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TITLE: Glutamate receptor and Kynurenine pathway functioning in the pathobiology of Gulf War Illness

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14. ABSTRACT

This project examines inflammatory pathway biomarkers obtained from cerebrospinal fluid (CSF) collected from 46 1990-1991 Gulf War veterans with (cases) and 23 without (controls) Gulf War Illness (GWI) (AIM 1), and test involvement of N-methyl-D-aspartate receptor (NMDAR) functioning in GWI by administering a single dose of ketamine to 19 cases and 19 controls (aim 2) while measuring EEG for NMDAR target engagement and assessment of GWI symptoms up to one week after the infusion. In 2019 we focused on subject recruitment for aim 1. Using the Gulf War Registry, advertisements and outreach to veterans at the local VA medical center, we recruited 16 subjects for the study in 2019. Of those, 13 (10 cases) completed study procedures, with 7 subjects (4 cases) providing a sample of CSF.

15. SUBJECT TERMS

Inflammation; kynurenine pathway; quinolinic acid; microglia; astrocytes; symptoms; Gulf War Illness; ketamine; cerebrospinal fluid; subject recruitment; protocol amendment

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1. INTRODUCTION:

This project has 2 aims: (i) examine the involvement in GWI of an inflammatory state and possible consequences on neuronal and glia functioning using biomarkers obtained from cerebrospinal fluid (CSF) in 1990-1991 Gulf War veterans with (n=46) and without (n=23) Gulf War Illness, and (ii) examine involvement of glutamatergic functioning in GWI testing the effect of a single infusion of 0.5 mg/kg of N-methyl-D-aspartate receptor (NMDAR) antagonist ketamine on gamma band EEG (for NMDAR target engagement) and symptoms of Gulf War Illness in 19 cases and 19 controls. Outcomes will provide evidence of an expected pro-inflammatory state in cases that could predispose to neuronal damage via NMDAR hyperactivation, and possible effects of temporarily blocking NMDAR hyperactivation with a subanesthetic dose (0.5 mg/kg) of ketamine.

2. KEYWORDS:

Inflammation; kynurenine pathway; quinolinic acid; microglia; astrocytes; symptoms; Gulf War Illness; ketamine; cerebrospinal fluid; subject recruitment; protocol amendment

3. ACCOMPLISHMENTS:

What were the major goals of the project?

Aim 1 is to determine biomarkers of central inflammation in cerebrospinal fluid (CSF), and associate those biomarkers with GWI symptoms.

The goal of sub-task 1 is to obtain approval of the human subject protocol by the Baylor College of Medicine (BCM) IRB, Michael E. DeBakey VA Medical Center (MEDVAMC) R&D, and DoD HRPO that had to be reached at the end of month 6 (the end of March 2018).

The goal of sub-task 2 is to start recruitment efforts in month 7 (April 2018) which continues to the end of month 28.

The goal of subtask 3 is to start research procedures in eligible veterans, which was projected start in month 7 and continue to end of month 28. At the end of September 2019 (end of month 30), the projected number of subjects enrolled in the study for aim 1 was 69.

Aim 2 is to evaluate involvement of NMDAR functioning in GWI.

The goal of sub-task 1 is to obtain approval of the human subject protocol by the Baylor College of Medicine (BCM) IRB, Michael E. DeBakey VA Medical Center (MEDVAMC) R&D, and DoD HRPO that had to be reached at the end of month 30 (the end of September 2019).

The goal of sub-task 2 is to start recruitment efforts in month 31 (October 2019) which continues to the end of month 41.

The goal of subtask 3 is to start research procedures in eligible veterans, which was projected start in month 30 and continue to end of month 41. At the end of December 2019 (end of month 33), the projected number of subjects enrolled in the study for aim 2 was 10.

What was accomplished under these goals?

AIM 1: determine biomarkers of central inflammation in cerebrospinal fluid (CSF), and associate those biomarkers with GWI symptoms.
Sub-task 1, and subtask 2 objective 1 were completed in 2018.

