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Effect of Vitamin K1 on Cell Growth Inhibition and Apoptosis on the U937 Cell Line

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ABSTRACT

This experiment was conducted in order to verify the role of Vitamin K1 as a cell growth inhibitor on the U937 cell line. This experiment was performed in two parts—one with a lesser concentration of Vitamin K1, and the other with a range of concentrations from low-to-high. Through the remaining number of U937 cells, as well as cell areas, it was concluded that the presence of Vitamin K1 reduces the number of cancer cells. It was also concluded that as Vitamin K1 concentration increases, so does the frequency and effects of apoptosis.

Keywords: Vitamin K1; U937 Cells; Cell Growth Inhibition; Apoptosis; Human Cancer

1. Introduction

1.1. Effects of Vitamin K on Cell Tissue Growth

Vitamin K is a hydrophobic vitamin that plays a role in blood coagulation, bone density preservation, and perhaps even the inhibition of various types of cancer [1]. Vitamin K is quickly becoming a targeted method of treating cancer cells due to its ability to slow cell growth. This ability is supported by numerous studies that have investigated the effects of higher levels of Vitamin K on the cell cycle. The results of these studies suggest that Vitamin K is effective in inhibiting cell growth in human cancer cells. In these experiments Vitamin K, specifically Vitamin K1, K2, and K3, has been used to treat cells isolated from various types of cancer. The cancer cells treated with Vitamin K experience a disruption in cell division and cell growth, thereby reducing their overall abundance [2].

When examining the effects of Vitamin K in the disruption of cancer cell growth, there are a number of previous studies that emphasize its effects. For instance, scientists at the University of Pittsburgh Medical Center synthesized a group of Vitamin K analogs. The Vitamin K analog that demonstrated the highest potency on cell growth was named compound 5 (Cpd 5). This analog acted by inhibiting protein tyrosine phosphatases (PTP). The inhibition of PTP by Cpd 5 led to cell growth arrest due to an alteration of cellular kinases, which are involved in signal transduction for cell cycle development. Thus, an analog of Vitamin K was shown to interrupt cancer cell development, verifying the role of Vitamin K in reducing the number and vitality of cancer cells [3].

The Thomas E. Starzl Transplantation Institute at the University of Pittsburgh conducted another study examining the effects of Vitamin K on cell growth inhibition. This study once again involved the use of the analog Cpd 5 in regards to induction of PTPases in a human hepatocellular cancer cell line (Hep3B) as a cell growth inhibitor. Like the previously mentioned study, this one also confirmed the role of Cpd 5 as an inhibitor of cancer cell growth. This confirmation further substantiates Vitamin K's role in the eradication of cancer cells [4].

1.2. Effects of Vitamin K on U937 Cells

The U937 model cell line is commonly used in a variety of biomedical research. This cell line was developed from the histiocytic lymphoma from the lung of a thirty-seven year old male in the 20th century [5]. U937 cells have become a standard target for research in regards to cell growth inhibition, due to both their ability to easily grow in a suspension, and their wide availability. The effects of Vitamin K on U937 cell growth inhibition have also been studied. These studies confirm the effects of Vitamin K on the inhibition of cancer cell growth. For instance, in a study performed at Hamamatsu Medical School in Japan, the effects of Vitamin K2 in combination with estradiol were studied in regards to tumor antigens. The study concluded that the utilization of a homologue of Vitamin K2, menaquinone 4 (MK4), and estradiol-17beta produced a drastic increase in the TM antigen levels in U937 cell line. The increase in TM an-

tigen levels reveals that Vitamin K2 plays a role in the inhibition of cancer cell growth, via antigen-mediated destruction of cancer cells. This verifies that Vitamin K2 acts as a cell growth inhibitor [6].

