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In vitro Model of the Potential Antibiotic Colloidal Silver Displays Cell Death and Growth Inhibition in SF-9 Insect Cells

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Abstract

In this project, we investigated the toxicity of Colloidal Silver, a pre-ampicillin antiseptic/disinfectant recently reported to be effective against several types of bacteria, including malaria, on Fall Armyworm (Spodoptera Frugiperda; SF-9) cells. We accomplished this goal by culturing healthy SF-9 cells, with different concentrations of Colloidal Silver.We observed the plates and refreshed the medium after 4 hours and 24 hours of incubation, and continued to observe the plates every 24 hours afterwards. The purpose of this experiment was to investigate the unknown effects of colloidal silver on eukaryotic cells (Insect), as it has mostly been studied on bacteria, to see the viability of Colloidal Silver as an efficient alternative to modern medicine. The results showed that the Colloidal Silver effectively weakened the cells and accelerated the cells' deaths. We conclude that the potency of Colloidal Silver makes it a promising viable alternative to modern medicine, as the silver effectively halts cell growth with minimal concentration. The relative small concentration effectively destroys cells and yet might not damage large organisms, such as humans. However, further research is needed to verify the results.

Introduction

Colloidal Silver is the name given to liquids that contains a certain size range of silver ion particles, from 1-1000 micrometer long. It has been believed for many generations to have antimicrobial properties. The mechanism that destroys prokaryote cells might also damage eukaryote cells. The purpose of the experiment was to test whether the SF-9, a kind of insect cell, would develop normally in the presence of colloidal silver or have their development hindered. Also, the experiment would help to shed light on the cutoff concentration for safe use in the environment with colloidal silver.

Firstly, the effect of colloidal silver in the environment was analyzed. At Hoseo University, Korea, a study analyzed the toxicity of silver nano-particles. The study utilized Daphnia magna, sensitive water organisms, to assess the potential toxicity of silver particles. Concentrations of silver particles above 0.001mg/L caused the Daphnia magna to swim abnormally. The organisms exhibited a dark pigment and upon examination it was discovered that the silver deposits caused the pigment change. Once the Daphnia magna were dissected large chunks of silver were discovered in the digestive track. The Daphnia magna could not utilize the silver for any metabolic purpose; therefore, organisms do not need silver.[1]

A previous study had determined a mechanism for the antimicrobial properties of silver. The enzyme Thioredoxin reductase stabilizes the DNA synthesis process and aids the production of adenine dinucleotide phosphate. The Thioredoxin reductase directly correlated with the enzyme activity of the cell because many enzymes rely on Thioredoxin reductase for electrons needed to utilize metal ions. The high concentration of colloidal revealed a disrupted Thioredoxin reductase activity and the synthesis of adenine dinucleotide phosphate was severely limited. The study utilized rat-cell cultures and pure Thioredoxin reductase to test the colloidal silver effect. The colloidal silver inhibited Thioredoxin reductase in both cases.[2]

A study of Guinea Pigs revealed that colloidal silver can potential harm large sections of tissue.[3] The study used Guinea Pigs to determine the toxicity of colloidal silver on the skin. Daily doses of colloid silver were given to the Guinea Pigs and the Guinea Pigs were put-down after two weeks. The study found that the entire cohort developed skin abnormalities, such as boils and swelling. Furthermore, the spleen and liver were affected by the consumption of colloidal silver. However, none of the cohort suffered serious disease or condition; and, the cohort all survived the experiment. The study found that the lowest concentration of colloidal deemed not to affect the tissue is 5 µg/mL.[3]

This study conducted herein attempts to determine the various affects that the concentration will have on SF-9 insect cells. The experiment will utilize concentrations around the 5 µg/mL established in the Guinea Pig study. Also, the experiment will utilize SF-9 insect cells because all animals have the enzyme Thioredoxin reductase.[4] Therefore, the SF-9 cells share in common with human cells the same Thioredoxin reductase enzyme that colloidal silver inhibits greatly.

