The Effect of Cytochalasin B on Average Pseudopodia Growth Rate in Amoeba

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Introduction:

In this study, I tested the hypothesis that Cytochalasin B would cause pseudopodia growth rate to decrease in Amoeba. Growth rate is measured by pseudopodia growth in micrometers per second. The affect on pseudopodia growth rate is important to study because pseudopodia are formed from actin filaments. Actin is a key protein in cell motility. White blood cells and other immune system cells must use actin to move through tissues to the sites of infection. Cell motility is also important when studying bacteria and other single celled organisms like *Amoeba proteus*.

Pseudopodia are used in phagocytosis and amoeba movement (Cooper, Hausman, 2007). They are formed from extending actin bundles. Actin bundles are formed from actin filaments that have been cross-linked together by actin bundling proteins (Cooper, Hausman, 2007). Two types of actin bundling proteins are fimbrin and alpha-actinin (Cooper, Hausman, 2007). In cells actin filaments are constantly being assembled and disassembled as the cell changes shape (Doera et al. 2008). Actin filaments form from actin monomers called globular (G) actin (Cooper, Hausman, 2007). Before G actin associates to form filaments they bind ATP, which increases their affinity for the actin filaments. Actin monomers add to filaments at the barbed end and dissociate from the pointed end (Cooper, Hausman, 2007). Once monomers are within the filament the ATP is hydrolyzed into ADP (Cooper, Hausman, 2007). Dissociation occurs at the pointed end because the monomers are bound to ADP, which does not increase its affinity for one another (Cooper, Hausman, 2007).

Cytochalasins are a class of drug that inhibits actin filament formation. It inhibits actin polymerization by binding on the barbed end of filaments (Cooper, Hausman, 2007). By binding at the barbed end G actin cannot bind to the filament to elongate them. To pull the Cytochalasin into cells it is dissolved in a solvent. Dimethyl Sulfoxide (DMSO) is used as a solvent for drugs because it is hydropathic. This means DMSO can carry its solute through cell membranes, which without the solvent would have been unable to penetrate the membrane ((Murata et al. 2003).

In this study, we exposed one slide of Amoeba to Cytochalasin B and another slide with pond water. Amoebas were then observed and photographed every fifteen seconds to determine the growth rate of pseudopodia. Amoebas were chosen because they can exceed 1mm in length. Their larger size makes it easier to measure length of pseudopodia. Since cytochalasin inhibits actin filament formation, pseudopodia are a good indicator for how the drug affects Amoeba.

Materials and Methods:

This experiment was conducted over a 3-hour period. Two trials were completed, each trial extended over a 2 minute period. For each trial one Amoeba was observed from the Control slide and the Cytochalasin slide. Slides were kept at room temperature and pond water was added as slides began to dry out. Slides were made by first, placing two slides on a large Petri dish. One slide was labeled A, for Cytochalasin and the second B for the Control. Then glass chips were placed and arranged on each slide using forceps. A drop of pond liquid containing 2-3 Amoebas was placed between the glass chips. A cover slip was then placed on top of the chips, and two parallel sides were sealed using VALAP. Premixed VALAP was placed on moderate heat to melt it.

All images were collected using a Digital interface Sony camera connected to Nikon Eclipse E200 microscope connected to Apple computers in the ICUC. When dealing with the Cytochalasin DMSO mixture gloves were used due to the membrane permeability of the solvent. Using a plastic disposable pipette 3 applications of either Cytochalasin or pond water was added to each corresponding slide so that the 150microliters of liquid in the flow chamber was replaced. The liquid was dispensed on one side of the slipcover, a Kim wipe was placed on the other side to pull the drops under the slipcover.

The slides were set-aside for 30 minutes, so Amoebas would have enough time to take in the solvents. The slide with the drug was observed first at 10x magnification and Kohler illumination. Using the program BTV a photo of the Amoeba was taken every 15 seconds for two minutes. The time interval was measured using a stopwatch. This procedure was then repeated for the Control and then again for both slides.

Using the program Adobe Photoshop the first image (time zero) was set at the background. The image was manipulated using gradient to turn it blue. A second layer was placed and the second image at time 15 seconds was placed on top. The opacity of the image was changed to 62% and the image was moved so the main body of the Amoeba overlapped. This step was repeated for each image in the 2-minute sequence, the background was always the time zero image. This process was repeated for each trial. Then for each trial the layers were flattened against the background, so 8 images were collected from the original 9 in each trial. The program Image J was then used to figure out difference between the main pseudopodium in the 2 overlapped images. Pseudopodia were defined as extensions of the Amoeba that were less wide then the main body of the Amoeba that changed in length as time went on. The length was found in number of pixels in the line from the tip of the pseudopodium in time zero to the tip of the pseudopodia at time 2. An image of this can be found in figure 2 at the end of the paper. This was done for each image using the same pseudopodium. This procedure was done for each trial. The data was analyzed using Excel

The amount of pixels at 10x in 100micrometers was found by taking a picture of a scale bar of 0.1mm. In Adobe Photoshop the distance marked in the scale bar for 100micrometers was copied and a new document was opened. The new document shows the number of pixels. In excel a table was made for the control and another for the drug. The difference in length was found by subtracting the previous image length from the current image. This gave the growth in pixels per 15 seconds, using the pixel number per 100micrometers the length of growth per 15 seconds was found in micrometers. This was then divided by 15 to find the rate of growth in micrometers per second. The average rate was calculated from both trails done. Once this procedure was completed for both the control and the drug a bar graph was made using the average rate of growth.

