

# The Effects of Nocodazole on Amoeba Pseudopod Counts

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## Abstract

This experiment was conducted on November 19, 2008 on the Wheaton College campus at the ICUC lab in the Science Center with a Ms. Sara Houlihan. The study was executed to support the hypothesis that pseudopodia count will decrease in amoeba when treated with the drug nocodazole. After pooling the control results in comparison to our experimental value it was found that the average pseudopodia count started at 4 pseudopods and decreased to 1 pseudopod after the drug nocodazole was dispensed to our amoeba. This was only a single instance, and repeated measures would need to be conducted to increase the power of the results. Other microtubule polymerisation inhibiting drugs or anti-mitotic drugs such as vincristine and colcemid could also be tested in comparison to nocodazole to help support this hypothesis in further research.

**Introduction:** In this study we tested the hypothesis that the number of pseudopodia of an amoeba treated with the drug nocodazole would decrease due to its ability to depolymerise the microtubules of the cell. Microtubules are essential to cells. They constitute one of the intricate fibers of the cytoskeleton that supports the cell before reaching the plasma membrane. They are very active in motility, due to their ability to rapidly polymerize and depolymerise their protofilaments, which consist of 13 tubulin dimers. These tubulin dimers consist of  $\alpha$  and  $\beta$  subunits giving them respective plus or minus ends, which determines which way they traffic vesicles. Vesicular transport is an important role of microtubules for the Secretory pathway and cell trafficking, and they are necessary for cell division when creating the mitotic spindle.

Dynamic cell functions such as the ones carried out by microtubules can be observed easily using the administration of drugs, to see if there is an effect if any on the targeted aspect of the cell. Nocodazole is an anti-

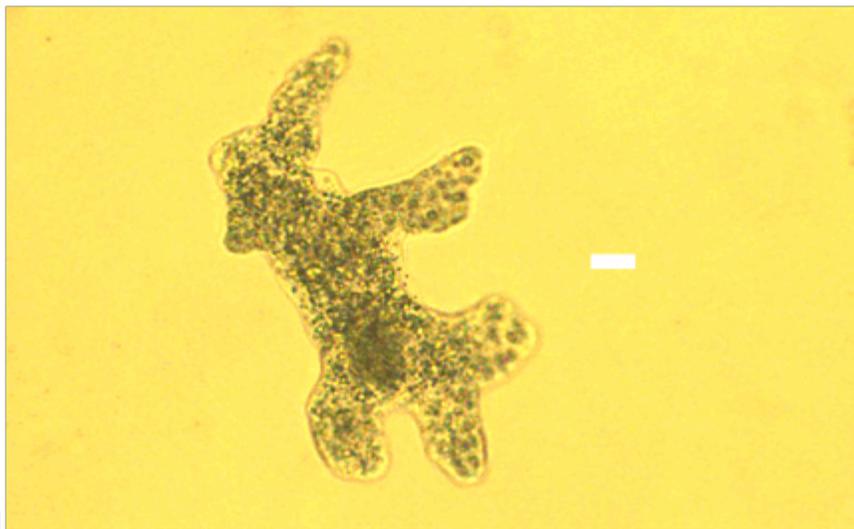
neoplastic agent that exerts its properties by depolymerising microtubules. Since microtubules are involved with the process of cell division, research related to targeting certain cells to stop dividing could be useful in respect to cancer treatments. There has already been research on nocodazole and its effects on lymphocytic leukemia cells, as well as other cancerous cells. This study however, focused on the effects of nocodazole on the pseudopodia count of amoebae.

Amoebae are members of the protozoa family and are classified into two different species amoeba dubia and amoeba proteus. They are large unicellular organisms that can range from 700 microns up to as large as a millimeter in size this makes them very easy to study using light microscopy. Amoebae have an exaggerated behavior when they use their microtubules for motility. The cell extends temporary projections called pseudopods or pseudopodia (Egmond, 2005). This is done by the reverse assembly of actin subunits into microfilaments, when a pseudopod stretches from the cell body the opposite end of the pseudopod contracts because it interacts with myosin. Pseudopodia are hard to classify since amoebae are very active and motile, for this experiment we concluded that a pseudopod must be at least 26 microns in length and 10 microns wide with two defined shoulders. A shoulder is defined by an angle that is greater than 45 degrees from the cell body. Pseudopods are recognizable usually by curved ended extensions that seem to be actively moving away from the cell body. Pseudopodia differ from filopodia, which are mostly clear ectoplasm material and lobopodia, which are short and blunt in formation. The parameter for what comprised a pseudopod was further developed due to a previous lab on November 12<sup>th</sup> that allowed us to become familiar with the amoebae and their characteristics. In this study we compared an amoeba that had been treated with nocodazole at a concentration of 2ug/ml via a wash through a flow cell to a control amoeba that was washed with water. The final number of pseudopods were measured after 30 minutes from the administration of the drug, and the cells behavior was observed throughout.