2. Purpose

The purpose of this experiment was to investigate the effects of Vitamin K1 on cell growth inhibition in U937 cells. This experiment was divided into two parts. The first involved the treatment of the U937 cell line with a lesser concentration of Vitamin K1, while the second consisted of the treatment of the cell line with four different concentrations of Vitamin K1. By examining the effects of Vitamin K1 on cell growth inhibition, its role in triggering apoptosis in cancer cells could be verified. It was hypothesized that Vitamin K1 would trigger apoptosis in the U937 cell line, resulting in a lower number of remaining cells and larger cell areas when treated with higher concentrations.

3. Materials and Methods

3.1. U937 Cells

The U937 Cells were supplied from the Biological Department at East Tennessee State University.

3.2. Media

The RPMI-1640 Media was created by Moore *et al.* at Roswell Park Memorial Institute, and is able to support many cell cultures, including human lymphocytes (Sigma-Aldrich, 2010). This media was equally distributed to each of the 6-well culture plates.

3.3. Treatment

Four varying concentrations of Vitamin K1 were used. Treatments were distributed equally amongst cell culture plates. Two cell culture plates were occupied by controls.

3.4. Cell Culture

An equal cell suspension of 3.9×10^5 cells/mL was added to each culture plate. In the second part of the experiment, an equal cell suspension of 5.0×10^5 cells/mL was added to each culture plate.

4. Procedure

The experiment was conducted under a sterile fume hood in the laboratory. A micropipette was used to prepare the cultures, which were placed in an incubator at 37˚C for a duration of 7 days.

The control consisted of equivalent amounts of RPMI-1640 media and U937 cell suspension containing 3.9×10^5 cells/mL. The treatment consisted of equivalent amounts of RPMI-1640 media, stock culture Vitamin K1, and a U937 cell suspension containing 3.9×10^5 cells/mL.

The cultures were placed into cell culture plates. In the first part of the experiment, the cell culture plates consisted of 2 controls and 2 of the 50.0 μM treated samples. In the second part of the experiment, the cell culture plates consisted of four controls, 2 of the 10.0 μM treated samples, 2 of the 50.0 μM treated samples, 2 of the 100.0 μM treated samples, and 2 of the 500.0 μM treated samples.

Once the cultures were incubated for 7 days, the cells were transferred from the cell culture plates to labeled test tubes. A sample from each tube was wet mounted onto a labeled slide. The cells were then placed under a microscope with a camera attached and four random images were collected per slide. A program called Image J was used to analyze the images. Using this program, the areas of the cells were obtained and graphed.

The remaining cells were transferred to a hemocytometer using a micropipette. The hemocytometer was placed under a microscope and the cell numbers were counted. Each cell count was conducted four times and averaged.

5. Results

The resulting cell counts for the first part of the experiment were collected and are shown in **Figure 1**. Each cell count is representative of 1.0×10^6 cells. The averages of the cell counts show that the treatment of the U937 cells with a Vitamin K1 concentration of 50.0 μM results fewer cells overall (**Figure 1**).

The resulting cell counts for the second part of the experiment are also shown in **Figure 2**. Again each cell count is representative of 1.0×10^6 cells. The averages of the cell counts show that as the treatment concentration of Vitamin K1 increases, the cell count decreases (**Figure 2**).

The cell areas obtained from the cell images using the program *Image J* were combined, arranged in increasing order, and graphed. **Figure 3** shows the areas of the cells in the control group, while **Figure 4** shows the areas of the cells treated with Vitamin K1. It is evident that the cell areas become larger after being treated with the 50.0 μM concentration of Vitamin K1.

The cell images reflect the results of the graphs. **Figures 5** and **6** are images of Control 1 and Treatment 1. Clearly a reduction in cell number and an expansion in cell area has occurred in the presence of Vitamin K1.

The treated cell areas obtained from the second part of the experiment were plotted against the control cell areas, and are shown below. **Figure 7** plots the 500.0 μM treat-

	Count 1	Count 2	Count 3	Count 4	Average
Control 1	144	180	172	136	1.58×10^6
Control 2	168	208	188	192	1.89×10^6
Treatment 1	148	128	140	152	1.42×10^6
Treatment 2	112	128	120	108	1.17×10^6

Figure 1. Cell counts of U937 cells—part 1.

