The Effect of Blue Scorpion Venom on *Borrelia burgdorferi*

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Abstract:
Increasing antimicrobial resistance in pathogenic bacteria such as *Borrelia burgdorferi*, the Lyme disease bacterium, has created the need to find novel biologically active compounds against these infectious agents. Various venoms, such as bee and snake venoms previously have been shown to have antimicrobial effects on different bacteria. In this project, the effect of Blue Scorpion Venom (BSV) was tested against the different morphological forms of *Borrelia burgdorferi*. The results show that the number of cysts and the size of biofilm forms can be reduced by BSV, however the spirochete form and the quantity of protective layers on the surface of the biofilm were not affected. In summary, BSV showed promising effect on some of the forms of *Borrelia burgdorferi*, but further research is necessary to fully describe its effect.

Introduction:

Lyme disease is a tick-borne, systemic disease caused by the spirochete *Borrelia burgdorferi* that has grown into a major public health problem since its discovery in the 1970s.[1] Patients often present with a combination of an expanding rash known as erythema migrans (EM), and other symptoms including fatigue, chills, fever, headache, muscle aches, joint aches, and swollen lymph nodes.[2] In the short-term, patients can experience facial palsy, and in the long term as many as 60% of patients experience bouts of arthritic symptoms.[2] Antibiotic therapy is the standard treatment for Lyme disease, but relapse of the disease often occurs when antibiotic therapy is discontinued. Even after treatment with antibiotics, approximately 10-20% patients present with symptoms that last months to years after the termination of treatment.[2]

The frontline treatment for chronic Lyme disease is administration of tetracyclines (e.g. doxycycline) or macrolides (e.g. clarithromycin). However, after 3 months of treatment with tetracyclines and macrolide, 50%-60% improvements are observed in patients, with a cure rate of only 20%.[3,4,5,6] Doxycycline treatment is presumed to be the best treatment for Lyme disease available today but the cure rate is still only about 20%.[2] Many studies have shown that in spite of continued and high-dose antibiotic therapy, chronic Lyme disease is rarely treated successfully. Once treatment is discontinued, relapse of the disease takes place. This means that even after antibiotic treatment, the host immune system fails to prevent recurrence. One possible explanation for this is the formation of round body and biofilm forms of *B. burgdorferi*, which may protect the organism from both the antibiotic therapy and the host's natural immune responses.

It has been suggested that this resistance and reoccurrence of Lyme disease might be due to the transformation of *B. burgdorferi* into several resistant forms during antibiotic therapy[7].The two well-known morphologies are classical spirochetes and round bodies. Round bodies form in response to environmental stress, including changes in pH, temperature, and exposure to antibiotics.[7] In order to successfully treat Lyme disease in the future, novel treatments must undermine the mechanisms *B. burgdorferi* uses to resist antibiotic therapy; therefore, we need to better understand the direct effect of antibiotics on the different morphological forms of *B. burgdorferi*.

The Lyme disease research group at the University of New Haven recently demonstrated that *Borrelia* can form a third, very organized structure called a biofilm[8.] A biofilm is a community of microorganisms encapsulated within a complex, extracellular matrix that forms when cell populations reach a certain cell density. In general, bacterial biofilms are known to show much greater resistance to antibiotics (up to 1000-fold) than free-living cells and the UNH research group has demonstrated that *Borrelia* biofilm is no exception [7, 9.] This research project will analyze the effects of the blue scorpion venom on biofilm-like colonies of *B. burgdorferi*.

