A Study of Cycloheximide’s Effect on the Cell
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Abstract

In this study, the effect of the drug cycloheximide was studied on the cell. Four major techniques were used including fluorescence microscopy, viability assay, migration assay, and western blot. Cycloheximide was also tested using three different doses, .25, .5 and 1 mM, as experimental treatments. It was generally found that cycloheximide affects the overall health of the cell organelles; the mitochondria, lysosomes, and cytoskeleton appeared less healthy than their control counterparts, although the nucleus did not have visible differences when compared to the control. The viability assay found that cells treated with cycloheximide were less active, and the higher the dose, the less activity. The migration assay found that cycloheximide affects the cells ability to migrate, especially when the dose was higher. Lastly, the western blot was not entirely conclusive because levels of beta-actin were not equal. However, the results pointed to the possibility that a higher dose of the drug was correlated with decreased concentration of BCL-2. Overall, these findings support that cycloheximide can lead to apoptosis, programmed cell death.

Introduction

Description & Function

Cycloheximide is an inhibitor of protein synthesis in eukaryotic cells that plays a role during the translocation step of protein synthesis, often resulting in apoptosis. It has a molecular formula of C_{15}H_{23}NO_{4} and a molecular weight of 281.35 g/mol. It’s IUPAC name is 4-[(2R)-2-[(1S,3S,5S)-3,5-dimethyl-2-oxocyclohexyl]-2-hydroxyethyl]piperidine-2,6-dione, and is most commonly seen
physically as colorless crystals. It is mostly heat and acid stable, and noncorrosive, but when it is heated to decomposition, nitrogen oxides are emitted, which are toxic. It is relatively inexpensive and works quickly; however, because of toxic side effects it is most commonly used for in vitro research. It was previously also used as a fungicide in the field of agriculture but because of its toxicity, this use has been decreasing. It has also been used as an anti-cancer drug, antibiotic, antifungal, and protein synthesis inhibitor, as previously mentioned.

**Mechanism of Action**

The full mechanism of action has not yet been fully uncovered. However, previous studies have found that it works to inhibit translation elongation via binding to the E-site on the 60S ribosomal unit and also interfering with deacetylated tRNA. The exact binding site, however, remains unknown. The concentration at which it is generally used and found to be effective in cells is 5-50 µg/ml for 4-24 hours. It is believed that an oral dose of 5-50 mg/kg or approximately 1 teaspoon will be lethal for an average 150 pound person.

**Previous Studies**

In the study, Establishment of Herpes Simplex Virus Latency in vitro with Cycloheximide, Kimiyasu Shiraki and Fred Rapp from The Pennsylvania State University College of Medicine indicate how they used Cycloheximide in their research. They began by infecting human embryonic lung cells with HSV, herpes simplex virus. The lungs were then exposed to 10 micrograms /milliliter of Cycloheximide for 24 hours and kept at 37 degrees Celsius. The temperature was then shifted to 40.5 degrees Celsius for different amounts of time (anywhere from 0 to 40 days) and at this temperature no Cycloheximide was provided for treatment. The experiment continued for 40 days. When the temperature was lowered, some cells were superinfected with human cytomegalovirus, HCMV, while some were not. Once returned
to 40.5 degrees Celsius, two different results were seen. In some cells, the virus suddenly reactivated after the temperature was lowered, and in some cells the virus was reactivated after the HCMV was added.

In a second study, *Molecular mechanism investigation of cycloheximide-induced hepatocyte apoptosis in rat livers by morphological and microarray analysis*, by Kazumi Ito et. al, Cycloheximide was also used. Male rats were intravenously given 6 mg/kg of Cycloheximide. Microarray analysis was performed on their livers after subsequent treatment after 1 hour, 2 hours, and then 6 hours after the treatment. Staining showed that after 2 hours, there was the greatest number of apoptotic hepatocytes. At the one hour interval, mRNA levels of ATF3 and CHOP genes were both visible at a greatly increased amount; these two genes have previously been linked to the ER stress-mediated apoptosis pathway. These results and others indicate that Cycloheximide treatment leads to hepatocyte apoptosis when ER stress is present.