	Count 1	Count 2	Count 3	Count 4	Average
Control 1A	162.0	175.0	160.0	158.0	1.64×10^6
Control 1B	155.0	184.0	163.0	169.0	1.68×10^6
10.0 μM	150.0	145.0	157.0	148.0	1.50×10^6
50.0 μM	142.0	139.0	132.0	128.0	1.35×10^6
100 μM	121.0	118.0	130.0	104.0	1.18×10^6
500 μM	101.0	95.0	111.0	99.0	1.02×10^6
Control 2A	173.0	162.0	178.0	154.0	1.67×10^6
Control 2B	170.0	159.0	165.0	161.0	1.64×10^6
10.0 μM	158.0	144.0	157.0	160.0	1.55×10^6
50.0 μM	133.0	146.0	127.0	131.0	1.34×10^6
100.0 μM	125.0	133.0	109.0	120.0	1.22×10^6
500.0 μM	100.0	115.0	98.0	96.0	1.02×10^6

Figure 2. Cell counts of U937 cells—part 2.

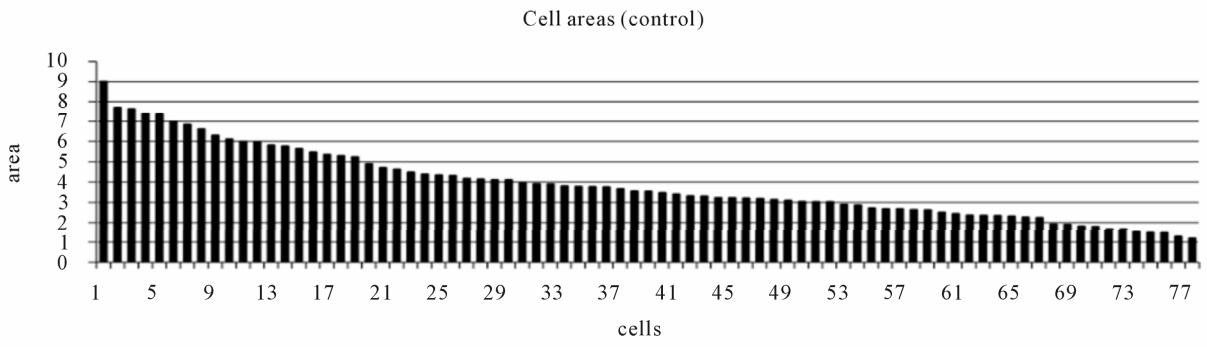

Figure 3. Control cell areas—part 1.

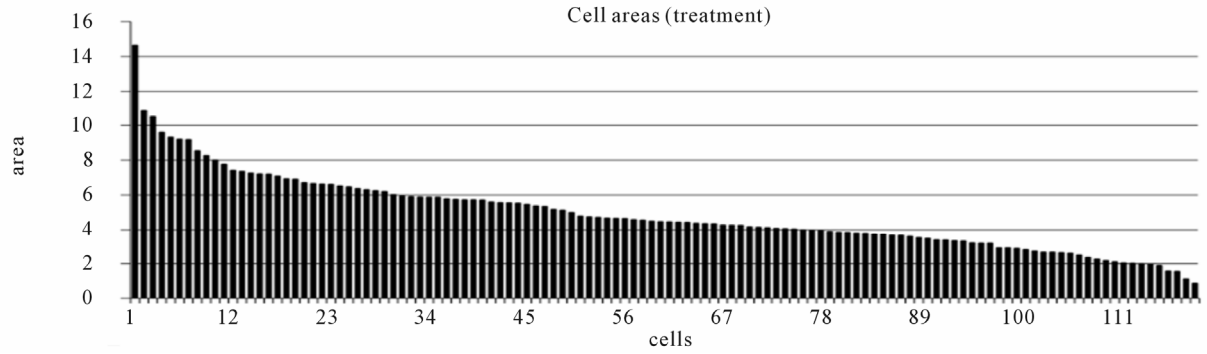

Figure 4. Treatment cell areas—part 1.