Colloidal silver has the potential to act as an antimicrobial. The colloidal silver was shown to have antibacterial properties; assays were preformed by Dr. Kira Morrill and her team of researchers in Southwest College of Naturopathic Medicine, analyzing interactions of Colloidal Silver with both Samonella typhimurium and Streptococcus pyogene. The colloidal silver effectively inhibited the bacteria in vitro[5]. Furthermore, colloidal appears to have a limited effect on some viruses. Small pox, for example, was mixed with Colloidal Silver, and observations were made stating that "colloidal silver is active towards smallpox

virus in the metallic state."[6] Therefore, colloidal silver can become a potential antimicrobial when used in safe quantities. The correlation between SF-9 cell deaths and colloidal silver concentration will determine a safe concentration for colloidal silver in cell cultures.

Materials and Methods

A culture of SF-9 cells was well-maintained and the medium changed often. Then, the flasks containing the appropriate amount of Colloidal Silver, 2 ul, 10 ul, 50 ul, 250 ul, and 1250 ul, were prepared (Table 1). The 6th flask was the control and contained no silver. The appropriate amount of Colloidal Silver was mixed with 3 ml of medium that contains SF-9 cells. By doing so, a minimum of 1 ppm Colloidal Silver solution is achieved in the flask with 1 ul of Silver Liquid, as the solid silver content in the original bottle was 15 grams, and the bottle contained 10 ml of Silver; thus the first flask contained a 5 part-per-million concentration. After the mixing is complete, flasks were incubated inside of an incubator for further observations. The condition of the cells were checked at least once per 48 hours. The cells were stained and counted at various time points and the cell numbers were counted using a hemocytometer.

Results

Figure 1 and Table 2 of the results reveal that the control (flask 6) of the experiment produced $163*10^4$ cells. The control had absolutely no silver and began with the same concentration of cells as the other flasks. The first flask, the one with the least silver, only 5 ppm, demonstrated 60% of the growth of the control. That suggests that the colloidal silver, with a minimal concentration, can reduce the reproductive rate of the SF-9.

The second culture illustrates the toxicity of the silver particles clearly. The cell culture only had $5*10^4$ cells. The cultures all began with $7.2*10^4$ cells. The cell count of the control to the second flask is 32.6; the second culture only grew 0.03 percent of the control. That vast drop in cell count can only be explained through the five-fold increase in silver. The silver not only stopped the growth of the cell culture but also destroyed 30% of the original cells.

The silver's high toxicity to SF-9 clearly demonstrates the potency of colloidal silver. The SF-9 cultures with colloidal all grew less than the control without silver. Furthermore, the high concentration of silver actually decreases the population of the SF-9 cells. The silver particles are clearly toxic to the SF-9 cells.

The colloidal silver actually exponentially increased the toxicity with each uL of silver added, over time (Figure 2). The "safe" concentration for colloidal silver becomes the line where the concentration of silver does decrease the cell growth. The 10 uL of silver choked the cell culture and did not allow growth to occur. Therefore, the 25ppm should be considered the threshold concentration. According to previous studies, the land environment can tolerate 30 ppm[1] of silver with ease. Therefore, 25 ppm should be considered a relatively low dosage, as a one-time use in some individuals cannot do significant harm to the environment.

Flask #	Amount of Silver Liquid (ul)	Concentration of Silver (ppm)	Cell Count (10^4)
1	2	5	7.2
2	10	25	7.2
3	50	125	7.2
4	250	625	7.2
5	1250	3025	7.2
6	0	0	7.2

Table 1. This table shows the amount of the silver, as well as the concentration of the silver, and the cell count, at the initial stage of the experiment. This is taken from hour 0 of the experiment, which validates the equal amount of cell count in every flask, regardless of concentration of the silver concentration.

Flask #	Concentration of Silver (ppm)	Cell Count (10^4)
1	5	91.25
2	25	5
3	125	0
4	625	0
5	3025	0
6	0	163

Table 2. This table shows the concentration of the silver and the cell count, after 144 hours. This table does not contain the amount of Silver Liquid.

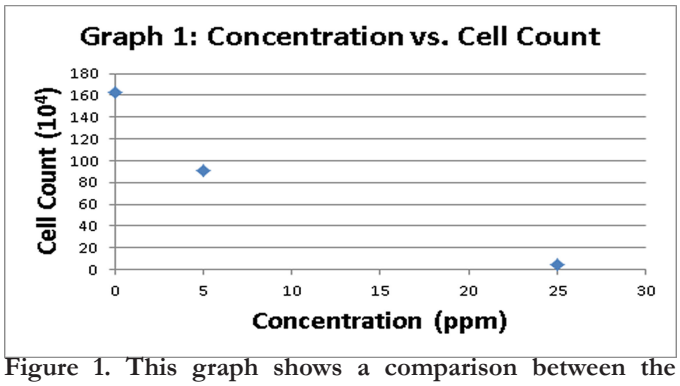

Figure 1. This graph shows a comparison between the concentration of the silver and the cell count, after 144 hours. This table does not contain flasks 3-5, as they reached 0 in cell count minutes after the experiment.