**Background of Fluorescence Microscopy**

Fluorescence microscopy imaging has been increasingly used in the last twenty years to examine cellular activity, the signaling between cells organelles, and to examine the regulation of organelles. This tool proved to be useful in the investigation of cycloheximide and how it affects cells. The different fluorescent dyes enabled the different components of the cell to be examined to better understand the drug (Dmitry et. al).

In this experiment, 3B-11 cells of a rat endothelial cell line were used. These endothelial cells came from the inner lining of blood vessels. These endothelial cells are beneficial for microscopy research, including drug research, because it is relatively easy to detect whether they are healthy or not. Healthy endothelial cells, because of their function in the cell, will stick to the bottom of the well and appear elongated (Alberts et. al).
The cells were exposed to cycloheximide, and then fluorescent dyes were used to examine the nucleus, lysosomes, mitochondria, plasma membrane, and cytoskeleton. DAPI dye was used to examine the nucleus because it stains the AT dense regions of DNA. Mito-tracker dye was used to examine the mitochondria of the 3B-11 cells. Mitochondria have a double membrane, and the dye interacts with the intermembrane area between the two membranes where the Kreb’s cycle of cellular respiration takes place. Lysotracker dye was used to detect the lysosomes of the cells. Lysosomes are very acidic because they break down different components of the cell that need to be recycled. The Lysotracker is able to cross the membrane and bind to the highly acidic organelles because of the fluorophore linked to a weak base that composes the dye. The last dye to be used was Phalloidin (in its modified version rhodamine) which binds to actin filaments, which enabled the observation of the cytoskeleton. It was expected that the four different stains would enable the observation of the specialized cell parts under the microscope. It was also expected that the cells may not all be healthy/normal because previous studies had found that cycloheximide often leads to apoptosis.

**Background of Viability Assay**

A viability assay was used to compare the cellular activity in the control cells, versus those treated with three different doses of cycloheximide. Viability assays can be very illuminating, especially ATP assays, which was used in this experiment. ATP assays are very sensitive and easier to use than some other types of viability assays. The luminescent signal, or “glo” reagent stabilizes within 10 minutes after it is added to the cells, and it typically glows with a half-life of over 5 hours (Riss). Because the “glo” reagent added to the cells is ATP-reactive, the fluorescence can be read by a plate reader, which will provide a measurement of the fluorescence in Relative Light Units. The higher the RLU, the greater presence of ATP,
indicating greater cellular activity. Because ATP is required for a multitude of cellular reactions, normal cells should be producing and using lots of ATP, so a higher RLU value would be expected with healthy cells. It was predicted that the viability assay would indicate more cellular activity (ATP) in the control cells, and increasing less in the experimental groups with a higher dose of drug.

**Background of Migration Assay**

Simultaneously with the viability assay, a migration assay was also performed to test cell mobility and migration. Cell migration occurs as a response to a chemical signal. It is a necessary function used in healthy functioning cells for wound repair, embryonic development, cell differentiation, and is also the basis of how tumors metastasize, although tumor metastasis is classified as cell invasion similar to migration. Cell invasion is also exhibited in cancer-free healthy cells as a response to inflammation ([Cell Migration, Chemotaxis and Invasion Assay Protocol](#)). Therefore, if cycloheximide decreases cell migration/invasion there would be an indication to research whether it could be used for cancer patients to decrease the metastasis of cancerous cells. In this experiment, it was predicted that the cells treated with cycloheximide would migrate at a slower rate, and would not migrate as much as the control cells.

**Background of Western Blotting and BCL-2**

The western blot is a procedure commonly used in cell biology to determine the concentration of a specific protein. Cells produce different proteins in response to their environment. The western blot requires the proteins to be isolated via washing and scraping them from the bottom of the flask. They then were placed in a hypotonic solution to lyse the cells, and once they are lysed the total protein concentration can be calculated with a spectrophotometer using a BCA assay. However, for this experiment, the specific protein of
interest was BCL-2. BCL-2 is a proto-oncogene. It is located on the inner mitochondrial membrane and is important for cell survival. Therefore to find the specific concentration of this protein, an antibody specific to BCL-2 was utilized to bind to the BCL-2. However, before the antibodies were used, protein electrophoresis was utilized to separate the proteins, and then the product of the protein electrophoresis was transferred from the polyacrylamide gel to a nitrocellulose membrane. Once the proteins have been transferred to the membrane, the membrane is incubated with the specific primary antibody and a secondary antibody which binds to the fc region of the primary antibody, and the subsequent chemical reaction can be captured by a camera for study. BCL-2 inhibits apoptosis. However, when it is phosphorylated, it is turned off and then apoptosis can proceed. Therefore, it was expected that because cycloheximide has been found to lead to apoptosis in other previous studies, the concentration of BCL-2 would be high in the control cells, and there would be a much lesser concentration of BCL-2 in the cells treated with cycloheximide.