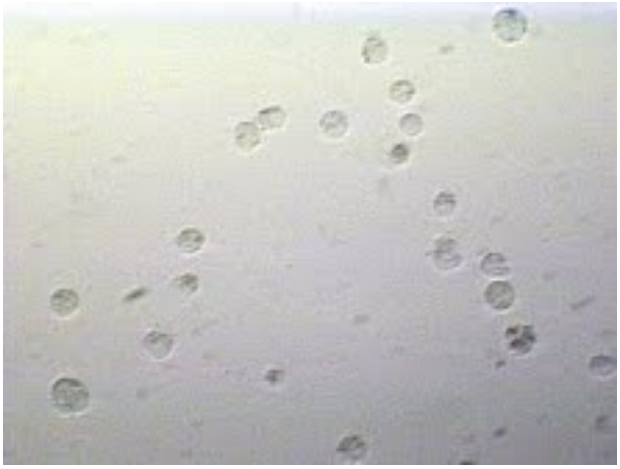

Figure 5. Control 1.

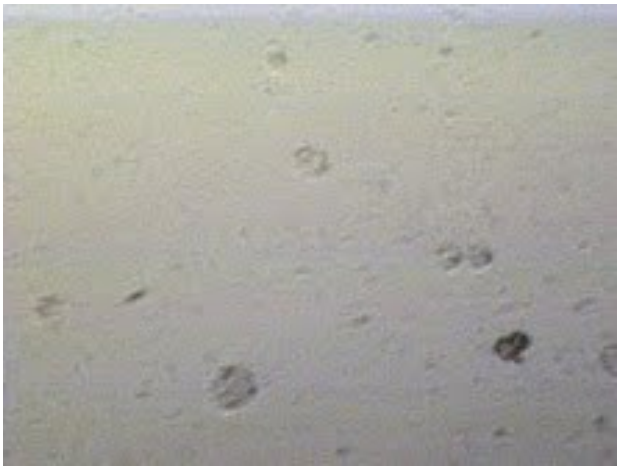

Figure 6. Treatment 1.

ment against the control. **Figure 8** plots the 100.0 μM treatment against the control. **Figure 9** plots the 50.0 μM treatment against the control. **Figure 10** plots the 10.0 μM treatment against the control. It is evident that as the Vitamin K1 concentration increases, the cell areas become larger (**Figures 7-10**).

6. Conclusions

To investigate the effectiveness of Vitamin K1 on U937 cell growth inhibition, U937 cells were grown with or without the treatment of a stock solution containing Vitamin K1. The results were supported by the results of former experiments involving Vitamin K1 and cell growth inhibition.

The cell counts in the first part of the experiment demonstrated that the treatment of the U937 cells with 50.0 μM of Vitamin K1 reduced the total number of cells (**Figure 1**). This can be understood in regards to the ability of Vitamin K1 to inhibit cancer cell growth. The reduction in the number of U937 cells in the treatment group indicates the occurrence of cell growth inhibition because of Vitamin K1.

The cell counts in the second part of the experiment demonstrated a reduction in the total number of U937 cells as the concentration of Vitamin K1 increased (**Figure 2**). The combined results in both tables demonstrate that cell growth is inhibited in the presence of Vitamin K1, and that as Vitamin K1 concentration is increased, growth inhibition becomes more pronounced. The cell images also support these results, as cell numbers are reduced in the presence of Vitamin K1 and cell areas are increased.

Figures 3 and **4** show that the treatment of the U937 cells with Vitamin K1 increases cell area, while **Figures 7-10** demonstrate that as Vitamin K1 concentration increases, so does the cell area. This increase in cell area is due to the activation of apoptosis in the U937 cells, which are undergoing cell cycle arrest. Thus, the presence of Vitamin K1 inhibits cell growth and triggers apoptosis. In addition, the increasing concentration of Vitamin K1 treatments increases the rate of apoptosis, triggering a more frequent cell death and increasing cell area.

