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International Journal of Plant Biology & Research

Research Article

Effect of Lawsone on Mitosis and Growth in Onion Roots

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Submitted: 11 July 2017

Accepted: 10 August 2017

Published: 12 August 2017

ISSN: 2333-6668

Keywords
- Henna
- Lawsone
- Mitosis
- Meristem
- Onion roots

Abstract

Lawsone is a compound found in the leaves of the henna plant (*Lawsoniainermis*). Lawsone has anti-mitotic properties that give it the potential for use as an anti-cancer drug. While most studies on lawsone have been done on animals, this series of experiments was designed to determine if lawsone has an anti-mitotic effect on plants specifically the meristematic cells in onion root tips grown in varying concentrations of this solution. First, the effect of lawsone on root growth and mitotic activity was determined. Next, the effect of higher concentrations on roots was investigated, and a concentration at which root growth is completely inhibited was discovered. Lastly, we ascertained which phase of the cell cycle is inhibited by lawsone, and whether the drug produces any chromosomal abnormalities. Suppression of root growth was found, indicating an anti-mitotic effect, which was supported by lowered mitotic indices. Cells treated with a relatively high concentration of lawsone (1600 µM) failed to divide, while cells exposed to lower concentrations exhibited low mitotic indices, but no noticeable chromosomal abnormalities. Lawsone was found to have a dose-dependent inhibitory effect on mitotic index. Cells at the highest concentration in the last test failed to reach metaphase even with a two-hour colchicine treatment, indicating that lawsone inhibits cell division at some point between interphase and prophase. This latter finding supports previous research that lawsone inhibits mitosis during the S phase of the cell cycle and helps strengthen its position as a potential anti-mitotic treatment and an anti-cancer alternative.

INTRODUCTION

One compound with the anti-mitotic potential to be a target-specific treatment for cancer is lawsone (2-hydroxy-1,4-naphthoquinone), which is one of the active ingredients in henna. Henna is a traditional dye derived from the henna plant (*Lawsoniainermis*), and it is used globally for body art and hair coloring because of its ability to stain proteins. Lawsone has been found to slow or stop the spread of cancerous cells by inhibiting S phase of the cell cycle [1], and therefore, preventing cell division. Because cells spend most of their time in interphase, lawsone is expected to have a negligible effect on non-cancerous cells. Up to this point, studies have mostly focused on how it affects animals. Studies on rodents and cell cultures have found it useful in the treatment and prevention of numerous cancers [2,3].

However, multiple studies have found that henna has inhibitory effects on other rapidly reproducing cells [4-7]. Rearing zebrafish embryos in henna solutions resulted in deformities and increased mortality [4]. Henna and its extracts (including lawsone) have been found to be useful in protecting organisms from pathogenic bacteria by inhibiting bacterial reproduction [5-7]. Despite the similarities between the meristematic cells of plants and these other rapidly reproducing cells, very few studies have investigated the effects of lawsone on plants. In one similar study, 3-d old roots exposed to lawsone for 3 h exhibited increasingly strong inhibitory effects (up to 50 %) on mitosis at relatively small concentrations (100 to 1000 µM, approximately) in a dose-dependent manner [8].

The meristem is an area of plant tissue found at the tips of roots and shoots that contains undifferentiated cells that reproduce often—nearly constantly. The high rate of mitotic activity in meristematic tissue makes it a convenient system for the study of factors that affect cell division, and therefore cancer cells. Onions (*Allium cepa*) are model organisms. They can be grown and manipulated easily, and there are standard methods for testing the effects of different substances on growth and chromosomes of onion root meristem cells [9].

In this study, a variation of this *Allium* Test [9] was used to examine the effect of lawsone on root length and mitotic activity of onion roots. Specifically, the number of cells found in each stage of mitosis and the overall rate of mitosis for root cells exposed to lawsone were recorded. If lawsone inhibits S phase, then DNA replication and the rest of the cell cycle would not occur. Because meristematic cells usually divide rapidly and repeatedly, the halting of their cell cycle should inhibit root growth. Therefore, it was predicted that root length of treated onions would be significantly shorter than that of the control treatment. Based on the results of the previous study [8], a dose-dependent decrease in both root length and mitotic activity was expected.