**Materials and Methods**

**Part One – Fluorescence Microscopy**

The first procedure utilized was fluorescence microscopy. The cells first had to be stained with different trackers. Four different stains were used: DAPI to identify the AT-rich regions of DNA, lysotracker to identify pH in the lysosomes, mito-tracker to identify the mitochondria, and phalloidin (using its modified version rhodamine) to identify the actin filaments of the cytoskeleton. 8 different wells of 3B-11 cells already treated with cycloheximide were used. First the media was removed from the wells using a pipette. Once all of the media was removed, growth media mixed with the mito-tracker was added to well 1 and 7. Growth media mixed with lysotracker was added to wells 4 and 6, and growth media mixed with phalloidin was added to
well 3. After the growth media and stain was added to the specific wells, they were incubated for 20 minutes. The wells were then washed twice with PBS paraformaldehyde, each time quickly removing the media. After the PBS, 0.1% Triton X-100 was added to all of the wells (except those treated with phalloidin), to make the cells permeable. The wells were then all washed with PBS. Stains were then added to the 7 experimental wells so that well once contained mito-tracker, well 2 contained phalloidin, well 3 contained DAPI, well 4 contained lysotracker, well 5 contained DAPI and phalloidin, well 6 contained DAPI and lysotracker, well 7 contained DAPI and mito-tracker, and well 8 remained the untreated control with no stains added. All of the wells were incubated for 20 minutes; media was then removed, washed with PBS twice. The wells were then removed from the slide, 50% glycerol in PBS was then added to the slide, and then a cover slip was placed on top.

**Part Two – Cell Culture and Counting**

The cell culture and staining portion of this experiment relied heavily upon sterile techniques so that contamination would be prevented. Two flasks were used; flask one has 200,000 cells and flask two had 100,000 cells. A sterile pipette was first used to remove the media from both flasks. Then PBS was added to both flasks, both flasks were then gently rocked and then the PBS was removed. Trypsin was then added to the flask. The flasks were then again rocked gently so that the cells detached from the bottom of the flask. Again, under the hood, RPMI AND 10%FBS was added. The cells were then suspended by pipetting up and down. A sample of each tube was then removed and placed into two clean microcentrifuge tubes, with trypan blue dye added to both tubes. The mixtures were then pipetted up and down to re-suspend the cells, and then tube 1 was pipetted into a hemocytometer, and tube 2 was pipetted into a second hemocytometer. The cells were then counted using the four 4x4 squares in either
hemocytometer. It was calculated that 200 microliters would be required to get 20,000 cells, so 200 microliters were added from the two combined flasks to a 6-well plate, and the remainder from the combined flasks was then placed in a T-25 flask. The wells and flask were then left to incubate.

**Part Three – Migration Assay and Viability Assay**

Three tubes were gathered with different doses of cycloheximide (.25, 5, and 2mM). Under the hood the growth media was aspirated from the four wells, growth media and drug was added to the experimental wells with the three different doses, and growth media without the drug was added to the control. Three scratches were then made down the wells using a 1, 10, and 200 microliter pipette tip.

The following day, the migration and viability assays were completed. For the viability assay, “glo” reagent was added to each of the 12 wells (3 wells for each dose, and 3 wells for the control). For the migration assay, the previously scratched wells were utilized. First, paraformaldehyde was removed, then PBS was added, then DAPI. The wells were incubated at room temperature for 10-15 minutes, then a microscope was used to made observations, and imaging (including Image J) was used to observe the migration assay and the fluorescence from the viability assay and calculate the RLUS.

**Part Four – Western Blot**

The western blot portion of this study was completed over a series of 4 days. The first day required the cell culture to be prepared. The media was first aspirated from the conical tube. DMEM was added to the tube and then the pellet was broken up. Cell suspension was added to each petri dish and then drugged media (in the three experimental dishes for each dose) or
control media (only for the control dish) was added to each dish. The cells were left to incubate at 37 degrees overnight.