The result of this experiment supports the hypothesis that Vitamin K1 can act as an inhibitor of cell growth in U937 cells. By averaging the cell counts from both parts

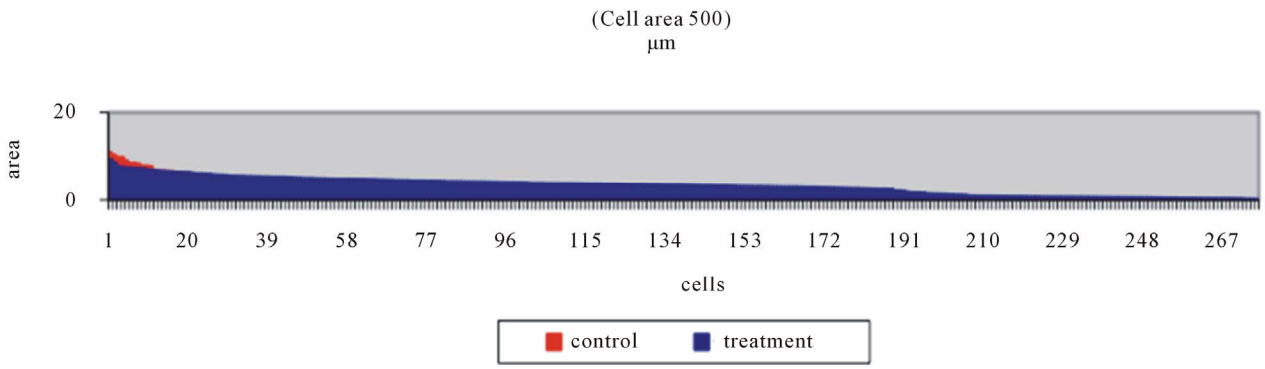

Figure 7. Cell Areas from 500.0 μM treatment vs. controls—part 2.

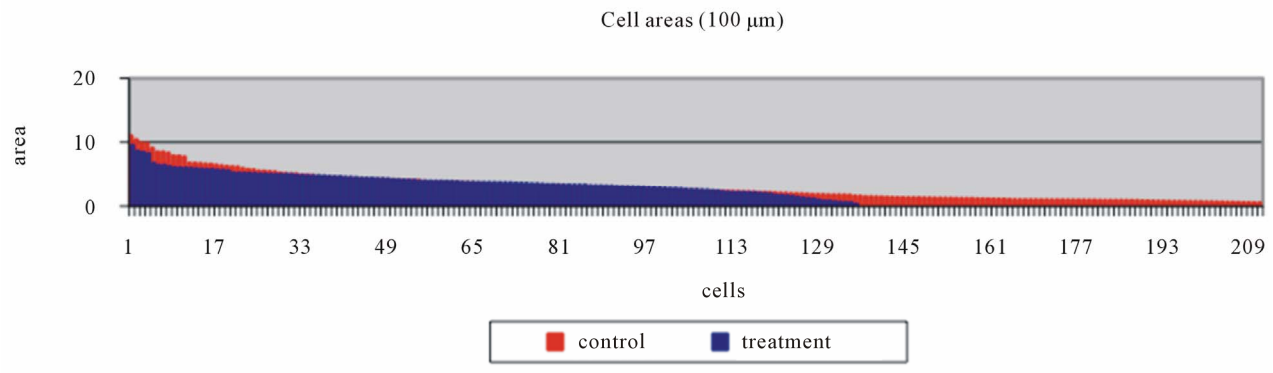

Figure 8. Cell areas from 100.0 μM treatment vs. controls—part 2.

