Evidence that the Drug Cytochalasin B Prevents the Generation of Pseudopodia in Amoebae

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Abstract

Introduction

In this experiment, I studied the effect of the drug cytochalasin B on the cellular behavior of the organism

Amoeba proteus. Cytochalasin B is a fungal toxin that has the ability to permeate cell walls and inhibit actin filament polymerization. The toxin can inhibit cell movement, stop glucose transportation, and prevent platelets from grouping. (Wikipedia, 2008) This information considered, the actin cortex plays a crucial role with the drug's interaction with the cell. Actin is made of G-actin monomers that form trimers that create a single strand of head to tail interactions known as filamentous actin. All of the monomers are oriented in the same direction and polymerization occurs at the barbed end and pointed end of the filament. The elongation of the barbed end occurs at a much faster rate than the pointed end because of the hydrolysis of Adenosine Triphosphate (ATP). The filament goes through a process called treadmilling, where the ADP actin snaps off of the pointed end and exchanges its ADP for ATP. Then the newly altered monomer adds itself on to the barbed end of the filament. Actin filaments often go on to construct actin bundles and actin networks. Bundles are cross-linked by special proteins and are essential to the plasma membrane structure, mechanical support, and movement of the cell. Networks, similarly, form three-dimensional frameworks that support the structure of the plasma membrane. (Cooper, 2007)

Cell movement is critical to the interaction and function of cells. Movement is caused by the extension of one part of the cell body away from its core. There are a couple of specific names for these extensions. Pseudopodia are moderately sized extensions that are formed by cross-linked actin filaments. Pseudopodia are responsible for both phagocytosis and amoeba locomotion. (Cooper, 2007) Lamellipodia are flatter, wider sheets of cellular extension containing a very similar actin network. Both extensions rely upon the polymerization and dissociation of actin filaments.(Cooper, 2007) Rapid polymerization in one direction causes the filaments to stretch the cell body in a specific way, allowing it to attach to other surfaces and release its attachments in the opposite direction. This process permits amoebae, single celled organisms themselves, to extend, attach, and retract their bodies to and from a substratum, resulting in a forward progressive movement. Amoebae use this locomotion to seek nutrients and healthier environments.

Because actin and actin filaments are essential to cell movement and structure, I wanted to observe the consequences of adding cytochalasin B to a cell to observe the effects on its locomotion. As previously noted, cytochalasin B caps off the barbed end of the actin filament, preventing it from being polymerized through the treadmilling effect. By using a single celled organism, Amoeba proteus, cellular behavior of a single cell and not a whole tissue could be easily assessed. In this study, I tested the hypothesis that fewer pseudopodia would be created by an amoeba if cytochalasin B was added to its environment. In this experiment, the motile behavior of an amoeba observed under controlled circumstances was compared to the behavior of an amoeba immersed in cytochalasin B.

Materials and Methods

Materials:

For this experiment, I needed access to a Nikon Eclipse E200 microscope, a Sony BTV camera and software, and an Apple Genesis computer. These electronic devices allowed me to view the amoebae at 100x magnification and capture images of its behavior over time. A supply of pipettes, amoebae, glass slides, cover slips, VALAP, brushes, Kimwipes, and cytochalasin B were needed for this study.

Methods:

To set up this experiment, I first had to create a chip chamber slide to put the amoeba on so that it was protected and it wouldn't get distorted by the pressure of the cover slip. This was done by crushing pieces of cover slip and placing them in several places on a clean slide. Amoebae were extracted from a collection glass under a microscope and put in the middle of the chip chamber slide. A clean cover slip was placed carefully on top. This slide was labeled Slide A. The process was repeated for a second slide called Slide B. Melted VALAP was painted along the top and bottom edges of the slide to create a flow chamber. The VALAP creates a pressurized chamber to which a new liquid can be added on one side and the older liquid can be removed from the other side using a Kimwipe. Both slide A and slide B were examined under a microscope under a 10x objective lens to assure one amoeba was in each slide. Then both amoebae were imaged under the BTV camera and pictures were taken at 100x magnification every five seconds for one minute. The purpose of this was to count the average number of pseudopodia created by the amoeba over one minute. For this study, a pseudopodium was defined as an extension away from the body that was at least 11 microns wide and 9.5 microns long. To accurately describe a pseudopodium, a horizontal line connecting the two sides of the body between the extension should be drawn with two parallel lines perpendicular to it running along the sides of the extension. The base is where the parallel lines meet the horizontal line perpendicularly.