On the second day, the protein was harvested and the BCA assay was prepared. Four tubes were gathered for the four petri dishes. The media was first removed from the cells and then washed twice with PBS. After all of the PBS was removed, M-PER with protease and phosphatase inhibitors was added to each dish. Each of the dishes were scraped and then transferred to their respective tubes. The contents of each tube were pipetted up and down several times, and then the four tubes were left to be incubated on ice for 35 minutes. The four tubes were then spun at 4 degrees Celsius for 10 minutes at 12,000 rpm. The supernatant was then added from each tube to 4 new tubes, and the pellet was discarded. BCA was then used; material from each tube was pipetted into a 96 well microplate, and a spectrophotometer was used to measure the absorbance. Each of the 4 protein samples (control, dose 1, 2, and 3) were then added to a well in row D, and working reagent was added to each. The plate was covered and left to incubate, and then the protein concentration for each was read.

On the third day, the gel was run and then transferred to a membrane. Lysate, MPER, and loading dye were mixed together using calculated amount of MPER and protein lysate depending on the BCA protein concentrations to get 20 microliters. 8 tubes were prepared all together (2 for each treatment/control). The tubes were then heated at 70 degrees Celsius for 10 minutes. The gel was then loaded with a sample from each of the 8 tubes, and a ladder, and was run at 200 V for 45 minutes. After the gel was run, the protein was transferred to a membrane. Nitrocellulose membrane and 4 pieces of filter paper were soaked in transfer buffer (methanol). The gel was then removed from the cassette. A gel sandwich was made with two pieces of filter paper on the bottom, then membrane, and the gel on top, and then two more pieces of soaked filter paper on
top of that. This enabled the proteins to flow toward the positive side at the bottom of the sandwich and get trapped in the pores of the membrane. The “sandwich” was then covered with a lid and run at 30 V for over an hour. After this time, the membrane was removed from the layers of filter paper and gel and placed in non-fat milk.

On the fourth day, imaging was performed. The membranes (previously cut into two) were placed into a box with TBST. Three washes of TBST buffer (each for 5 minutes) was performed. The secondary antibody (1:2000 dilution in 5% milk) was added to the box after the TBST was removed and left to incubate for 45 minutes. Again 3 washes with TBST were performed after secondary antibody was removed. Chemiluminescence was prepared from peroxide solution and luminol solution. The membranes were placed on plastic wrap and the Chemiluminescence was poured on top of the membranes, and rocked for a few minutes. Plastic wrap was then placed on top to keep the membranes moist. The membranes were then imaged so that the banding patterns could be observed.

**Results**

**Fluorescence Microscopy**

Fluorescence microscopy was used to observe the organelles of the control and treated cells. Figure 1 shown below compares the control cells with the two treatments of mito-tracker. Mito-tracker was used by itself, as shown in image B to illuminate the mitochondria of the treated cells, and also along with DAPI in image C to compare the mitochondria and DNA of the treated cells.
As shown in figure 1, the staining was successful therefore the cells are living. Image A showed the cells that were not treated with the drug. They appear relatively healthy, and although they were not stained with DAPI, DAPI is still present. In image B, there were a number of brightly fluorescent cells that were clumped together suggesting a number of unhealthy cells. Image C indicates that in the treated cells, the mitochondria and DNA are present together, although there is visible clumping again indicating the cells are not entirely healthy.

Figure 2, below presents the cells that were treated with phallotoxin to illuminate the cytoskeleton, in comparison with the control cells.
Above, in Figure 2, image A again serves to represent the healthy control cells. Images B shows the actin filaments of the cytoskeleton illuminated. A number of cells appear healthy because of how they appear squashed and stuck to the bottom of the well. However, there are also a number of cells that still look circular, and because they did not stick to the bottom of the well, they are most likely unhealthy. Image C indicates that there is much more DNA present (blue) than actin filaments (green) so although most of the cells with the drug treatment have DNA, not all have actin filaments, or at least they weren’t all indicated by the stain.

