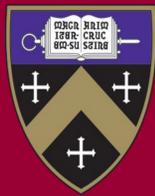


Evaluation of mosquito (*Aedes aegypti*) organic anion transporters through microinjection of sulfonate group dyes.

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Abstract

The Gates Foundation estimates that every year, 750,000 people die from mosquito-borne diseases. During a blood meal, mosquitoes voluntarily ingest a large variety of foreign substances, some are essential to metabolic function (metabolites) and others are potentially harmful and need to be excreted through the urine (xenobiotics). Their effective xenobiotic transport system has made the development of mosquitocides challenging. The relationship between mosquito excretory transporters (specifically organic anion transporters) and the structures they preference could yield valuable insight into pesticide development. Previous work has shown that charge plays a significant role in xenobiotic transport. In our study we injected mosquitoes with a variety of compounds that differ in their number of sulfonate groups (R-SO₃⁻). We found that the addition of a sulfonate group significantly decreased the toxicity of the compound to the mosquitoes. While it is unclear if this decrease in mortality is due to preferential excretion, this work does yield promising results for the development of adjuvants to current mosquitocides.

Methods

- Mosquitoes were injected with a 345 nl bolus (a conservative estimation for the volume of a blood meal) of dye at various concentrations using a micro-injector. Mosquitoes were then monitored for 24-hours and a dose-response curve was generated.
- Controls were a Phosphate buffer solution (1X) with 5% Dimethylsulfoxide (DMSO)

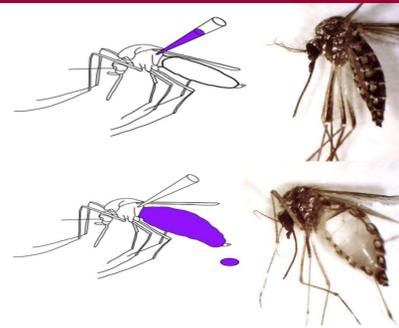


Figure 2. Diagram of micro injection.

Dose Response

Dose Response For Phenylxanthenes

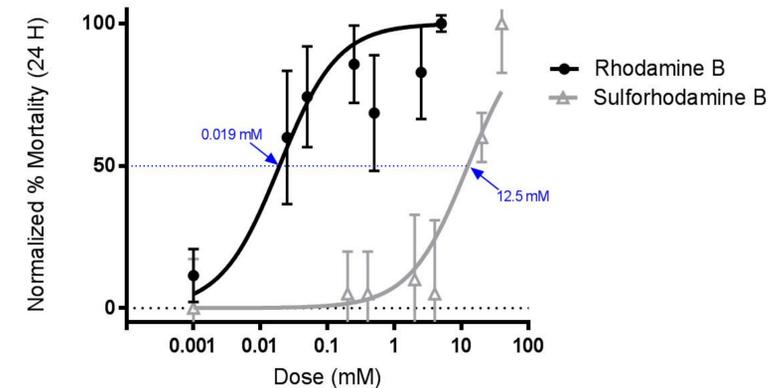


Figure 5. Dose-Response curve for mosquitoes after injection with Sulforhodamine B and Rhodamine B. Controls were injected with both PBS and a mixture of PBS/5%DMSO. Experimental groups were monitored for 24 h/hrs after injection (ANOVA, $R^2_{Rhod} = 0.35$, $R^2_{Sulfrhod} = 0.75$, $p > 0.001$).

Background

A study on the grasshopper *Schistocerca gregaria* observed a collection of sulfonate chlorophenol red (CPR) in malpighian tubules, indicating the presence of organic anion transporters (OAT's). Competitive inhibition of the OAT has been observed through the inhibition of fluorine by the sulfonate indigo carmine in the cockroach *Blaberus giganteus*. In *Aedes aegypti*, competitive inhibition of other excretory transporters has been linked to an increase in toxicity of the pesticides.

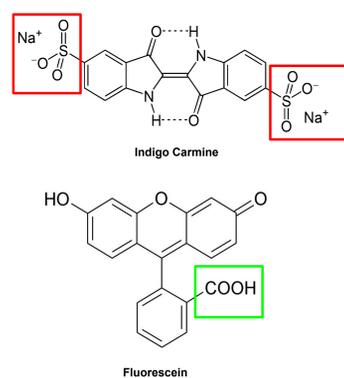
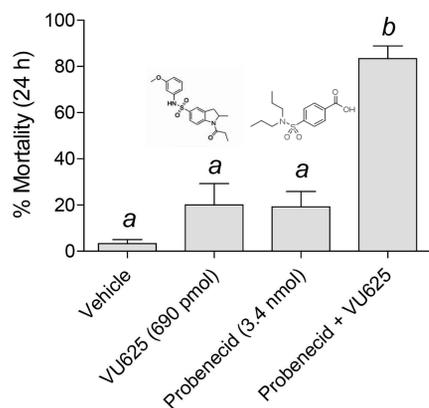


Figure 1A. Effects of probenecid and VU625 on the in vivo excretory capacity of adult female mosquitoes (*Aedes aegypti*). Figure 1B. Structural Diagram of Fluorescein and its proposed competitive inhibitor indigo carmine.

