

Peer Review

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Application ID:
19PRE34450056

PI Name: Joshua Wesalo
Institution: University of Pittsburgh
Project Title: Spatially controlled Nox2 inhibitors for the targeted treatment of reperfusion injury in myocardial infarction

Reviewer 1

Brief Summary of the Proposal

This proposal is submitted by Joshua Wesalo, a third-year graduate student in the Medical Scientist Training Program, Department of Chemistry at the University of Pittsburgh. The applicant's career goal is to become a translational MD/PhD in cardiology. The research strategy proposes a novel approach for treating myocardial ischemia reperfusion (I/R) injury that uses a boronic ester-based mechanism to activate a Nox2 inhibitor that can be targeted to tissues with oxidative stress such as occurs with I/R injury. Aim 1 proposes to optimize H₂O₂-responsive Nox2 inhibitors and test three of them in cardiomyocytes isolated from male Sprague-Dawley rats. Aim 2 proposes to test the most promising inhibitor developed in aim 1 in a mouse model of myocardial I/R injury.

Potential Impact of Proposed Work on the AHA Mission

High: If successful, the proposed studies have the potential to significantly impact the mission of the AHA as the study is designed to develop a treatment with minimal side effects that is designed to limit ischemia reperfusion injuries.

Proposal Criteria Evaluations

Criterion 1 - Applicant:

The applicant's goal is to become an MD/PhD. He has completed his preclinical training in medical school and the USMLE step 1 and he now plans to complete the proposed studies for his dissertation. The applicant's academic record is excellent and his letters of recommendation are exceptionally strong.

The applicant developed the idea for the current proposal based on his prior research experiences. There is concern that the proposed studies may be overly ambitious for a two-year award as the applicant has only one manuscript in preparation (as second author) from his prior undergraduate studies at F&M and two publications in preparation from his current doctoral research. This slow rate of productivity may be attributed to his high courseload during his preclinical training period, the difficulty in isolating and purifying compounds as noted by one recommender, as well as the nature of the study; requiring intellectual property protection prior to publication.

Criterion 2 - Sponsor/Training Plan and Environment:

The primary sponsor is Dr. Alexander Deiters, a Professor at the University of Pittsburgh specializing in developing chemical tools to address biological problems. Drs. Dutta and Weber have also agreed to collaborate with Joshua on the proposed studies.

The sponsor has the expertise to direct the proposed research training and has an excellent funding and publication record (>125 publications). The sponsor also has an excellent record of mentoring with 16 current students and 13 past doctoral students in addition to 2 past MS students and 3 postdoctoral fellows in the past 3 years. Funding is available to support the proposed studies.

The sponsor has developed a training plan consistent with the applicant's goals of becoming an MD/PhD. Included in the training plan are weekly lab meetings, presenting research at conferences, enrollment in a Medical Scientist Training Program and a Longitudinal Clinical Clerkship program.

The research environment is excellent and includes collaborative research with Drs. Dutta and Weber, which is a strength of the proposal.

Criterion 3 - Research:

The goal of the proposed research is to develop a novel drug that can specifically target tissues with elevated H₂O₂ and turn on an inhibitor of Nox2 only in those tissues, thereby limiting side effects. The drug will be caged by boronic esters that are only removed when exposed to elevated H₂O₂, thus unmasking the active drug that inhibits Nox2. Letters from collaborators indicate willingness to train and assist with the experiments involving cell cultures and mouse models of I/R injury. The applicant will be responsible for completing all studies with assistance from the collaborators.

Aim 1 proposes to use 6- week old Sprague Dawley rat pups for the development of primary cardiomyocyte cultures and aim 2 proposes to use 10-12 week-old C57BL/6J mice for in vivo experiments. The proposal would be strengthened by including an explanation as to why the same model was not used for each aim. References should be included for justification of only including male animals in the study design. The proposal would also be strengthened by including justification for the low number of animals proposed in aim 1. Consideration for other off-target sites of the inhibitor should also be mentioned.

While a strength of the proposal is the consideration of alternative approaches should aim 1 prove challenging, a significant weakness of the proposal is that aim 2 depends on the development of an inhibitor in aim 1. Thus, there is additional concern the project may be overly ambitious for a 2-year award.

Summary of discussion

Reviewer 2

Brief Summary of the Proposal

Potential Impact of Proposed Work on the AHA Mission

Although percutaneous intervention can improve blood flow in infarcted myocardium in patients with acute myocardial infarction, reactive oxygen species production during reperfusion can induce additional cardiomyocyte death, arrhythmias, and heart failure. This proposal will optimize hydrogen peroxide (H₂O₂)-responsive Nox2 inhibitor for selectively preventing acute and chronic cardiac injuries. This application is a highly interesting study and has significant impact to the AHA mission.