Below, in Figure 3, the cells that were treated with lysotracker, to illuminate the lysosomes of the cell are presented. The cells treated with lysotracker only (image B) show the organelles very dimly, indicating either that the staining was not entirely successful, or that the lysosomes are not entirely healthy. In image C, it is interesting to note that the lysosomes are visible, but the DNA is nowhere to be found.

The final stain used was DAPI, which was used to show the nucleus of the cells, because it binds to the DNA, particularly to the regions of DNA that are AT rich. Compared the other organelles previously mentioned, the DNA in the treated cells did not look particularly unhealthy. The fluorescence in images B and C are rather bright, there doesn’t appear to be much
clumping, and there is lots of DNA present.

*Figure 4 – Wells Treated with DAPI to illuminate the DNA*

Image A is of the cells that were not treated with cycloheximide, or stain, although some DAPI is still visible. Image B depicts the cells treated only with DAPI, so the DNA is illuminated. Image C depicts the cells treated with mito-tracker and DAPI (already pictured in figure 1) so the DNA is indicated by the blue fluorescence and the mitochondria are indicated by the red. The DAPI fluorescence channel was used for image A, B, and C, and the TRITC channel was also utilized in image C.

**Viability Assay**

A viability assay was performed to observe the effect cycloheximide has on cells in different doses. In particular, the viability assay aimed to observe the amount of cellular activity, measured by the amount of ATP in the cells. Below, figure 1 summarizes the data gathered from the viability assay.

*Figure 5 – Data from Viability Assay*
Legend: Figure A indicates the 12 wells used for the viability assay. The first row of three wells had no drug added, the second row was treated with a dose of .25mM, the third row was treated with a dose of .5mM, and the fourth row was treated with a dose of 1mM. Figure B is a graph of the average RLU values for each treatment (control, dose 1, etc.) plotted against the dosage. Figure C is a graph of the gold of control, and was calculated by dividing the average control RLU by all four average RLUs calculated in figure B.

In part A of figure five it appears that the control cells in row one are the brightest. Below in row 2, the cells treated with dose 1 are also bright but slightly dimmer. In row three, the cells treated with dose 2 are visibly less bright in comparison to the untreated control cells and those treated with dose 1. Lastly, in row 4, the cells treated with dose 3, the highest dose, the cells are similar in brightness to the cells treated with dose 2. From this image, there is a visible trend that as a greater dose of cycloheximide was added, the cells became less bright, which indicates less cellular activity.

In part B of figure five above, the Relative Light Units for each well was calculated. The average RLU was calculated for each treatment (control, dose 1, dose 2, dose 3) and plotted against the dose of cycloheximide previously added to the cells. The graph reflects the observations made in part A. The control cells, with no drug added were the most bright and had the highest average RLUs, 18051.7. The cells treated with dose 2, 0.25mM, were slightly less bright with an average of 16140.4 RLU. The cells treated with dose 3, 0.5mM, were visibly much less bright and had an average of 12512.8 RLU. Lastly, the cells treated with dose 3, 1.0 mM, had an average of 10686.6 RLU. The standard deviations were also calculated, indicated by the error bars located on all 4 points on the graph. The greatest standard deviation was for the control group with 1734.2, next were the cells treated with dose 1 and had a standard deviation of 1432.8. The next highest standard deviation was for the cells treated with dose 3 (1205.5), and the smallest deviation was for the cells treated with dose 2 (802.8).
Lastly, a fold of change graph was made for the viability assay data, and is shown in part C of figure five. The fold of change graph followed the same descending trend that was visible in part B. The fold of change was greatest, for the control, then with dose 1, dose 2, and was the smallest for dose 3. This graph indicates how much the RLU$s changed from an initial to final value.

**Migration Assay**

In addition with the viability assay, a migration assay was also performed. The same treatments were used, no drug for the control group, and then .25, 5, and 1 mM of cycloheximide for the three experimental treatments. The cells were allowed to incubate for a period of time until they covered the bottom of their respective wells, then 3 different sized pipette tips were used to make scratches through the wells. Below is figure six, which depicts the amount of cell migration after the scratches were made.

*Figure 6 – Brightfield Images of Migration Assay*

Legend: Scratches were made July 21, 2015. The migration assay was observed one day later, on July 22, 2015. Figure A shows the cells that were not treated with any drug. Figure B shows the cells that were treated with .25
mM, figure B shows the cells that were treated with .5 mM, and figure D shows the cells that were treated with 1 mM of cycloheximide.