Dyes

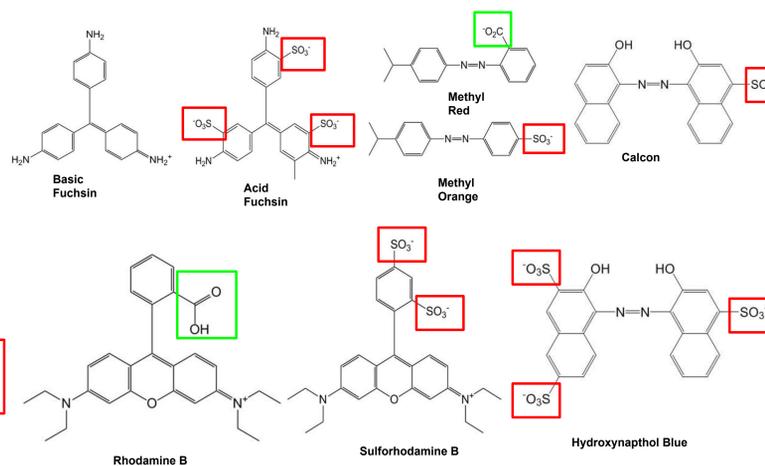


Figure 3. Structural diagrams of dyes used to characterize the OAT transport of *Aedes aegypti*. Green box indicates carboxyl group. Red Box indicates sulfonate group.

Results

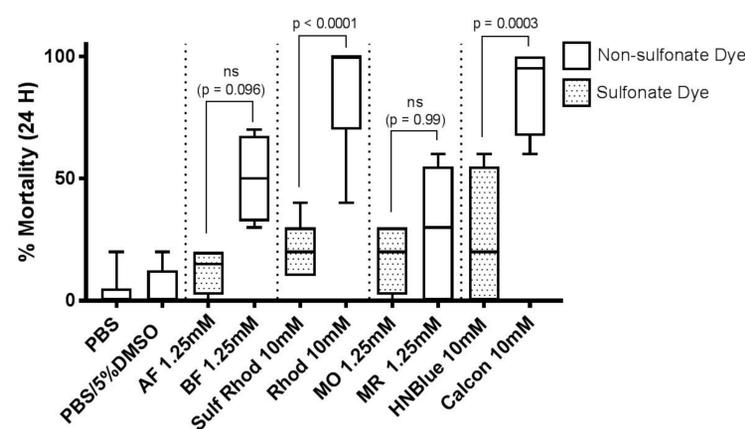


Figure 4. Response (Percent mortality) of mosquitoes after exposure to dyes. PBS: Phosphate Buffer solution, PBS/5% DMSO: PBS with 5% dimethyl sulfide, AF= Acid Fuchsin injected at 1.25mM, BF= Basic Fuchsin injected at 1.25mM, Sulf Rhod = Sulforhodamine B injected at 10mM, Rhod = Rhodamine B injected at 10mM, MO=Methyl Orange injected at 1.25mM, MR=Methyl Red injected at 1.25mM, HNBBlue: Hydroxynaphthol Blue injected at 10 mM and Calcon: injected at 10mM. (ANOVA, $F=12.416$, $p < 0.01$).

Conclusions and Future Aims

- Pilot studies on saturated dye solutions indicate the addition of a sulfonate group significantly decreased the compounds mortality in a variety of parent compounds.
- The dose-response curves for rhodamine B and sulforhodamine B support that the substitution of the carboxyl group for a sulfonate group significantly decreased xanthene toxicity.
- More cases are required to determine whether this finding is a blanket trend for all dyes.
- Injection of parent xanthene is required to quantify the effect of Sulfonate groups on toxicity.
- Excretion trials are needed to identify if the decrease in toxicity is due to an increase in excretion preference.
- It is our expectation that the anions with a greater amount of sulfonate groups will be transported with greater efficiency by the OATs and therefore enter into the mosquito's Malpighian tubules more readily.

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References

- Halberg, K.A.; Mobjerg, N. First evidence of epithelial transport in tardigrades: a comparative investigation of organic anion transport. *J. Experimental Biology* 2011, 215, 497-507.
- O' Donnell M.J. Too much of a good thing: how insects cope with excess ions or toxins in the diet. *J. Experimental Biology* 2008, 212, 363-372.
- Chahine S.; Seabrooke S.; O' Donnell M.J. Effects of Genetic Knock-Down of Organic Anion Transporter Genes on Secretion of Fluorescent Organic Ions By Malpighian Tubules Of *Drosophila Melanogaster*. *Archives of Insect Biochemistry and Physiology* 2012, Vol 81, No 4, 228-240
- Raphemot R, Rouhier MF, Swale DR, Days E, Weaver CD, et al. (2014) Discovery and Characterization of a Potent and Selective Inhibitor of *Aedes aegypti* Inward Rectifier Potassium Channels. *PLoS ONE* 9(11): e110772. doi:10.1371/journal.pone.0110772
- Raphemot R, Rouhier MF, Hopkins CR, Gogliotti RD, Lovell KM, Hine RM, Ghosalkar D, Longo A, Beyenbach KW, Denton JS, et al. Eliciting renal failure in mosquitoes with a small-molecule inhibitor of inward-rectifying potassium channels. *PLoS ONE* 2013;8(5):e64905.
- Hemingway J, Beaty BJ, Rowland M, Scott TW, Sharp BL. The innovative vector control consortium: Improved control of mosquito-borne diseases. *Trends Parasitol* 22(7):308-12
- "The Spread of Zika Virus." *The Economist*. The Economist Newspaper, 26 Jan. 2016. Web. 01 May 2016.

Goals

- Identify whether the organic anion transporter, like OAT, can effectively transport anionic dyes in the Malpighian tubules of the model mosquito species (*Aedes aegypti*).
- Investigate the relationship between sulfonate groups and toxicity to determine an appropriate concentration for excretion assays.
- Investigate if there is a relationship between an organic anion's number of sulfonate groups and its ability to be transported by the OAT.