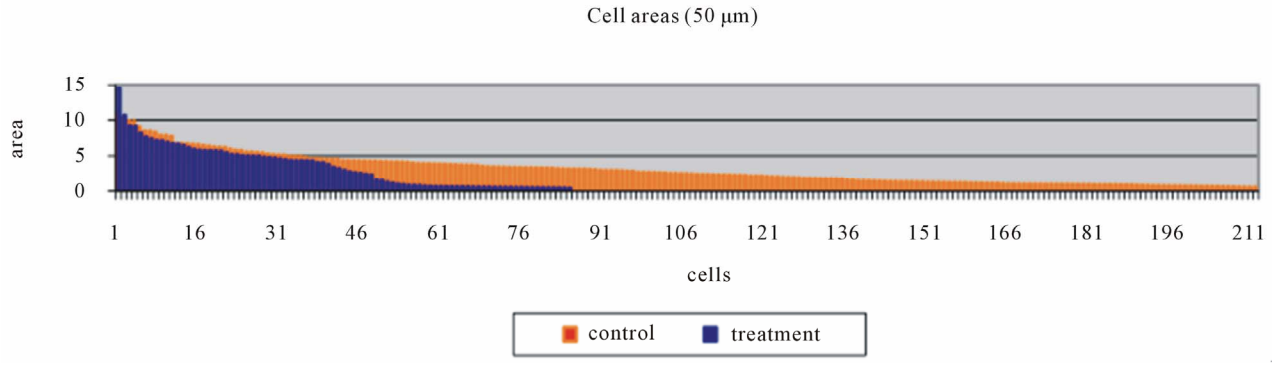

Figure 9. Cell areas from 50.0 μM treatment vs. controls—part 2.

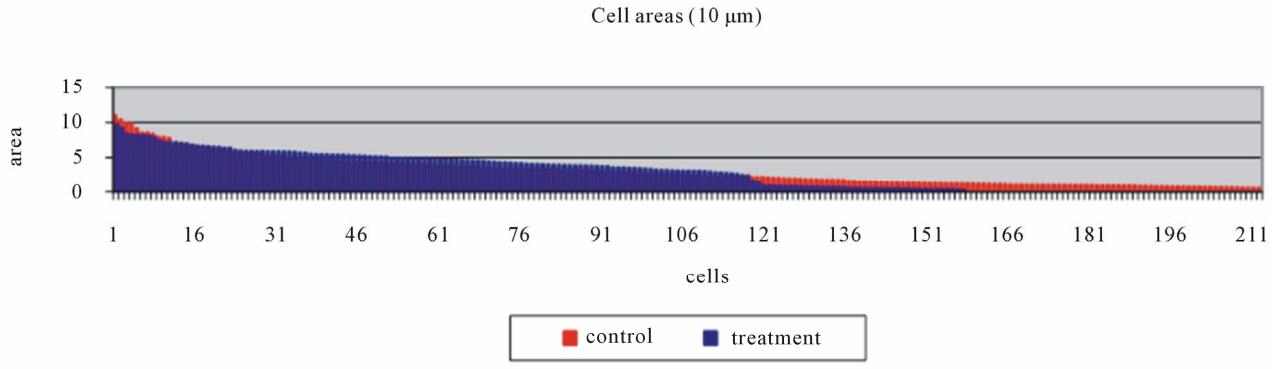

Figure 10. Cell areas from 10.0 μM treatment vs. controls—part 2.

of the experiment, it is evident that the presence of Vitamin K1 triggers cell cycle arrest, resulting in an overall lower abundance of cells. It is also evident that increasing Vitamin K1 concentration results in lower cell abundance as the concentration increases. By analyzing cell areas for both parts of the experiment, it becomes obvious that the presence of Vitamin K1 activates apoptosis, illustrated by the increasing cell areas. It is also obvious that increased concentrations of Vitamin K1 generate a more frequent apoptotic rate, indicated by increasing cellular areas, as the treatment concentration increases. Through this experiment, Vitamin K1 is again verified as being capable of reducing the abundance of cancer cells and increasing the rate of apoptosis.

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