A-D in figure six each show one of the scratches made for that particular treatment. Part A shows that after a day cell migration was nearly 100%. There bottom of the well is not visible where the scrape once was, the only slight indication that a scrape was once made is the slightly different pattern the cells made to fill in the space. In figure B there is still a region of space where the bottom of the well is visible, where the scrape was made. However, it is evident that some new cells have begun to migrate into the area of the scrape, perhaps about 40% migration. In part C, there is not much cell migration at all, approximately 10%. The scrape is still very prominent. Lastly, in part D there is also barely any migration, perhaps only 5% migration.

**Western Blot**

As indicated above in previous sections, a western blot was performed to find the concentration of protein in question, BCL-2. Below, in figure 7 is an image after the gel was run. On the left are the very dim results of the BCL-2, and on the right are the much more visible bands of beta-actin.

*Figure 7 – Results of Protein Electrophoresis*

Left: BCL-2. Right: Beta-actin

These results show that Beta-actin was much more present, in a higher concentration than BCL-2.

The relative amount of BCL-2 protein and Beta-actin protein was also calculated for each of the four treatments (control, dose 1, 2, and 3). Below, in figure 8 is a graph depicting these values.
The values depicted in figure 8 reflect the observations made in figure 7. Beta-actin was present in a greater concentration compared with BCL-2.

**Discussion**

**Fluorescence Microscopy**

Fluorescence microscopy is an important tool for observing cellular activity and organelles. The purpose of this experiment was to observe the 8 different wells of cells treated with cycloheximide under the microscope. It was expected that the cell staining would be successful so that when observed under the microscope, the different parts of the cell would be illuminated, depending on which stain(s) were added, and it was also expected that some of the cells would be unhealthy because of the ability of cycloheximide to inhibit protein synthesis in eukaryotic cells, during the translocation step of protein synthesis.

The eight different wells indicated a range of healthy and unhealthy cells. It has been shown that the presence of the drug cycloheximide often results in eventual cell apoptosis. Therefore, because of the number of cells that were living in the eight different wells, it is possible that they were at different stages of development or perhaps the drug needs a certain number of hours/days before it begins to work full force on the cells.
The healthy cells often appeared elongated and adhered to the bottom of the well, while the unhealthy cells appeared circular and clumped together. Using fluorescent microscopy, it was apparent that the staining worked in all of the wells. In all seven wells, excluding the control well, there was a mixture of healthy and also unhealthy cells. In well 3, figure 4, it was interesting to note that the AT regions of the nucleus were more bright. Also interesting to note is that in well 4, figure 5, the lysosomes only appeared to be present in adhering, healthy cells. In well 8, the control, DAPI still appeared, even though none was intentionally added.

As in any experiment, there is always the potential for error. In this lab, it is possible that the staining protocol was not performed correctly. There were a number of steps, and a number of different dyes to be used. Therefore, this would most likely be the root of any error in this experiment.

**Viability Assay**

The results of the viability assay indicated that cellular activity declined as a greater dose of cycloheximide was used to treat the cells. Because a brighter glow indicated more ATP, the control cells that were brightest with greater fluorescence were most likely functioning more normally because more ATP indicates more cellular activity, although it could also indicate more cells. The experimental cells treated with different doses of cycloheximide were less bright, most likely indicating less cellular activity, or perhaps less cells indicating that the treatments of cycloheximide lead to cell death. Because of the values of the RLUs and fold of change, there seems to be evidence supporting that cells treated with cycloheximide produce and use less ATP. However, because there are two possible explanations for this finding (less cells or less cellular activity) it is possible that cycloheximide decreases cellular activity, or that cycloheximide leads
to cell death. Because cycloheximide is an inhibitor of protein synthesis, both of these explanations could be plausible.

Another interesting note to make about the data, returning to figure one, is that the standard deviation was highest for the two treatments that were brightest, the control and dose 1, and the standard deviation was much lower for the two less bright treatments, dose 2 and 3. A lower standard deviation indicates more confidence with the data so perhaps this is an indication of some possible experimental error. As in any experiment, there is always the possibility of experimental errors. For the viability assay, it is possible that due to human error, the incorrect dose was added to the wrong treatment.