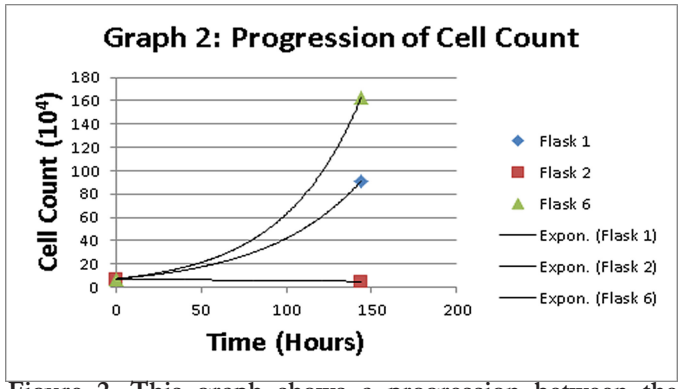

Figure 2. This graph shows a progression between the silver and the cell count, after 144 hours. This table does not contain flasks 3-5, as they reached 0 in cell count minutes after the experiment.

Discussion

The complaints that the Federal government received before they banned the advertising and usage of Colloidal Silver as an alternative to other treatments, can be attributed not to the harm that the particles of silver actual produce, but the additives that they need to add in order to keep the production cost low, as well as the rough process of making the silver particles.

As to the concentration of that silver that allows complete normal growth simply does not exist. The silver's toxicity damages the cells and hinders their growth; therefore, the slightest damage done early in the cell culture greatly impacts their growth because the cells grow exponential.

As suggested in the introduction, it is hypothesized that the silver inhibits the Thioredoxin reductase of bacteria. This discovery implied that a method of silver toxicity includes Thioredoxin reductase inhibition. Moreover, the silver probably inhibited the Thioredoxin reductase of SF-9 cells, although further study is needed. However, continuing the assumption that it did inhibit the Thioredoxin reductase, then the silver affects the SP-9 Thioredoxin reductase type. According to another study, all animals share the same Thioredoxin reductase[4]. Therefore, the silver would very well inhibit the same Thioredoxin reductase in human cells as SF-9 cells. The colloidal silver could be assumed toxic to human cells as well other animal cells, as supported by the guinea pig study[3].

Ultimately, the effects of Colloidal Silver on humans are not known. However, based on this experiment and previous results, it is speculated that Colloidal Silver is a viable alternate to modern drugs, such as antibiotics.

References

1. Asghari, Saba, Seyed Ali Johari, Ji Hyun Lee, Yong Seok Kim, Yong Bae Jeon, Hyun Jung Choi, Min Chaul Moon, and Il Je Yu, 2012, Toxicity of Various Colloidal Silver concentration in dapnia Magna, J Nanobiotechnology ; 10: 14.

2. Srivastava, Milan, Sanjay Singh, and William T. Self, 2012, Exporse to Colloidal Silver Inhibits Selenprotein Synthesis and the Activity of Thioredoxin Reductase, Environ Health Perspect; 120(1): 56–61.

3. Korani, M., SM Rezayat, K. Gilani, S. Arbabi Bidgoli, and S. Adeli, 2012, Actute and Subchronic Dermal Toxicity of Colloidal Silver in Guinea Pig, Int J Nanomedicine; 6: 855–862.

4. Hirt, D., and S. Müller, 2012, The Diversity and Evolution of Thioredoxin Reductase, Trends Parasitol;18(7):302-8.

5. Kira Morrill, Kathleen May, 2012, Spectrum of Antimicrobial Activity Associated with Ionic Colloidal Silver. The Journal of Alternative and Complementary Medicine.

6. N. E. Bogdanchikova, A. V. Kurbatov, V. V. Tret'yakov, P. P. Rodionov, 1992, Activity of colloidal silver preparations towards smallpox virus, Pharmaceutical Chemistry Journal, volume 26, Issue 9-10, pp 778-779.