After the controlled pseudopodia generated were averaged, slide A was flushed of its original water environment with 3 washes of cytochalasin B through the flow chamber. Three separate flushes were needed to completely exchange the fluid under the cover slip. The amoeba was found under the scope and observed for another minute, again, taking digital photographs using the BTV camera every five seconds. The number of pseudopodia was counted. Then the same procedure was done for slide A. The final number of pseudopodia was averaged for slide A and B. Slide A and B were examined again after 30 minutes of exposure to cytochalasin B and photos were taken in the same way to observe final effects of the drug on the organism.

Results

Because results were measured quantitatively by taking pictures of the amoeba every five seconds over one minute, the findings measured the number of pseudopodia per one minute's period of time. Figure 6 shows the average

number of pseudopodia generated over this interval for both the control and the cytochalasin B immersed amoebae.

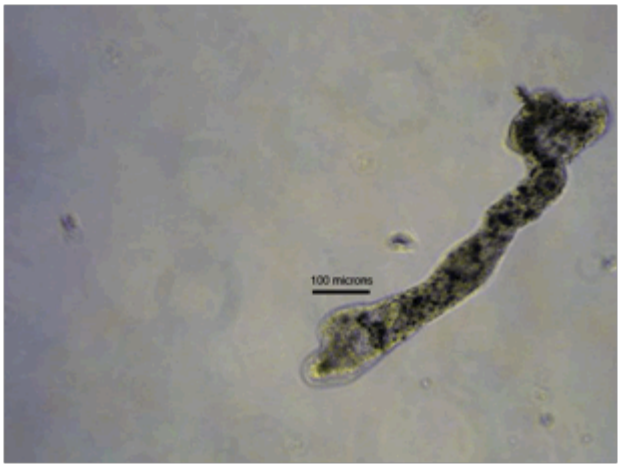

Figure 1: This figure shows the amoeba from slide A, the drug-free slide. The pseudopodia were counted and then subsequent pictures were taken, one every five seconds for a minute. Figure 2 shows the same amoeba after a one minute lapse. Picture was taken at 100x magnification (under the 10x objective lens) at 3:03 pm.

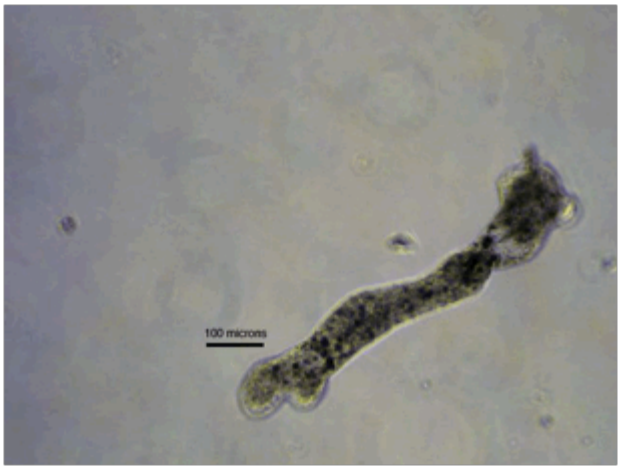

Figure 2: Picture of an amoeba from slide A, the control slide. This picture was taken at 3:04 pm at 100x total magnification.

The first figure shows that there are two distinct pseudopodia. Over a minute, two more are formed, and one disappeared (as seen in Figure 2). This makes the total number of pseudopodia generated over a minute: two. After the cytochalasin B was added, a qualitative change in behavior was noted. Not only did the amoeba cease to extend itself to the degree that it had before, it also slowed its creation of new pseudopodia. Figure 3 and 4 indicate this change in behavior.