Venoms are complex mixtures of proteins, including a variety of enzymes, as well as enzyme inhibitors, nucleotides, lipids, mucopolysaccharides, and biogenic amines.[10] These components also include a variety of antimicrobial compounds that we are just beginning to discover. Escozine is a product derived from the venom of the Caribbean Blue Scorpion. Escozine is primarily sold as a supplement for individuals with cancer, and is something humans can consume orally. Other scorpion venoms are being studied for use in humans as well, and other venoms, from bees in particular, have been shown promise against the spirochete form of *Borrelia burgdorferi.*[10, 11, 12]

Materials and Methods:

Borrelia burgdorferi B31 was grown in BSK-H complete media (SIGMA, 6% rabbit serum) for 6 days with treatment beginning after 4 days of growth. $5x10^6$ cells were plated in a 1ml total volume on a 48 well plate and allowed to grow at 32^oC, 12% CO_2. The amount of biofilm was quantified by either standard crystal violet staining or the total carbohydrate assay; the results were presented as the optical density of each treatment alongside the blank which received distilled water or phosphate buffered saline. Each experiment was done in three independent trials in replicates of six. For images the same growth and treatment protocols were followed and BacLite Live/Dead staining was used to visualize the potential effects. For the analysis of the free forms of *Borrelia*, seeding was conducted at the same concentration of $5x10^6$ cells/mL in a total volume of 2mL. Treatment began after 1 day of growth, and continued for a total of 3 days. The day after the last treatment, the cells were counted, separately accounting for both cysts and spirochetes.

Results:

BSV treatment showed dramatic effects on *Borrelia* biofilms by microscopic methods at all concentrations studied (Figure 1). The sizes of the biofilms were significantly reduced and some of the *Borrelia* biofilms showed dead (red) cells. Additionally, biofilms appeared to be less tightly compacted following treatment. Using direct microscopy method, BSV also demonstrated significant effect on the cyst forms of *Borrelia*, the round structures seen within and around the biofilm.

Quantitatively, BSV seemed to have no effect on the spirochete forms when evaluated by direct counting (Figure 2, top), but it did appear to have an effect on the number of cysts found (Figure 2, middle). BSV also did not appear to have much effect on the extracellular matrix (ECM) of polysaccharides that is measured by the total carbohydrate assay (Figure 2, bottom). These findings are based on three independent

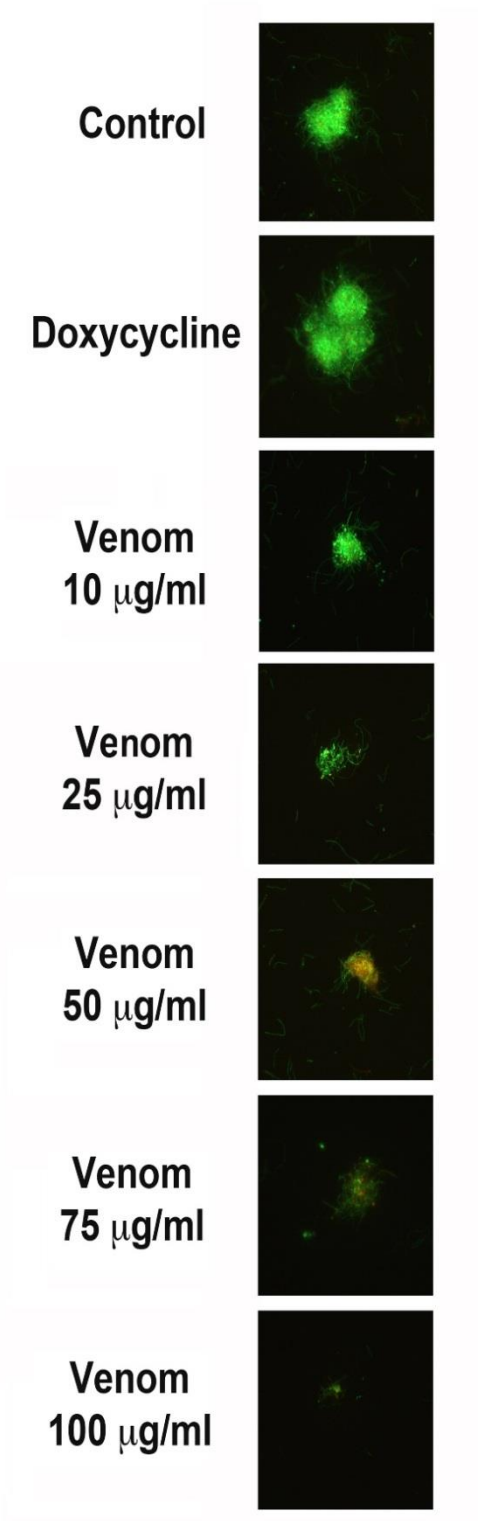
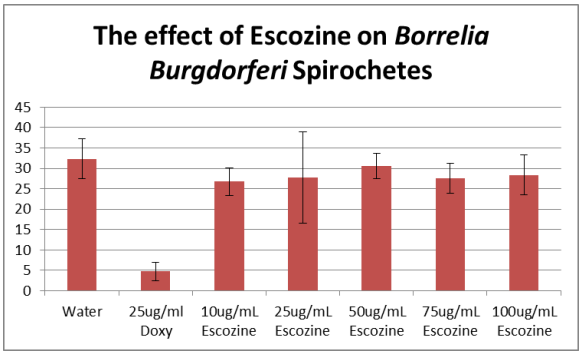
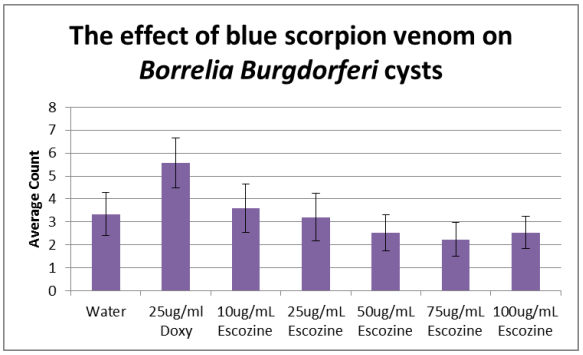