**Migration Assay**

Figure two, the brightfield images of the cells migration is another illuminating observation of cycloheximide at work. It was a general trend that the greater the dose of cycloheximide, the less migration, or the slower the rate of migration. Because all of the cells were observed after one day, it remains unknown whether 100% migration would have been achieved in all four wells, given enough time. Cell migration is an integral part of healthy cell functioning, leading to a healthy multicellular organism. Cell migration is involved in wound healing, embryonic development, and more, so if cells aren’t able to migrate properly to specific locations, the organism is at risk. The treatment of cycloheximide seemed to prohibit, or greatly slow down cell migration which could suggest a number of properties of cycloheximide, one of which could be that cycloheximide inhibits a particular chemical or mechanical signal, but more experimental procedures would need to be done to support this theory.
For the migration assay, it is very possible that there were some incomplete and partial scrapes, but this error was very difficult to completely avoid. In cell biology experiments, there is also always the concern for contamination, so sterile techniques were closely followed, but it is still possible that contamination was a source of error.

**Western Blot**

The specific protein in question was BCL-2. Beta-actin was used as a control to ensure that the proteins were loaded correctly. In order for any assumptions to be made about BCL-2, the concentration of Beta-action should have been constant in all of the lanes. If BCL-2 was elevated it could have turned into an oncogene. However, because the beta-actin bands were not exactly consistent (indicated best by figure eight), it is more difficult to conclude that the differences in the BCL-2 bands is because of different doses of drug. Instead, because the bands of Beta-actin were not entirely consistent, it is possible that there may have been errors in loading samples into the dye.

**Overarching Conclusions**

At the commencement of this study it was previously known that cycloheximide is an inhibitor of protein, often leading to apoptosis. Because of this, it was predicted that in general, the treatment of cycloheximide would lead to less healthy and less active cells, and this was generally found to be true. It was a general trend that the treatment of cycloheximide resulted in less healthy organelles, less viable cells, their migration was decreased, and the concentration of BCL-2 was decreased. Therefore, the hypotheses stated in the introduction were supported. Moving forward, it would be interesting and beneficial to perform a similar experiment with the
same drug, using slightly higher doses, and it would also be beneficial to perform the western
blot procedure again because the findings from that portion were not entirely conclusive.

References

Science; 2002. Blood Vessels and Endothelial Cells. Available from:
http://www.ncbi.nlm.nih.gov/books/NBK26848/

Cell Migration, Chemotaxis and Invasion Assay Protocol. New York: Corning Inc. (accessed
Aug. 6, 2015). Retrieved from
http://www.level.com.tw/html/ezcatfiles/vipweb20/img/img/34961/2-
an_Chemotaxis_protocol.pdf

Cycloheximide. National Center for Biotechnology Information. PubChem Compound Database;

http://www.cellsignal.com/product/productDetail.jsp?productId=2112

Dmitry B. Zorov, Evgeny Kobrinsky, Magdalena Juhaszova, Steven J. Sollott (2004). Examining
Intracellular Organelle Function Using Fluorescent Probes. Circulation Research, 95(1), 239-252. DOI:
10.1161/01.RES.0000137875.42385.8e
http://circres.ahajournals.org/search?author1=Dmitry+B.+Zorov&sortspec=date&submit=Submit

Ito, Kazumi (2006). Molecular mechanism investigation of cycloheximide-induced hepatocyte apoptosis
in rat livers by morphological and microarray analysis. Toxicology (Amsterdam), 219(1), 175 -
186. (ISSN: 0300-483X)

Loukovaara, Mikko. (1995). Regulation of sex hormone-binding globulin secretion and gene expression
by cycloheximide in vitro. The Journal of steroid biochemistry and molecular biology, 54(3), 141-
146. (ISSN: 0960-0760)

Riss TL, Moravec RA, Niles ALet al., authors; Minor L, editor. Cell Viability Assays. 2013 May
Guidance Manual [Internet]. Bethesda (MD): Eli Lilly & Company and the National Center for
Advancing Translational Sciences; 2004-. Available from:
http://www.ncbi.nlm.nih.gov/books/NBK144065/

Inhibition of Eukaryotic Translation Elongation by Cycloheximide and Lactimidomycin. Nature
Chemical Biology, 6(3), 209–217. doi:10.1038/nchembio.304