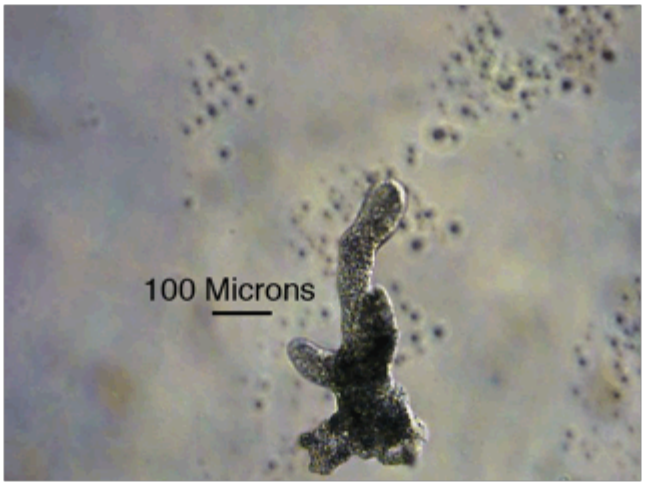

Figure 3: Amoeba in slide B after the cytochalasin B was initially added. There are 6 remarkable pseudopodia on this amoeba at this time, 3:50 pm. The amoeba is magnified by 100x.

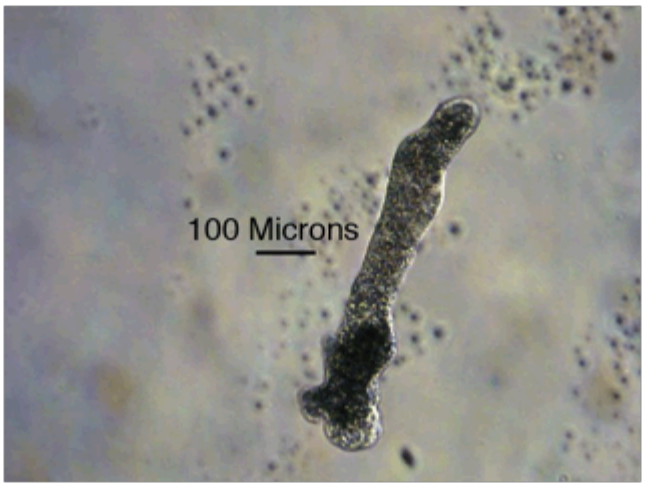

Figure 4: In this case, after one minute there are fewer pseudopodia present. The number of new pseudopodia, the variable in this case, is zero. Picture was taken at 3:51pm at 100x total magnification.

The amoeba was tested again after 30 minutes exposure to the drug and three complete flushes of cytochalasin B. It now resembled merely one circular amoeba, no longer producing any pseudopodia and its organelle streaming had slowed to a minimum.

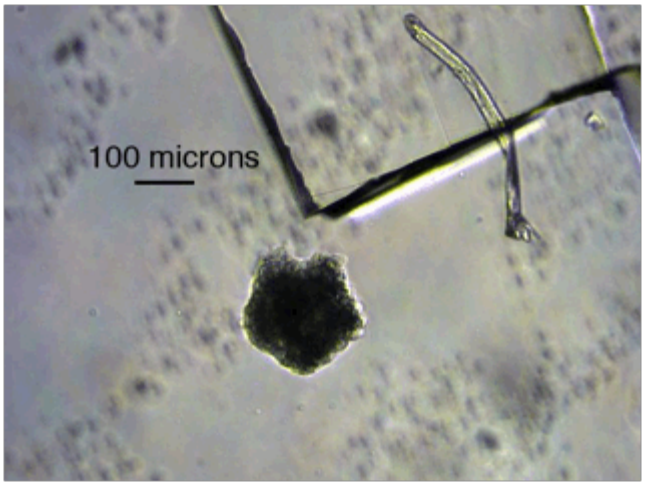

Figure 5: The amoeba, immersed in cytochalasin B, shows none of its former shape and produces no new pseudopodia after 30 minutes. The amoeba is 100x magnified.