Sub-task 2

Objectives 2 and 3: Veteran recruitment and identification, and research procedures. Key outcomes: We recruited no subjects in 2018. In 2019 we focused on subject recruitment for aim 1. In 2019, we send 180 recruitment letters to veterans who are in the Gulf War Registry. Fifteen agreed to participate, but 11 failed the phone prescreen. Four subjects (2%) were enrolled. As in the last period, recruitment is slow, in particular amongst controls, because individuals do not want to consent to the lumbar puncture to collect CSF. We addressed this concern by (i) amending procedures so that subjects can provide a blood sample without a CSF samples in which biomarkers in blood will be used as a proxy for inflammatory processes in the brain, (ii) placing an advertisement in Splash Clinical to complement subject recruitment through the Gulf War Registry, (iii) outreach to veterans with flyers at the Houston VA medical center, and (iv) entering into a Material Transfer Agreement (MTA) with Dr. Sullivan who collected CSF from GWI cases for her studies.

We ran an IRB-approved recruitment advertisement in Splash Clinical between September 29, 2019 and October 19, 2019. We received 75 phone calls. We were able to contact 42 veterans, 6 (14%) of whom were enrolled. Five additional veterans have scheduling conflicts. Outreach at the VA resulted in the enrollment of six subjects. Of the 16 enrolled individuals, 13 (10 cases) completed study procedures for blood and 7 subjects (4 cases) for CSF in 2019. Transfer of 5 CSF samples (all cases) will occur in 2020 when the MTA is approved by HRPO. This means that we will have 9 CSF samples for cases and 3 for controls of the projected 69 (46 cases).

We will continue recruitment for aim 1 until September 2020, the end date of the current grant. We will apply for a 12-month non-cost extension so that we can continue recruitment for aim 1 until July 2021.

AIM 2: Evaluate involvement of NMDAR functioning in GWI.
Sub-task 1.

Objective 1: Obtain approval of the human subject protocol by the Baylor College of Medicine (BCM) IRB, Michael E. DeBakey VA Medical Center (MEDVAMC) R&D, and DoD HRPO. Key outcomes: The IRB approved of aim 2 study procedures on December 11, 2019. Request for approval is submitted to the R&D and HRPO in January 2020.

Sub-task 2:

Objective 1: Subject recruitment. Key outcomes: We have a list of cases who expressed interest in the ketamine infusion for aim 2 and who consented to be contacted for new studies. These subjects will be contacted as soon as HRPO has approved the study procedures. As noted, procedures for aim 2 have been amended from the procedures described in the original study proposal of GW160077. The major change is to test

NMMDAR functioning with ketamine only in cases. We are readying the amendment for DoD approval. We apply for a non-cost extension to complete aim 2 procedures.

What opportunities for training and professional development has the project provided?

Nothing to report.

How were the results disseminated to communities of interest?

Nothing to report.

What do you plan to do during the next reporting period to accomplish the goals?

Aim 1.
1) I am in the process to apply for a non-cost extension to the end of September of 2021 to continue subject recruitment for aim 1 until the end of the non-cost extension period. This period will now overlap with the period that aim 2 study procedures will be performed. We will also place another Splash Clinical advertisement in February 2021, and we continue outreach at the Houston VA.

2) Together with co-I Dr. Lea Steele I continue to research the possibility to obtain CSF samples from existing sources. This source is likely the biobank set up under BBRAIN. In addition, samples that are left over after our analyses have been completed at the end of the non-cost extension period (the end of September 2021) will all be submitted to BBRAIN.

Aim 2.
1) We will apply for a non-cost extension for the completion of aim 2 study procedures.

2) Aim 2 procedure amendments will be submitted for DoD approval in January 2020. In January 2020 we will also submit the procedures for MEDVAMC R&D and for HRPO approval.

3) Because cases who completed aim 1 procedures have expressed enthusiasm to be enrolled in a medication study, cases who will enroll for aim 2 overlap those who completed or will complete aim 1 procedures.

4. **IMPACT:** D*escribe distinctive contributions, major accomplishments, innovations, successes, or any change in practice or behavior that has come about as a result of the project relative to:*

What was the impact on the development of the principal discipline(s) of the project?

Nothing to report.

What was the impact on other disciplines?

Nothing to report.

What was the impact on technology transfer?

Nothing to report.

What was the impact on society beyond science and technology?

Nothing to report.

