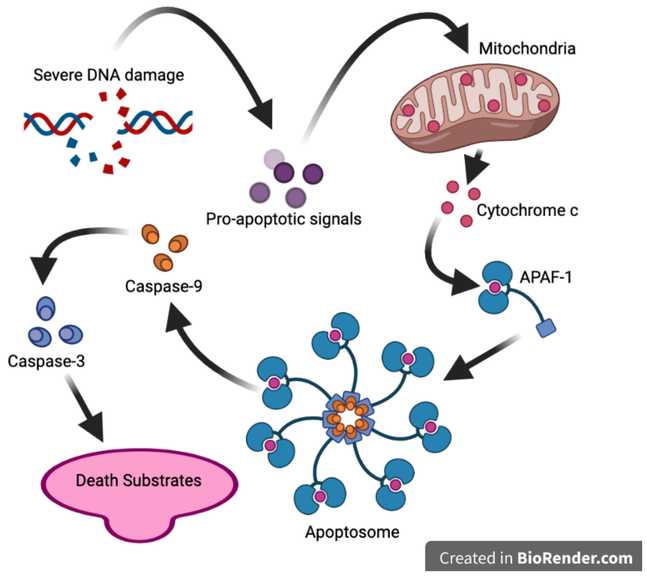

Figure 1: The signaling cascade of mitochondrial apoptosis (Susin et al., 2000).

Proton pump inhibitors (PPIs) inhibit gastric acid secretion by inactivating proton pumps (Shin, 2008). Currently, PPIs are utilized as supportive therapy in patients undergoing chemotherapy. Prior research has shown that omeprazole directly induces caspase-dependent apoptosis in Jurkat cells (Scaringi, 2004), but the capability of PPIs to act as standalone cancer treatments remains largely unevaluated. PPIs could present a viable, less-toxic alternative to chemotherapeutics.

Experimental

Temporal Dynamics Study

- Cells incubated with 100mM PPI, complete medium, & D_2R at 37℃ and 5% CO_2 for 6 hours
- Cells re-suspended in complete medium and D_2R fluorophore
- White light & fluorescence microscopy images taken of aliquots from sample every 3 hours for a total of 30 hours
- Images processed with ImageJ
- Fluorescence threshold established

Microfluidic Device Trials

- Incubation repeated from prior study
- Microfluidic devices (Fig. 2A) coated with antibody ladder (Fig. 2B)
- Cells adhered, white and green light images taken for pre-wash photos
- 1x PBS wash for 5 minutes
- Post-wash images taken
- Imaging repeated every 6 hours
- Images processed with ImageJ
- Binding & apoptotic cell % determined

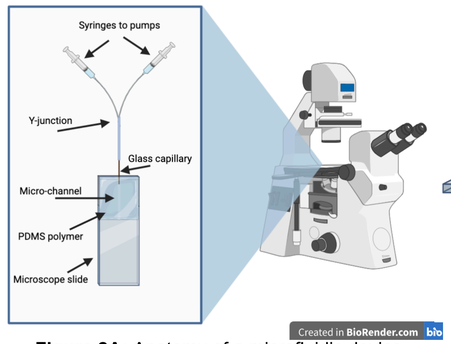

Figure 2A: Anatomy of a microfluidic device attached to an inverted microscope.

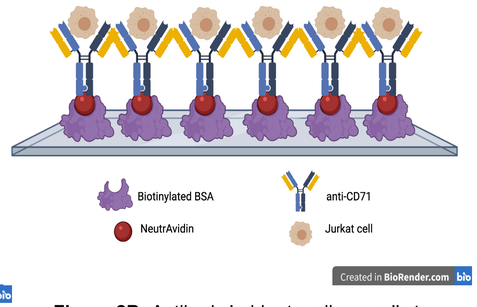

Figure 2B: Antibody ladder to adhere cells to the microfluidic device for observation.

