TOWARDS A MECHANISM OF ACTION OF AN ANTISCHISTOSOMAL DRUG: MEASURING GRANULOCYTIC ACTIVITY

PROJECT DESCRIPTION

A. Description of Project:
Schistosomiasis is a tropical disease which leads to vital organ damage and causes among the highest incidences of parasite-related mortality worldwide. The causative agent is a family of schistosome parasites, namely *Schistosoma mansoni*, *S. haematobium*, and *S. japonicum*, which infect freshwater bodies and are capable of directly penetrating the skin. This disease affects over 200 million individuals around the world, primarily in poverty-stricken areas of Africa. It is, therefore, one of the most significant helminths (parasitic worms) in regards to socio-economic impact and public health, second only to malaria, and is considered a neglected tropical disease by the World Health Organization. Treatment currently consists of two doses of the drug praziquantel (PZ), which is effective only against the adult worm. The use of two separate doses leads to pill sharing among family members to preserve costs. This results in uncleared infections that may cause parasite resistance against PZ. Although resistance is not yet confirmed, there are areas with low cure rates, suggesting the possibility of future resistance. A new, single-dose, orally administered drug would be beneficial to combat resistance of praziquantel, avoid dose-sharing among family members, prevent improper sterilization technique during intravenous drug delivery, and to eradicate juvenile infections early to lower damage inflicted by the worm.

Previous studies have discovered and tested an antischistosomal drug termed Ro 13-3978 which has been shown to have a substantially higher efficacy against both the juvenile and mature parasite in mice (published) and monkeys (unpublished) when compared to PZ. Moreover, Ro 13-3978 is safe to deliver orally in a single dose to mouse models, and it has a lower median effective dose (ED_{50}) compared to that of PZ. This effectiveness, however, does not transfer to *in vitro* (outside of the host) experiments. This indicates that Ro 13-3978 is host-dependent, but the mechanism by which it functions *in vivo* remains unknown.

For this drug to further advance into clinical trials in humans, a confirmed mechanism of action is highly desirable. Prior explanations, including the hypothesis that Ro 13-3978 is metabolized into an active form *in vivo* (inside the host), were disproved. The aim of this study is to investigate a likely mechanism of Ro 13-3978 as a drug which facilitates the activation of the host innate immune system. The body’s natural reaction to helminths includes the stimulation of eosinophils, a type of cell which specializes in attacking the worm. The migration, proliferation, and activation of eosinophils at the site of the infection allows for the release of cytotoxic granule proteins, like major basic protein (MBP) and eosinophil cationic protein (ECP), proven to be effective at killing *S. mansoni*. However the immune system is often overwhelmed by the infection and is unable to clear it fully. Preliminary results from our lab indicate that Ro 13-3978 acts to enhance the granulocytic activity of eosinophils in mice previously exposed to the drug, evident by a measurable increase in the granule proteins eosinophil derived neurotoxin (EDN) and superoxide anion compared to control groups (unpublished). Although increased levels of EDN and superoxide anions show evidence that the eosinophil is granulating more, these proteins are not proven to be effective against helminthic worms, as the parasites usually have sufficient mechanisms by which to protect themselves against superoxide anions and EDN is a neurotoxin with little toxicity to the tegument of the schistosome worm. Moreover, many hypothesize that eosinophils differentially release only specific granule proteins (a process unique to Figure 1. Structure of the antischistosomal aryl hydantoin Ro 13-3978, discovered in the 1980s by Hoffmann La-Roche.)
eosinophils and mast cells called piecemeal degranulation) despite the fact that they are all stored in the same granule. Therefore, proteins proven to be potent against the worm, like MBP and ECP, should be tested as well. Additionally, if Ro 13-3978 does enhance eosinophil’s ability to granulate, there needs to be a comparison between the experimental drug and other known eosinophil activating factors (EAF).

B. Activities/Methodology:
Major basic protein accounts for approximately 50% of eosinophil granule proteins, and has previously been shown to cause detachment and subsequent ballooning of the parasite’s outer membrane. Each granule also contains eosinophil cationic protein at a lesser concentration, but ECP is more toxic than MBP and mediates full fragmentation of the parasite. Additionally, both of these have been shown to enhance the adhesion of the eosinophil to the worm. To test for MBP and ECP, ten male BALB/c mice will be treated with Ro 13-3978, while another ten will serve as controls. No mice will be infected with Schistosoma parasites to confirm that any observed eosinophil activation is caused by Ro 13-3978, not by a natural immune response against the parasite. Sixteen hours post-treatment, eosinophils will be isolated from mouse spleen cells by magnetic separation. It’s necessary to observe the effects of the extracted eosinophils without the worm present to ensure that protein concentrations are a result of the eosinophil and not the parasite.