**Methods:** For this experiment we observed two amoebae, one to serve as the control, and the other the experimental subject. First two flow cell slides were created one for each of the amoeba. Flow cells allow the scientist to wash the cells that they are studying with drugs as well as allow oxygen to pass through 2 sides of the cover slip. These were made by preparing shards of cover slips by fragmenting them using a Kimwipe and placing them where the outer edge of the top cover slip will be placed. This places the top cover slip on a mount so oxygen may still pass through and drugs can be washed through the flow cell slide. Next the amoeba were placed in the middle of the square arrangement of chips and covered with a cover slip. By carefully using a pair of tweezers and placing an edge down first this reduces air bubbles that will interrupt viewing later on. When the top cover slip is placed on the cover slip chip arrangement the top and bottom sides of the cover slip are sealed with VALAP, which is a mixture of Vaseline, Lanolin

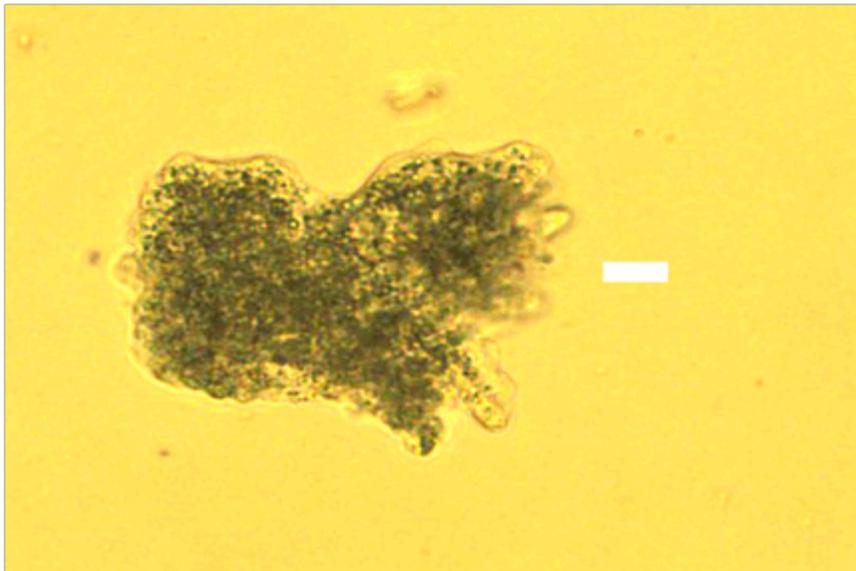
and Paraffin reduced to create a quickly sealing adhesive for aqueous specimens. Once this is complete for the control and experimental amoeba pictures were taken using the SPOT insight camera, and loaded into Adobe photo shop for the fitting of the scale bar that would constitute what a pseudopod was. We used an E200 Nikon Eclipse camera light microscope at the 10X objective, throughout the experiment. Once pictures were taken of the control and experimental amoeba we washed the cells over a period of 30 minutes 3 times at 10 minute intervals. The control was washed with water from its natural environment and was kept undisturbed, while the experimental specimen was treated with 2ug/ml of nocodazole. Washing an aqueous solution under the flow cell can be done by first applying an amount of the solution on one side of the cover slip and then gently touching the corner of a Kimwipe to the other side for a short period of time. After 30 minutes we took a picture of the experimental amoeba and compared it to the control and the experimental data that was obtained earlier (Gilpatrick, 2008).

**Results:** According to our description of what defined a pseudopod our control amoeba had 5 pseudopodia and our experimental amoeba before treatment had 3. The cells are very active on their own and were constantly stretching their pseudopods to move. Taking the average of the amoebae pseudopod count before any drug was administered on average we got 4 pseudopods. The experimental amoeba's pseudopod count decreased to 1 after 30 minutes of being exposed to nocodazole. The control picture below was taken at 3:20 p.m. (Figure 1.1) The picture of the experimental amoeba before drug administration was taken at 3:35p.m. The final image was taken at 4:12 of the amoeba that was washed with nocodazole. (Figure 1.2) Comparing these results we took the average of our control and experimental before drug treatment and looked at the differences of our final experimental pseudopod count. This bar graph is shown below as figure 1.3.



