In Vitro Impact of Triatomine *Salivary* Glands Extracts Introduced to Endothelial Cells

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Abstract

Chagas Disease (AKA Trypanosomiasis) is caused by biting/feeding behavior from the arthropod vector Triatoma (subfamily of Reduviidae family), that house the endoparasite *Trypanosoma cruzi*, which can then be passed to human and mammalian hosts (Schmidt, et al., 2011). Resources are currently being utilized to help minimize the effects and susceptibility of Chagas within endemic areas. Previous research has demonstrated that there are biochemical interactions between specific Triatoma salivary proteins and host cells (Ribeiro, Assumpção, Van Pham, Francischetti, & Reisenman, 2012).This study examined the interactions made from salivary proteins procured from the *T. sanguisuga* and *T. indictiva* species with the expression of two glycoproteins, fibronectin (angiogenic) and thrombospondin (antiangiogenic) when exposed to Human Umbilical Vein Endothelial Cells (HUVECs).

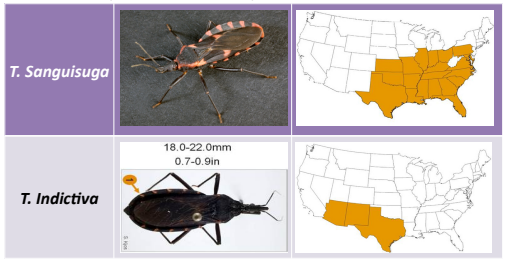
Questions and Hypotheses

Question: Do Salivary Gland Extracts (SGE) obtained from *T. Sanguisuga* and *T. indictiva* species have an influence on the expression of glycoproteins when exposed to HUVECs?

Hypothesis: Human Umbilical Vein Endothelial Cells (HUVECs) will have differential expression of the glycoproteins fibronectin-1 and thrombospondin-1 after the exposure to *Triatoma Sanguisuga* and *Triatoma indictiva* salivary gland extracts.

Study System and Background

Reduviid insects, commonly known as assassin bugs due to their piercing/ sucking mouthparts used to prey feed on other insects, contain a subfamily of triatomines, commonly known as kissing bugs, which instead procure blood meals from animal hosts (Schmidt, et al., 2011). Triatomines subsequently are carriers for the blood parasite, *Trypanosoma cruzi* (Schmidt, et al., 2011). Even though triatomines feed on a multitude of animals, only mammals are known to be adversely affected by the *T. cruzi* parasite (Bern, Kjos, Yabsley, & Montgomery, 2011). *T. Cruzi* is transferred to the host from an infected triatomine (result from ingesting a blood meal from an infected animal) via the insect defecating near a human/animal, and this material entering the mucus membranes or breaks in the skin (CDC, 2017). This study utilizes two species that are reported within the United States. *T. Sanguisuga* species is reported in 23 states, covering much of the south and south east, and *T. indictiva* species is only reported in Arizona, New Mexico, and Texas (Schmidt, et al., 2011).



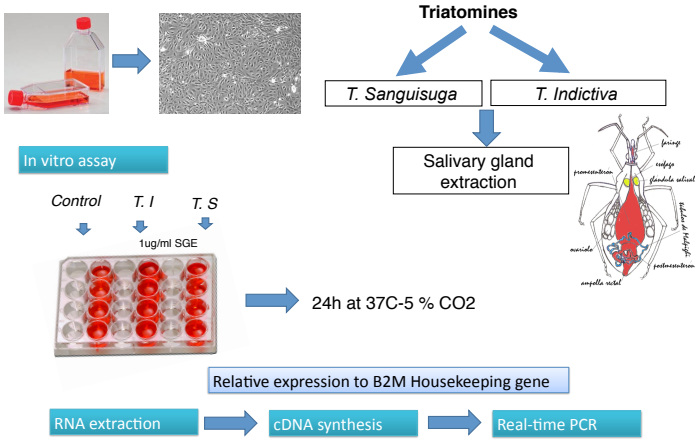
The glycoproteins fibronectin-1 and thrombospondin-1 were selected from the salivary extract for this study based on their ability to influence wound healing. Fibronectin has angiogenic properties (aids the development in new blood vessels) (Astrof & Hynes, 2009). In contrast, thrombospondin-1 has anti-angiogenic properties (antagonizes the development of new blood vessels (Bradshaw, 2014).

Methods and Experimental Design

This study uses salivary gland extracts obtained from *T. Sanguisuga* and *T. indictiva* species and applied them to Human Umbilical Vein Endothelial Cells (HUVECs) that are being cultured in the laboratory. HUVECs were chosen for their ability to replicate pathological conditions related that involve human endothelial cells (Cell Applications, n.d.). Fibronectin 1 and Thrombospondin 1 results were analyzed using real-time PCR (ran in triplicate), which were compared to the relative baseline expression of the B2M Housekeeping gene.

HUVEC exposure to *T. Sanguisuga* and *T. Indictiva* SGE

HUVEC cell line culturing



Results

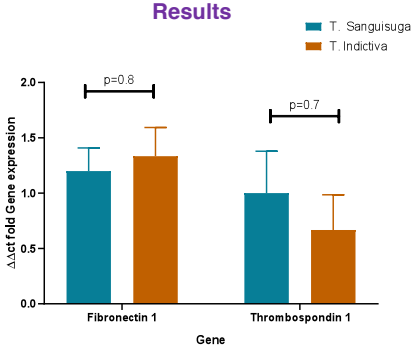


Figure 1. Fold gene expression of Fibronectin-1 and thrombospondin-1on HUVEC cells after the treatment with T. Sanguisua (blue) and T. indictiva (brown orange) SGE.

Conclusions

The hypothesis that fibronectin-1 and thrombospondin-1 have a different expression on HUVEC cells was supported by the data. This demonstrates that the salivary glycoproteins have a net potential to stimulate wound healing. Even although no statistical difference was found between both species, antigenic activity (more fibronectin and less thombospondin 1) could be less affected in *T. Sanguisuga* as compare with *T. Indictiva*. Lastly, this research can also have a larger impact in science with regards to the biochemical interactions that take place in vivo with regards to immune response, serological testing, and changes in host cells, etc.

Future Directions

- Separation of Salivary gland extracts into proteins to test individual proteins.
- Testing SGE from other Triatomines species in more skin and immune human cells.
- In vivo wound healing studies using the SGE and isolated proteins.

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Acknowledgements

Thank you to the staff and students within the Vector Biology laboratory at Kansas State University. In particular, thank you to Dr. Lidia Montenegro-Cadena and Dr. Berlin Londono-Renteria for closely mentoring me throughout this project and other ongoing research projects. Also, thank you to Dr. Jeremy Marshall from the Kansas State University Entomology Department for putting in long hours to organize the Undergrad Research Experience on campus.