Figure 1: Qualitative analysis of *Borrelia burgdorferi* biofilms treated with Blue Scorpion Venom. BacLight Live/Dead viability staining after 72 h BSV treatment on 4 day old biofilms at 40x magnification. Green staining shows live cells, orange staining shows dead cells. As the concentration of BSV increases, the amount of green staining decreases, as does how tightly packed the biofilm is itself.

replicates. Combined with the microscopic data, this suggests that while the ECM does form in treated biofilms, the BSV is still capable of getting into the biofilm to cause damage, and the cyst direct counting data explains why there are dead cysts seen in BacLite staining (Figure 1).





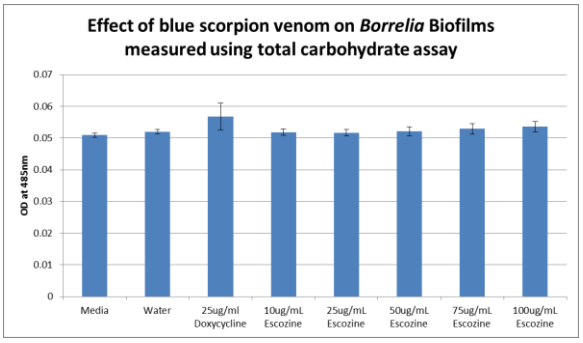


Figure 2: Quantitative analysis of *Borrelia burgdorferi* biofilms treated with Blue Scorpion Venom. The above data represent the means of three independent experiments in which each data point represents 6 samples. Error bars represent the standard deviation of three separate trials.

Conclusions and Future Work:

The results show promising effect of BSV on some of the morphological forms, but more studies are necessary to fully evaluate whether BSV would be an effective agent for the treatment of Lyme disease. More sensitive assays are needed to determine the effect BSV has on the viability of biofilms. Various metabolic assays are currently being developed by the Lyme Disease Research Group, which will hopefully help to understand the effects of BSV on *Borrelia*. Additionally, further direct counting using fluorescent methods will help to confirm these results and prepare this data for further publication outside the University.

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Biography:

Christina Kling is a rising senior at the University of New Haven majoring in Biotechnology. She plans to pursue a Ph.D. in Molecular Biology and ultimately wants to do medical research for unmet medical needs.