Results – Temporal Dynamics

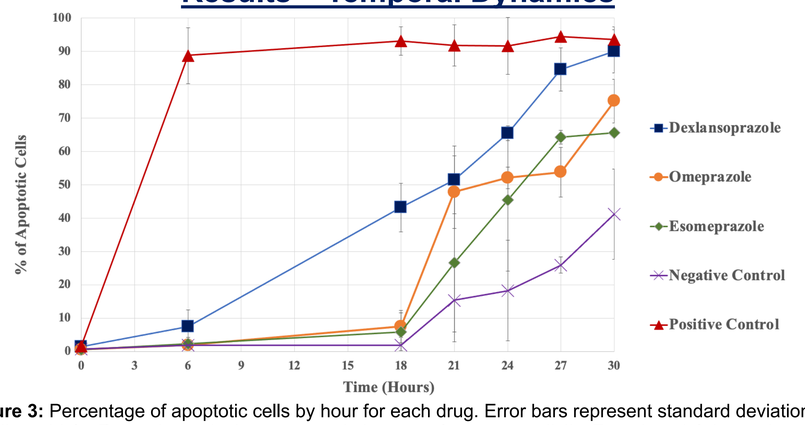

Figure 3: Percentage of apoptotic cells by hour for each drug. Error bars represent standard deviation for three trials. Percent apoptosis corresponds to general caspase activity. Experimental drugs show increased caspase activity over time, with dexlansoprazole showing comparable activity to the positive control drug, doxorubicin (100μM), at hours 27 and 30.

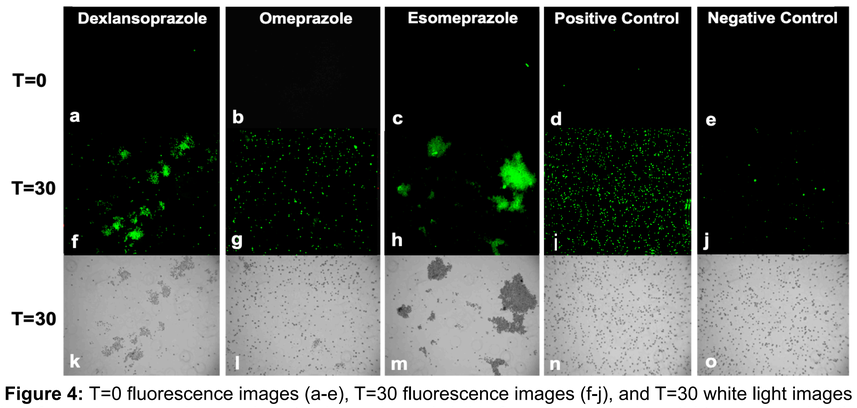

Figure 4: T=0 fluorescence images (a-e), T=30 fluorescence images (f-j), and T=30 white light images (k-o) for each sample. All experimental drugs showed visible fluorescence by hour thirty, compared to the negative control. Dexlansoprazole and esomeprazole showed a high degree of aggregation that was not observed with the other samples. Fluorescence images enhanced for clarity.

Results – Microfluidic Device Trials

Initial microfluidic device trials seeking to observe caspase activity were severely limited by low cell count. A study was performed to determine if PPI treatment reduced binding affinity.

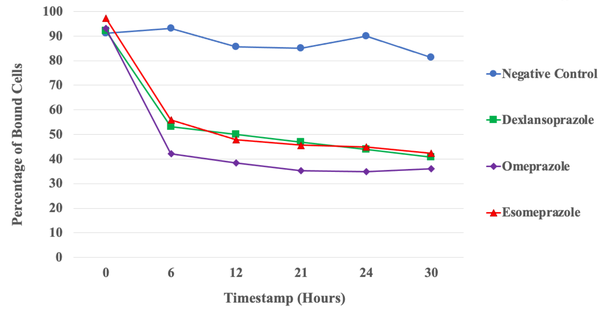

Figure 5: Percentage of bound cells for each drug by hour. PPI-treated cells all show a dramatically reduced binding affinity after the 6-hour incubation period.

Conclusions

All three experimental drugs were shown to induce general caspase activity in Jurkat cells within twenty-four hours of exposure, with dexlansoprazole exhibiting comparable apoptotic activity to doxorubicin at hour 27. Omeprazole and esomeprazole showed a slower onset, but still reached 60% apoptotic cells by hour 30. These results indicate that the tested PPIs induce late-onset apoptosis in Jurkat cells following treatment with 100mM of drug for 6 hours. Microfluidic device trials revealed that drug-treated cells bind to the affinity surface at a much lower percentage than the negative control cells, suggesting that PPIs alter the cellular surface in some capacity.

Future Work

Future studies may seek to:

- Verify cell death due to apoptosis via Annexin V staining
- Extend the window of observation beyond 30 hours
- Assess the apoptotic effects of other PPI drugs
- Characterize temporal dynamics of unique caspases
- Evaluate PPIs for their ability to induce caspase activity in other cancer cell lines
- Explain disruptions to surface receptors following exposure
- Identify a suitable antibody for microfluidic device trials

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Acknowledgements

We thank the Irene Piscopo Rodgers '59 and James D. Rodgers Student Research Fellowship I for the generous funding for this project. We also thank the UMW Department of Chemistry and Physics and the UMW Biology Department for supplies and resources. Special thank you to Shreya Murali (UMW '23) for assistance with data collection and processing.