5. CHANGES/PROBLEMS:

Changes in approach and reasons for change

Few prospective subjects are consenting to the lumbar puncture, the primary medium to examine biomarkers of brain inflammatory pathways in this study for aim 1. To address this concern we amended the protocol and informed consent form, making it possible for enrolled individuals to opt out of the lumbar puncture. This decreased risk for subjects who do not want to give CSF. 2) We have amended the design of the study proposed for aim 2 to test NMDAR involvement in GWI. We changed the design from 2 groups (cases and controls) getting a sub-anesthetic dose of NMDAR antagonist ketamine (40 min intravenous infusion of 0.5 mg/kg) to a group of only cases. Ketamine can have anti-inflammatory effects, a major contributor to GWI. Based on a power analysis we need to test 21 cases to find an effect of ketamine on fatigue and pain. Involvement of NMDAR functioning in GWI is tested by changes in scalp EEG gamma band power as a non-invasive measure of engagement of ketamine with the NMDAR, as well as by testing changes at 1 day, 2 days and 7 days after a single infusion in symptoms of pain, fatigue, cognition and other GWI-related complications. This design allows us to answer the same question that we originally

envisioned with two groups, and provides important pilot data for future grant applications to treatment of GWI.

Actual or anticipated problems or delays and actions or plans to resolve them

Few prospective subjects were consenting to the lumbar puncture, the primary medium to examine biomarkers of brain inflammatory pathways in this study for aim 1. To address this concern we continue to look for more partners who can share CSF obtained from cases with GWI or veteran controls. The BBRAIN biobank is one possibility.
We will apply for HRPO and DoD approval for the change in design. This could delay starting study procedures for aim 2. We currently have a list of cases who competed aim 1, consented to be recontacted for future GWI studies, and who expressed interest in enrolling in a ketamine study.

Changes that had a significant impact on expenditures

We do not foresee a significant impact on expenditures

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents

Nothing to report.

Significant changes in use or care of human subjects

Nothing to report.

Significant changes in use or care of vertebrate animals

Nothing to report.

Significant changes in use of biohazards and/or select agents

Nothing to report.

6. **PRODUCTS:** *List any products resulting from the project during the reporting period. If there is nothing to report under a particular item, state "Nothing to Report."*

Publications, conference papers, and presentations

Journal publications.

Nothing to report.

Books or other non-periodical, one-time publications.

Nothing to report.

Other publications, conference papers and presentations.

Nothing to report.

Website(s) or other Internet site(s)

Nothing to report.

Technologies or techniques

Nothing to report.

Inventions, patent applications, and/or licenses

Nothing to report.

Other Products

Nothing to report.

7. PARTICIPANTS & OTHER COLLABORATING ORGANIZATIONS

What individuals have worked on the project?

Name:	Bylinda Vo-Le, MS. No change

Has there been a change in the active other support of the PD/PI(s) or senior/key personnel since the last reporting period?

Nothing to report.

What other organizations were involved as partners?

Organization name:	Michael E. DeBakey VA Medical Center
Location of organization:	Houston, TX
Partner's contribution to the project:	Facilities: study staff uses the partner's facilities for subject recruitment and project activities. Collaboration: we collaborate with partner's staff who also have GWI projects for bimonthly meetings to discuss subject recruitment and study progress.
Organization name:	Michael E. DeBakey VA Medical Center
Location of organization:	Houston, TX
Partner's contribution to the project:	Facilities: study staff uses the partner's facilities for subject recruitment and project activities. Collaboration: we collaborate with partner's staff who also have GWI projects for bimonthly meetings to discuss subject recruitment and study progress.

8. SPECIAL REPORTING REQUIREMENTS

COLLABORATIVE AWARDS: *For collaborative awards, independent reports are required from BOTH the Initiating Principal Investigator (PI) and the Collaborating/Partnering PI. A duplicative report is acceptable; however, tasks shall be clearly marked with the responsible PI and research site. A report shall be submitted to* ***https://ers.amedd.army.mil*** *for each unique award.*

QUAD CHARTS: *If applicable, the Quad Chart (available on https://www.usamraa.army.mil) should be updated and submitted with attachments.*

9. APPENDICES: *Attach all appendices that contain information that supplements, clarifies or supports the text. Examples include original copies of journal articles, reprints of manuscripts and abstracts, a curriculum vitae, patent applications, study questionnaires, and surveys, etc.*