MATERIALS AND METHODS

For our variation of the *Allium* Test [9], 16 large (25 mm diameter) test tubes were filled with four replicates each of a DI water control and three experimental concentrations of lawsone. The concentrations used were 0, 40, 70 and 100 µM. These concentrations were chosen based on previous literature: namely, that 40 µM was found to be capable of 50 % tumor suppression [1] and 100 µM resulted in deformities in zebrafish embryos [4]. A third, intermediate concentration was chosen midway between 40 and 100 µM. Pearl onion bulbs were placed atop the test tubes with their husk and dried roots removed, and with their basal plates submerged in the solution (Figure 1). The test tubes were kept in a location away from direct sunlight.

Each day, the onions and their root growth were photographed. Following the fourth day, the roots were severed and fixed in a 3:1 (v/v) solution of absolute ethanol : glacial acetic acid. The five longest roots from each replicate were measured to the nearest 0.1 mm. Roots were stored in the refrigerator at 37 °F for a minimum of 24 hrs prior to slide preparation.

For slide preparation, roots were treated with 1N HCl for 3 min to soften the cell walls and then stained in aceto-orcein for 30 min. The roots were rinsed in a 45 % glacial acetic acid solution for 10 s before being placed onto a microscope slide. The 2-4 mm meristematic root tip region (discernible by its opaque and/or darker coloration) was removed by severing with a sharp scalpel. The remainder of the root was discarded. Two drops of the rinse were placed onto the root tip followed by a coverslip that was squashed by placing a paper towel on top of the coverslip and pressing firmly straight down with the thumb. This produced a monolayer of meristematic cells between the coverslip and slide. The edges of the cover slip were sealed with clear nail polish. Five slides were prepared for each of the four replicates of each treatment, with the two showing the best monolayer and most mitotic activity chosen to be used for data analysis.

For ease of categorizing and counting cells, a photograph was taken of a microscopic field at 400X containing a minimum of five cells in mitosis in the field of view. Cells in each phase of mitosis as well as interphase were counted and these data were used to calculate mitotic index (mitotic index = number of cells in mitosis divided by total cells counted and multiplied by 100).

In a second experiment, two onions were grown for several days in the same manner as the first experiment. The first onion was a DI water control, while the second was grown in a far more concentrated solution of lawsone (1600 µM). The control onion grew normally, but the treated onion never grew measurable or observable roots, even when left for a week.

These results were then used to design a third experiment at a range of intermediate doses: 250, 500, 1000 and 1500 µM (n = 1 per treatment). Onions were treated for 2 h with 0.05 % colchicine to accumulate chromosomes at metaphase where they may be optimally studied for chromosomal abnormalities. The roots were fixed, measured, and processed as described above. Because of the use of colchicine, an accurate mitotic index could not reasonably be determined, but a "metaphase index" was calculated to compare the activity of the samples (metaphase index = number of cells in metaphase divided by total cells counted and multiplied by 100).

ANOVA was used to compare treatment groups for variation in root length, while regression was used to evaluate the relationship between lawsone concentration and mitotic index.

RESULTS AND DISCUSSION

Root length

In the first experiment, all groups grew at least some visible roots within 24 h. Differences in root lengths between experimental and control groups were noticeable within 48 h. Onions treated with lawsone were observed to have fewer, shorter, and misshapen roots, stained by the lawsone. ANOVA revealed significant variation between treatment groups ($F_{3, 76}$ = 38.24, $p < 0.001$) (Figure 2). Post-hoc pair-wise comparison tests showed that all treatment groups were statistically different from the control. The 40 and 70 µM groups were not statistically different from each other ($p > 0.05$), but the 100 µM group was statistically different from each ($p < 0.05$ and 0.001, respectively).

Root length decreased significantly from even a very small concentration of lawsone. Our findings are consistent with previous data in demonstrating that lawsone inhibits growth in cancerous and other rapidly reproducing cells [1-7]. This appears to be consistent across a broad range of eukaryotic and prokaryotic kingdoms.