Proposal Criteria Evaluations

Criterion 1 ' Evaluation of the Applicant

The applicant, Joshua Wesale obtained his bachelor degree at Franklin & Marshall College in 2013. In 2014, the applicant enrolled in the MD/PhD program at the University of Pittsburgh, School of Medicine. Now the applicant is a third-year PhD student. His long-term goal is to become a translational physician-scientist in cardiology. The applicant has an excellent academic record. In last three years, the applicant was trained to build some skills for developing new chemical approaches to solve biological problems. The applicant has presented his studies in local conferences 9 times. The applicant did not have the publications as the first-author or co-author. Considering the lack of the applicant's background on the cardiovascular research field, the applicant invited two collaborators on the proposal. There are the strongest support letters from three referees. The sponsor, Dr. Alexander Deiters thought the applicant is a highly qualified candidate for the AHA fellowship award and possesses all the qualities and skills to become an independent physician-scientist.

Criterion 2 ' Sponsor/Training Plan and Environment

The sponsor, Dr. Alexander Deiters is currently a professor at University of Pittsburgh. Dr. Deiters's research is focusing on developing innovative chemical tools to address biological problems. Dr. Deiters has a good and clear track record for publications and research support. Dr. Deiters's research Currently is supported by an R01, an R21, and one funding from national science foundation with one-year remaining on them. The sponsor has a history of training predoctoral trainees (41 predoctoral trainees) and postdoctoral fellows (12 trainees). The sponsor clearly understand the applicant's long-term goal to be a translational physician-scientist in cardiology. The comprehensive training plan is well described. Two collaborators will guide and assist the applicant to conduct proposed cell culture experiments and cardiac functional measurements in cardiac ischemia-reperfusion model. Facilities and resources are suited for the execution of the specific aims in this proposal. Considering 16 current trainees in the sponsor's laboratory, one concern is whether the sponsor has enough time to guide the applicant smoothly to complete this proposal on time.

Criterion 3 ' Evaluation of the Proposal

Percutaneous intervention (PCI) is an effective treatment to recover blood flow in the infarcted myocardium in patients with acute cardiac infarction. However, a big problem is that reactive oxygen species overproduction during ischemia-reperfusion also induce additional cardiac injury, cardiac arrhythmias, and heart failure. In the proposal, the first specific aim is to optimize H₂O₂-activated Nox2 inhibitors and test them in cardiac myocytes. The second specific aim is to test H₂O₂-activated Nox2 inhibitors in preventing acute and chronic cardiac injury through selective target of the infarcted myocardium in cardiac ischemia-reperfusion mice. This predoctoral application will explore hydrogen peroxide-activated Nox2 inhibitor to locally reduce ROS production and prevent acute and chronic cardiac injuries. This is a well written and very interesting proposal. Selectively targeting ROS production in the infarcted myocardium is innovative. The hypothesis to be tested is clearly defined and well supported by the preliminary data. The proposed approach including the experiments at the chemical, cellular, and integrative levels insures the expected results and broad interpretation. The applicant's training and participation of two collaborators ensure the feasibility of the planned approach. There are a few concerns: 1) other sources of ROS production such as mitochondria respiration, and xanthine-xanthine oxidase activation, and uncoupling of nitric oxide synthase are ignored; 2) to achieve

robust and unbiased results, the applicant should mention to use male and female mice in the proposal. 3) After H₂O₂-activated Nox2 inhibitor blocks Nox2 activity and decreases H₂O₂ level in the infarcted myocardium, how is the Nox2 inhibitor activated and the effect of the Nox2 inhibitor remained? Is all of the Nox2 inhibitor activated by initial H₂O₂ overproduction?

Summary of discussion

Reviewer 3

Brief Summary of the Proposal

See Reviewer 1

Potential Impact of Proposed Work on the AHA Mission

The proposal has a potentially high impact on the AHA mission by studying a new therapy for ischemia reperfusion injury after myocardial infarction.

Proposal Criteria Evaluations

Criterion 1 ' Evaluation of the Applicant

The applicant is an exceptional MD PhD student at Un of Pittsburgh. He is very accomplished with multiple honors. The letters or recommendations are exceptional and outstanding. The candidate has worked on a prior research project to treat GM3 synthase deficiency in the Amish. Only weakness is the absence of any publication thus far but this has been addressed by the mentor.

Criterion 2 ' Sponsor/Training Plan and Environment

Excellent environment and training plan. Only concern is the large number of predoctoral trainees in the laboratory of the sponsor.

Criterion 3 ' Evaluation of the Proposal

Strengths:

- Interesting concept
- Appropriate collaborations to ensure success
- Innovative
- Logical layout of experiments

Weaknesses:

- proposed inhibitor has not yet been developed
- no expertise in the IR injury mouse model
- not clear how the dose of the proposed molecule for the in vivo experiments will be determined
- off target effects will be evaluated at 24 hours. It seems that this time point is chosen for convenience with no other rationale or preliminary data to justify this particular timepoint
- rationale for the chronic treatment unclear

Summary of discussion

There was substantial enthusiasm for this application. The application was considered of high impact with its focus on myocardial infarction. The applicant was considered an exceptional 3rd year medical student enrolled in the Medical Scientist Training Program at the University of Pittsburgh. The Sponsor was considered excellent as well with appropriate expertise to direct the research and train the applicant and is well funded to support this research. Concerns were raised regarding the high number of trainees in the laboratory and if this will negatively impact the training environment. Weaknesses were noted in the research plan as outlined by the reviewers. Significant concern was raised about the connection between the Aims since if an adequate inhibitor is not developed in Aim 1, then Aim 2 cannot be performed.