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Measuring Immune Responsiveness in *Xenopus laevis* using Phytohaemagglutanin (PHA)

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Abstract

Phytohaemagglutanin (PHA) is a red kidney bean extract that has been used to stimulate an immunological T cell response in a variety of animals. This procedure has become very popular method to analyze an individual animal's ability to trigger an immunological response.(Brown 2014) PHA skin testing has been used in a variety of avian, mice, lizard, and amphibian species where it is injected into the muscle, skin, or webbing of an animal and then recording the amount of swelling before and after the injection.(Smits 1999) We are using the amphibian Xenopus laevis to test 1) their immune reaction under normal conditions and 2) to test how stress effects their immune reaction. In our control non-stress experiment to discover if PHA actually causes swelling, we found that significant swelling occurs at all time points in comparison to the saline injected foot. In the stress versus non-stress PHA experiments, we found no significant variation between the stressed versus non-stress, but still a significant difference between PHA versus saline injection.

Introduction

Phytohaemagglutanin (PHA) is a red kidney bean extract that has been used to stimulate an immunological T cell response in a variety of animals. This procedure has become very popular method to analyze an individual animal's ability to trigger an immunological response.(Brown 2014) PHA skin testing has been used in a variety of avian, mice, lizard, and amphibian species where it is injected into the muscle, skin, or webbing of an animal and then recording the amount of swelling before and after the injection.(Smits 1999) We are using the amphibian *Xenopus laevis* to test 1) their immune reaction under normal conditions and 2) to test how stress effects their immune reaction. We are led to believe that stressing the frogs should lead to some kind of change in the frogs' reaction to the PHA because stress has found to delay or inhibit immune responses in other animals, such as humans. This type of effect could be seen as a slower reaction to the PHA, a slower decline of swelling after reaching peak thickness, or a much less intense reaction to the swelling completely. Such methods have yet to be utilized to examine the immune function of *Xenopus laevis*.

Methods

We took eight frogs and anesthetized them in .1% concentration of MS-222. Then we gave them surgical markings on their ventral side in order to identify the individuals. While the frogs were still asleep we measured each frogs' middle clawed toe three times with a caliper and recorded the thickness. Then we injected 50 microliters of saline into the left foot and 50 microliters of 10 mg/ml concentration of PHA solution into the right foot. Afterward, we allowed the frogs to come out of the anesthesia and measured and recorded each toe's thickness at 4, 6, 24, and 48 hour time points. In order to test the effect of stress on the PHA reaction, we took one group of 6 frogs and placed into 50-ml conical centrifuge tubes (with ventilation holes drilled in them) and then placed them on a stirring platform at 100 rotations per minute for approximately 2 hours in order to stress this group of frogs. We also did a separate stress group of 6 frogs that were manually stirred in their tank for 45 minute intervals. They would be stressed roughly three times per week at random time intervals in order to prevent the frogs from habituating to a routine stressor. Once the frogs were stressed they were anesthetized in .1% concentration of MS-222. Then each frogs' middle clawed toe on each foot was measured three times with a caliper

Then the left foot was injected with 50 microliters of saline and the right foot with 50 microliters of 10 mg/ml concentration of PHA solution. Afterwards the frogs were measured at 4, 8, 12, 24, and 48 hour and then these data were entered into an Excel spreadsheet. The measurements of swelling were standardized by the change in swelling relative to the individual frog's size (XtX0, where Xt= thickness at time t and X0= thickness before injection). The data were then plotted as graph that compared the stressed to non-stressed frogs.