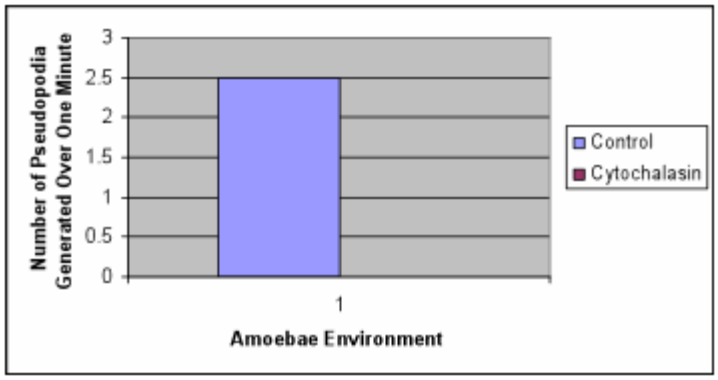

Figure 6: This graph shows the average number of pseudopodia generated by an amoeba in a given environment over one minute. The control amoebae generated about 2.5 pseudopodia per minute whereas the cytochalasin B amoebae created no new pseudopodia.

Discussion

Given the data above, my hypothesis, that Cytochalasin B would result in the creation of fewer pseudopodia, was supported. The amoeba, after 30 minutes exposed to the drug, ceased movement entirely except for some cellular streaming. The graph in Figure 6 shows the stark contrast between the control amoebae and the experimental amoebae. In the first slide, Slide A, there was no cytochalasin B added. The normal activity of an amoeba was noted as producing at least 2 new pseudopodia per minute. When cytochalasin B was added, the behavior and the number of pseudopodia created drastically changed. The amoeba went from producing an average of 2.5 pseudopodia per second to producing none. In a relevant experiment done by Kueh and Charras, a similar substance, Cytochalasin D, was used to observe the creation and destruction of actin filaments. The results showed that the cytochalasin family is a family of drugs that cap the barbed end of the actin filament, whereby ceasing the creation of new segments of actin. Cytochalasin D also served to inhibit the disassembly on the pointed end of an actin filament.(Kueh, Charras, 2008) This research was pertinent to

the results gathered above. The lack of pseudopodia generated can be explained by this information. The cytochalasin B stops the treadmilling effect of the actin that is essential for cell motility by capping the barbed end of the filament. Actin polymerization at the plus end of the filament extends pseudopodia. If there is no way for the G- actin trimers to add on to the barbed end, there will be no extensions of the cell body, therefore, there will be no pseudopodia. This supports the data collected from this experiment. Other groups in the lab carrying out the same experiment, or experiments very similar to this, achieved like results. In collaboration with Ashley Gabree, cytochalasin B had identical behavioral effects measured in similar quantities. The data that she collected supported the data collected here.

Potential sources of error for this experiment come from the DMSO that was used to dissolve the cytochalasin B in to administer to the amoebae. Because the solution was composed of DMSO and cytochalasin B, the effect of the DMSO on the organism cannot be separated from the wanted results from the cytochalasin B. To refine this experiment, a solution of solely DMSO should be used as a control in contrast to the pond water used in this experiment. Other sources of error could come from the health of the amoeba. Since they were older amoebae and put under harsh light and with little food, the source of this perceived new behavior could be due to age or health and not the addition of cytochalasin B.

In future experiments, fresher and healthier amoebae should be used. The experiment should also be repeated many more times for consistency of data. Another change would be to increase the time of observation to 5 or 10 minutes to get a better sense of how many pseudopodia are actually generated. Studies done at different concentrations of cytochalasin B could indicate other results. What is the threshold for actin polymerization in this environment? What if the length and rate of extension of pseudopodia was examined as well? These future experiments could well determine further effects of cytochalasin B on amoebae and their actin cortexes.

References

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