To quantify both MBP and ECP production, half of the cells will be incubated with Ro 13-3978 and/or interleukin-5 (IL-5), a cytokine released in vivo which is known to promote eosinophil activation, according to Table 1. Following incubation, MBP and ECP levels will be determined by two separate enzyme-linked immunosorbent assays (ELISA).

In order to compare the experimental drug to other known eosinophil activating factors, cultured eosinophils from untreated mice will be incubated and stimulated with increasing concentrations of platelet-activating factor (a known eosinophil activating factor) or Ro 13-3978 according to Table 2. Concentrations of eosinophil peroxidase (EPO) can be quantified by ELISA, and expression of key proteins by quantifying gene expression used in degranulation, like Raf-1 or the membrane-associated protein 3, can be measured by quantitative polymerase chain reaction (qPCR). If there is a significant difference between the treated and control groups at lower concentrations of PAF, it is likely that Ro 13-3978’s host-dependent mechanism mediates the reduction of eosinophil’s trigger level.

C. Project Timeline:
Day 1 and 2: All ten mice will be treated simultaneously, MBP and ECP ELISAs performed 16 hours post treatment. Day 3-6: Eosinophils from untreated mice will be magnetically separated, and EPO levels measured.

D. Student/Mentor Roles:
I will be completing each step of this experiment, including mouse treatment, magnetic separation, cell culture, functional assays, and ELISA, under guidance from Dr. Paul H. Davis. In the pursuit of excellence and professionalism, I will analyze all results and review procedures with Dr. Davis weekly during lab meeting. Additionally, Dr. Davis will advise me during the preparation of the final report and presentation.
BUDGET JUSTIFICATION

Student stipend: $500
This stipend will help to support the student to complete the work on this project.

Mice: $500
This project will require at least 20 male mice. Two additional mice will be purchased in case of accidental death or any other unforeseen issues during treatment.

Cell separation supplies: $500
Sample separation will be performed using the autoMACS Pro Separator in the Flow Cytometry Research Facility of the University of Nebraska Medical Center. The requested amount will be used to purchase the reagents necessary to isolate eosinophils for subsequent analysis. Additionally, we will purchase the columns, buffer reagents, and antibodies for the instrument.

Tissue culture and mouse supplies: $1000
Suspensions of Ro 13-3978, the supplies to administer the drug safely to the mice (including oral gavage syringes, filters, and pipettes), cell culture supplies (including media and flasks), and the assay kits are essential to maintaining healthy eosinophils which can be further assayed.

All other supplies, including those to provide personal safety (namely gloves and disinfectants), and any costs incurred exceeding the amount provided by this grant will be provided by Dr. Paul H. Davis’ existing laboratory funds. Additionally, Dr. Davis will provide the space and instruments to perform all assays.

Total Amount Requested: $2500
REFERENCES

This letter is written in support of SAMANTHA SACK, who is pursuing a FUSE project in my laboratory. I have known this student for 7 months, and am confident in their ability to perform work in my laboratory.

Samantha joined my lab in May 2017, shortly after I hired her as one of the Health Careers Resource Center interns. She was unsure of her choice in medicine, and wanted to experience biomedical research prior to applying to medical school. In her time since, she has decided against medicine and has committed to her lab work. Recently completing a large scale experiment wherein she utilized many of the necessary skills outlined in this proposal, I am confident she has the ability and motivation to perform the described project. Additionally, she performed the beginning portion of this experiment, wherein she determined both EDN and superoxide anion levels of separated eosinophils as described in the introduction.

As the lab PI, I will ensure that all appropriate safety measures are taken, and that the student will have adequate training, both safety and topically, to complete the work they propose to do. I have read the Project Description, drafted by the student, and I believe it accurately captures the project goals and methods, and is achievable by the student within the stated timeframe. I assert that the student was the primary author of this proposal (except for the budget section), and received only editorial assistance from me throughout the writing process.

As indicated in the budget section, if FUSE funds are insufficient to complete the project, my lab has sufficient resources to cover additional expenses.

I fondly support the FUSE concept, its administrators, and its students, and I am most appreciative of the opportunity to engage with undergraduate students in important and original scientific research. I believe it is a prominent attraction to UNO. Please contact me with any additional questions.

Sincerely,
Paul H. Davis, Ph.D.
Assistant Professor of Biology
University of Nebraska at Omaha
pdavis@unomaha.edu
402-554-3379