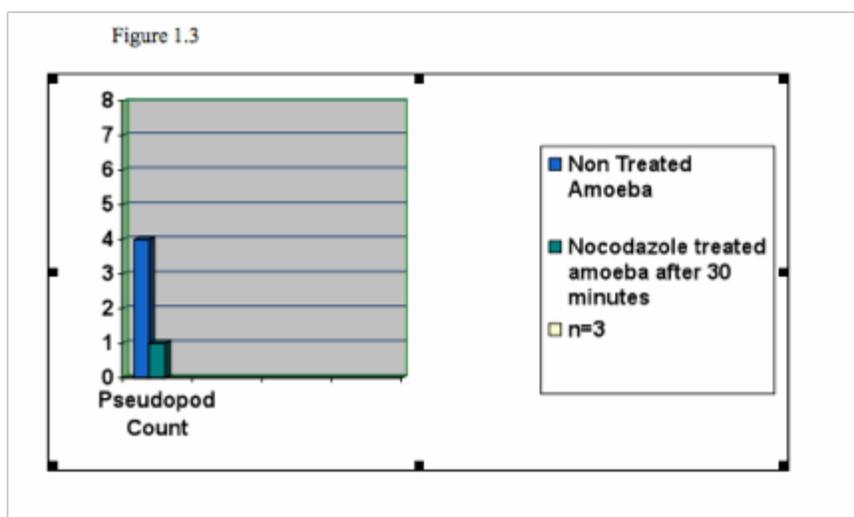
**Figure 1.1**

Control Amoeba scale bar = 10 microns wide 26 microns long



**Figure 1.2**

Experimental Amoeba after 30 minutes of Nocodazole treatment scale same as previous figure.



**Figure 1.3**

Figure 1.3 depicts a bar graph showing the average number of pseudopodia before treatment and the final experimental pseudopod count of one. N=3

**Discussion:** The results we obtained support our hypothesis that nocodazole treated amoebae will show a decrease in the number of pseudopodia on a cell. This makes sense because for a cell to be motile it needs to be able to utilize the filaments of its cytoskeleton or flagellum. Microtubules need to have GTP at the plus and minus end for the subunits to form protofilaments. When the  $\beta$ -tubulin undergoes addition it is exposed at the plus end and makes contact with the  $\alpha$ -tubulin, this causes the hydrolysis of GTP. The  $\alpha$  or minus end can serve as a GAP (GTPase activating protein) for  $\beta$  tubulin of neighboring dimers in a protofilament. Microtubules, just like actin use treadmilling to efficiently build and depolymerise their protofilaments (The Cell, 2007). When observing the cell it seemed as if the cell was trying to

extend pseudopods yet could not and would contract back to the cell body. Eventually the cell became more compact and contracted than it had originally been as well as compared to the control amoeba.

Two studies that highlight the direct chemical pathways that nocodazole effects when it is administered to different structures in the cell help explain how nocodazole works. The first was a study done in 1997 that looked at the effects of nocodazole-treated fibroblasts and Golgi dispersion and describes it as a kinesin driven process. (Minin, 1997.) This experiment treated cells with nocodazole and concluded that the depolymerization of microtubules in cells by nocodazole at concentrations ranging from 1 mM to 10 mM leads to the dispersal of Golgi material throughout the cytoplasm. The study also found that there are microtubules that are more stable and can withstand higher doses of nocodazole near the centrioles of the cell as opposed to microtubules in the ECM. The study also suggested that nocodazole blocks the building process of microtubules early on in the construction of a microtubule.

The other study looked at nocodazole induced myoblasts and myotubes (Musa, 2006.) Myoblasts minus ends of microtubules are embedded in the centrosome and it is been observed that  $\gamma$ -tubulin in the MTOC (microtubule organizing centre) as well as other factors nucleate microtubules. This study induced myoblasts with 2.5  $\mu\text{g/ml}$  for 45 min at 37°C (Musa, 2003.) The results showed the tenacity of microtubules and how efficient they really are. After the nocodazole treatment the microtubules were not able to sustain  $\alpha$ -tubulin and depolymerized however, when microtubules were allowed to re-polymerise, they grew back almost immediately in just over a minute. The site close to the nucleus which was associated with  $\gamma$ -tubulin and pericentrin was the location where the microtubules started to grow back first. This study suggests that nocodazole may directly inhibit  $\gamma$ -tubulin and cause microtubules to depolymerize right down to the nucleus. The centrioles are located near the nucleus and contain the minus ends of most microtubules, so it would make sense the when they are split apart that is the location they build back from. The speed in which they rebuild is astonishing and shows the effectiveness of microtubule construction.

In conclusion, this experiment had a very small sample size, but the inhibitory response that supported our hypothesis may be due to nocodazole targeting the plus ends to de-polymerise all the way down to the initial minus ends near the centrosome and keep them at that spot by blocking  $\alpha$ -tubulin. Nocodazole may also affect the GTP reactions when dimers are building on one another to cease them to build and reverse this process. Further studies should be conducted to increase the strength of our results to support our hypothesis that nocodazole decreases pseudopodia counts. By increasing the statistical sample size of this hypothesis we increase the power and hopefully aim to decrease the variance of our results.

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(S. Houlihan, results obtained through collaboration with Ms. Sara Houlihan, November 19, 2008).