Mitotic activity

Mitotic indices calculated for each group revealed a 10-15 % decrease in mitotic activity at each increase of concentration ($F_{1,30}$ = 9.297, $p < 0.005$) (Figure 3) with statistically significant differences between any two non-adjacent concentrations at the $p < 0.05$ level and an even greater difference between the control and 100 µM groups ($p < 0.01$). There was a significant regression (R^2 = 0.24, F = 9.297, p = 0.00476) with mitotic index decreasing by 0.022 for each micromolar of lawsone.

Figure 1 Comparison of control and experimental groups at higher concentrations of lawsone (0, 250, 500, 1,000, and 1,500 µM from left to right)

Figure 2: Mean ± 1SE root length of five longest roots after 4 d at varying concentrations of lawsone (n=4)

Figure 3: Mean ± 1SE mitotic index calculated for each treatment of lawsone (n=8)

Figure 4: Mean ± 1SE metaphase index calculated for each treatment of lawsone (n=1)

Table 1: Total cell counts and number of mitotic phases for each lawsone treatment (n=8).

Concentration (µM)	Interphase	Prophase	Metaphase	Anaphase	Telophase	Total Cells Counted	Mitotic Index
0	2437	71	43	35	22	2608	6.56
40	2452	62	41	22	21	2598	5.62
70	2360	39	35	28	22	2484	4.99
100	2208	44	19	21	16	2308	4.33

The roots exposed to high concentrations of Lawsone in the third experiment showed very little growth, but all roots exhibited at least some growth. Most roots treated with lawsone that did grow were stained black. As expected, the colchicine resulted in metaphase cells being the only mitotic ones. Examination of karyotypes from these metaphase cells did not reveal any noticeable abnormalities. The metaphase index decreased sharply from control to 250 µM and again from 250 to 500 µM (Figure 4). At 500 µM, the metaphase index was near zero, but increased slightly before dropping to complete inactivity at 1500 µM.

These findings that lawsone has an inhibitory effect on mitotic activity support the previous finding that it blocks S phase [1]. Due to experimental constraints, it was impossible to determine whether the cells were in S phase, but the number of cells in interphase did increase. The decrease in mitotic index appears to be dose-dependent. For this experiment, the relationship was approximately a 12 % decrease in mitotic activity for every 30 µM increase in concentration of lawsone.

Our results had some consistencies with previous work on *Allium* [8]. A significant inhibitory effect on mitotic activity was found, even at low concentrations. Larger differences were found in this study than the previous study, which could be attributed to length of treatment (four days as opposed to three hours). Such a short-term treatment of 3 h may not have manifested effects in cells that were not at the stage of the cell cycle that is targeted. This study was more sensitive to detecting how cells respond to long-term, rather than short-term exposure.

CONCLUSION

This experiment established lawsone's inhibitory effect on plant root growth and mitotic activity. Our preliminary results demonstrate the critical concentration range of biological activity. Our data further corroborate lawsone's anti-mitotic properties and potential as a target-specific cancer treatment or prevention alternative. However, these experiments were limited by the categorical, rather than continuous, concentrations of lawsone used. A more continuous range of concentrations could elucidate the relationship between lawsone and mitotic inhibition. Future studies based on these results may include the effect of lawsone on gut bacteria (a subject that may be greatly affected if lawsone is taken as an oral supplement), on less rapidly reproducing somatic cells (a potential side effect if it is used in cancer treatment), and on other areas of plant physiology including the shoot meristem and non-meristematic tissue.

ACKNOWLEDGEMENTS

This project was funded by the NSF REU program. We would like to thank all the members of the REU program at Samford University for making this project possible, especially Betsy Dobbins, Jim Duchesneau, Malia Fincher, Mary Anne Garner, and Andrew Hataway. We also extend our thanks to Brian Wisenden and Chris Graf for their critical review. Special thanks to Mary Anne Garner and Andrew Hataway for their continued support throughout the course of this study.

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Cite this article

Lueders MB, Howell WM, Dovlatabadi H (2017) Effect of Lawsone on Mitosis and Growth in Onion Roots. Int J Plant Biol Res 5(3): 1071.