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]

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

replicates.