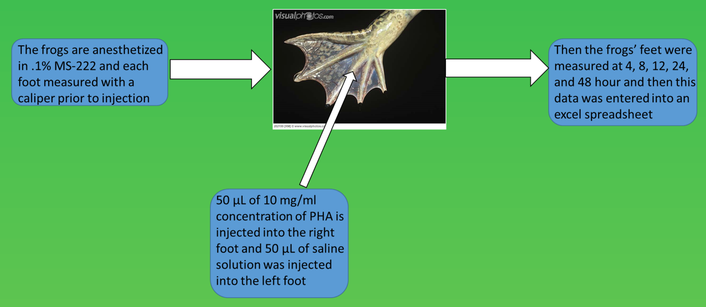

Figure 1: The standard outline of the procedure for injecting the *Xenopus* with PHA

Results

A paired t-test showed that there was in fact a significant difference between the PHA and saline injected feet. This simply stands as our proof of principle that PHA induces significant swelling in *Xenopus laevis*. Then in the following two experiments we implemented chased and restraint stress. We found that chased stress(Figure 3) showed no significant swelling between the stress and non-stress PHA swelling, but still retained a significant difference between the PHA and saline injected feet. The restraint stress experiments(Figure 4) concluded with no significant results, but we did find measurements approaching significant at time point 4. (p=.067) There was also a significant difference between the PHA and saline injected feet.

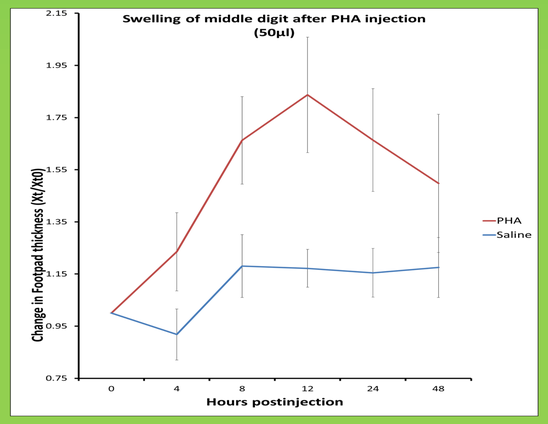

Figure 2: Non stress PHA and saline injection of the right and left foot.

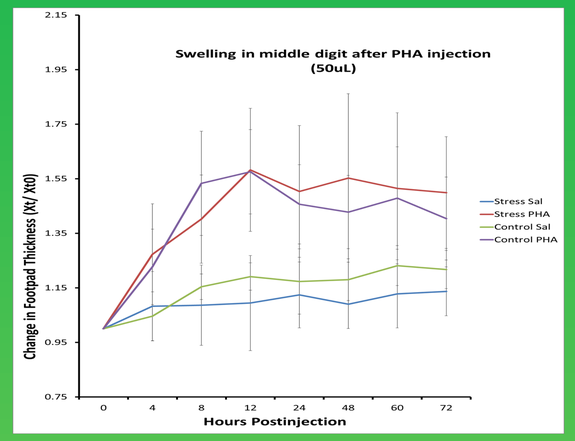

Figure 3: Chased stress PHA and saline feet measurements.

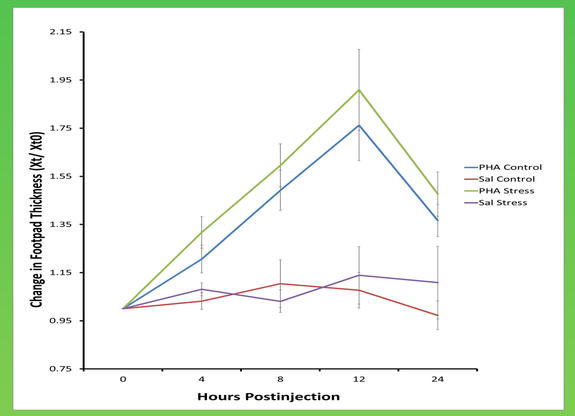

Figure 4: Restraint stress PHA and saline feet measurements.

Discussion

Our test results gave us no significant support of our hypothesis, that stress would have some form of influence in immunological response in *Xenopus laevis.* This may suggest that stress does not influence the *Xenopus*'s immune system, or it may suggest that our physical stressors were not intense enough to stimulate a measurable difference in their response to the PHA. Seeing that at time point 4 of the restraint stress approached significant, this my suggest that we need to focus on differences between swelling at even earlier time points at shorter intervals.

Acknowledgments

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Refferences

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