Results:

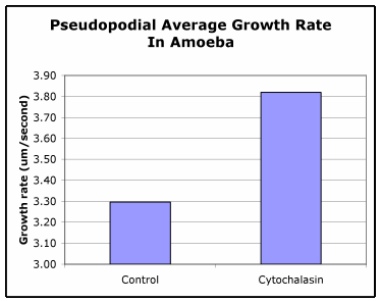

Figure: The average growth rate for the control and Cytochalasin was found using a sample size of 16.

Figure 1 shows the average growth rate of pseudopodium in Amoeba. The control was found to change in length by 3.30µm /second. The Amoebas exposed to Cytochalasin grew at a rate of 3.82µm/second. While Amoeba treated with Cytochalasin B were found to have a higher pseudopodial growth rate the data points ranged from 0.36µm /second to 11.74µm /second. The control had less variability ranging from 0.11µm /second to 6.53µm /second.

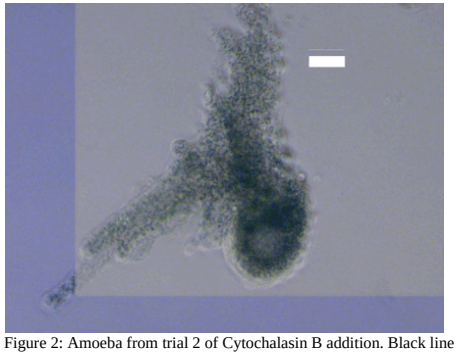

Figure 2: Amoeba from trial 2 of Cytochalasin B addition. Black line represents how measurements were taken using Image J. Scale bar is equal to 167pixles to 100µm.

Discussion:

The data collected did not support the hypothesis that pseudopodial formation rate would decrease with addition of Cytochalasin. The results show that Cytochalasin had a formation rate of 3.80µm/second and the Control had a formation rate of 3.20µm/second. These results would suggest that the growth rate is increased by Cytochalasin B. Since Cytochalasin blocks actin filament formation it would be assumed that pseudopodium would not continue to grow. In an experiment conducted by Brown and Spudich in 1979 similar results were found when high concentrations of Cytochalasin were added to purified Dictyostelium discoideum, actin polymerization was found to increase (Brown, Spudich, 1979). Cytochalasins have also been found to be chemo-attractants; when the drug is applied to the tips of cells lamellipodium extend toward the drug (Bereiter-Hahn, 2005). This find can help to explain why the pseudopodia grew with Cytochalasin present. While it could have been the drug that increased formation rate it is also possible the experiment was affected by other variables. An indication of this was seen by how much the amoeba pseudopodial growth rate varied with Cytochalasin. There was a difference of 11.48µm /second between the slowest and fastest rate. For the control amoeba there was only a difference of 6.42µm /second in pseudopodial growth rate. These results suggest something else could have affected the growth rate.

Many factors could have affected the outcome of this experiment. First the control did not have DMSO added, which could have affected Amoeba in the slide with the drug. Also because plastic pipettes were used to add the liquids to the flow chambers the exact amount could not be determined. This means the drug slide may have had a concentration that was two low to affect the Amoeba. Because only two trials were done and very few Amoebas were used, the results may not reflect the affect of the drug but that of the Amoeba. The measurements also could have been off because it was hard to determine where the tip of the pseudopodia was. It was also hard to determine where main body of the amoeba was, because all the surfaces of the amoeba changed within the 15-second period. All these factors could have significantly affected the results of the experiment.

In future experiments a more precise pipettement should be used to apply a more exact amount of each solution the flow chamber. DMSO should also be added to the control slide, to make sure it had no affect on the Amoebas. More trials and Amoeba should also be used if this experiment were to be rerun. A possible experiment for future study could be to add different concentrations of Cytocalasin and see how it affected the Amoeba. This would be especially interesting because of the finds by Brown and Spudich, which found that at higher concentrations actin polymerization increased. Another possible experiment would be to measure pseudopodia deformation rate, since Cytochalasin affects actin filament formation.

Amoebas treated with Cytochalasin B were found to have a higher average growth rate, 3.82µm /second, then Amoeba exposed only to pond water, 3.30µm /second. The average growth rates had wide deviation within them, which would suggest Cytochalasin might not have been the only variable at work.

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Introduction: