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14. ABSTRACT

The effect of fumaric acid esters (DMF/MMF) on the CAA outcomes after TBI is postulated to be positive, though several discrepancies remain. Nrf2 is one of the master regulators of redox and inflammation. DMF and its metabolite MMF hold anti-oxidative and anti-inflammatory by activating Nrf2. Thus, we expect that understanding the unique and respective roles of DMF and MMF on Nrf2 on CAA neuroprotection is essential, and its validation in TBI would strengthen their potential use for the veterans and active military people. It is unclear whether DMF has superior beneficial effects over MMF or vice versa. Furthermore, whether the therapeutic window would defer from acute TBI over a repetitive concussion-like brain insults leading to CAA needs to be tested. Considering these knowledge gaps, we aim to start answering these questions using preclinical models. For this quarter, we successfully maintain/renewed the animal protocols approved by the Institutional IACUC. During the institutional COVID-19 Lab shutdown, we had to sac mice and stopped the breeding; we have recently restarted the knockouts and the generation and characterization of the new cre-flux inducible conditional knockouts.

15. SUBJECT TERMS

NONE LISTED

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1. Introduction

Subject: The effect of fumaric acid esters (DMF/MMF) on the CAA outcomes after TBI is postulated to be positive, though several discrepancies remain. Optimizing their respective effectiveness in both acute severe and mild repetitive head trauma is essential for the design of optimal TBI trials. Nrf2 is one of the master regulators of redox and inflammation. DMF and its metabolite MMF hold anti-oxidative and anti-inflammatory by activating Nrf2, and has been approved for multiple sclerosis and psoriasis. Thus, we expect that understanding the unique and respective roles of DMF and MMF on Nrf2 on CAA neuroprotection is essential, and its validation in two complementary TBI models would strengthen their potential use for the veterans and active military people. Hypothesis and Purpose: Preclinical studies raised questions, and it is unclear whether DMF has superior beneficial effects over MMF or vice versa. Furthermore, whether the therapeutic window would defer from acute TBI over a repetitive concussion-like brain insults leading to CAA needs to be tested. Considering these knowledge gaps, it is essential to determine the optimal DMF and MMF therapeutic regiment and validate their respective effectiveness in two complementary TBI models. Goals: The Aim 1 is to determine whether DMF/MMF treatment attenuates A.D. and/or CAA neurobehavioral and pathophysiological outcomes following TBI. The Aim 2 is to test whether the DMF/MMF associated CAA neuroprotective mechanisms after TBI is mediated through the Nrf2 upregulation, using global $Nrf2^{-/-}$. Thus far, we got the animals protocols approved by the Institutional IACUC, and then by the ACURO, we got the CCI protocol standardized, we started the breeding to generate enough the global knockout and also started the generation and characterization of the new cre-flox inducible conditional knockouts for the Aim 3. Though, during the institutional COVID-19 Lab shutdown, we had to sac all mice and stopped the breeding, we have only recently restarted the generation of knockouts and characterization of the new cre-flux inducible conditional knockouts.

2. Keywords

Alzheimer, Cerebral amyloid angiopathy, Fumaric acid esters, Transcription factor

3. Accomplishments

- What were the major goals of the project?

AIM 1

Subtask 2: Treat animals with DMF/MMF after TBI, do behavioral, harvest brains, and brain slicing

AIM 2

Major Task 2: Repeat the optimal conditions in the Nrf2-/- mice

AIM 3

Major Task 3: Based on the result from Aim 2, select the first cre mice to breed with the Nrf2fl mice (and compare results with matched controls)

- What was accomplished under these goals?

AIM 1

So far, we have used 212 mice for AIM 1. CRND8 mice have a tendency to spontaneously die, so many mice did not survive the length of the entire experiment. 160 CRND8 mice were successfully genotyped. We used 77 TgCRND8 and 83 NTgCRND8 mice. We used 50% male CRND8 mice and 50% female CRND8 mice. 181 CRND8 mice received LFAO injection, vehicle injection, or no injection. Of these mice, 149 received CCI and 100 mg/kg body weight MMF daily for 3 months. Of the mice who received CCI and MMF, 45 were survived for 3 months post-surgery, and 22 were survived for 6 months post-surgery. In total, 54 brains were harvested from CRND8 mice who underwent LFAO/control injection, CCI, and 100 mg/kg MMF treatment for 3 months. These brains will be sliced, stained with Cresyl violet, and quantified in Fall 2020. In the future, we will use more CRND8 mice to administer LFAO or vehicle injection, perform CCI, sham surgery, or no surgery, and administer methylcellulose control daily for 3 months. In the future, we will use more CRND8 mice to administer LFAO or vehicle injection, perform sham surgery or no surgery, and administer MMF or DMF daily for 3 months. Also, 16 CRND8 mice received P0/P1 10 uM LFAO ICV injections, double perfusion was performed, and their brains were harvested. Of these 16 mice, 8 were transgenic, and 8 were non-transgenic. These brains will be silced and stained with s-thioflavin and quantified to assess the development of CAA.

Furthermore, 9 CRND8 mice received LFAO injections of different concentrations at 2 months of age. 6 of these mice received ICV injections, and 3 of the mice received hippocampal injections. Their brains were harvested 2 months later and were sliced and stained with S-Thioflavin to assess the development of CAA.

These brains will be quantified, and CAA development will be correlated with LFAO injection concentration in Fall 2020.

Subtask 2: Treat animals with DMF/MMF after TBI, do behavioral, harvest brains, and brain slicing.
We treated 5 mice with the vehicle, 5 mice with DMF, and 5 mice with MMF.

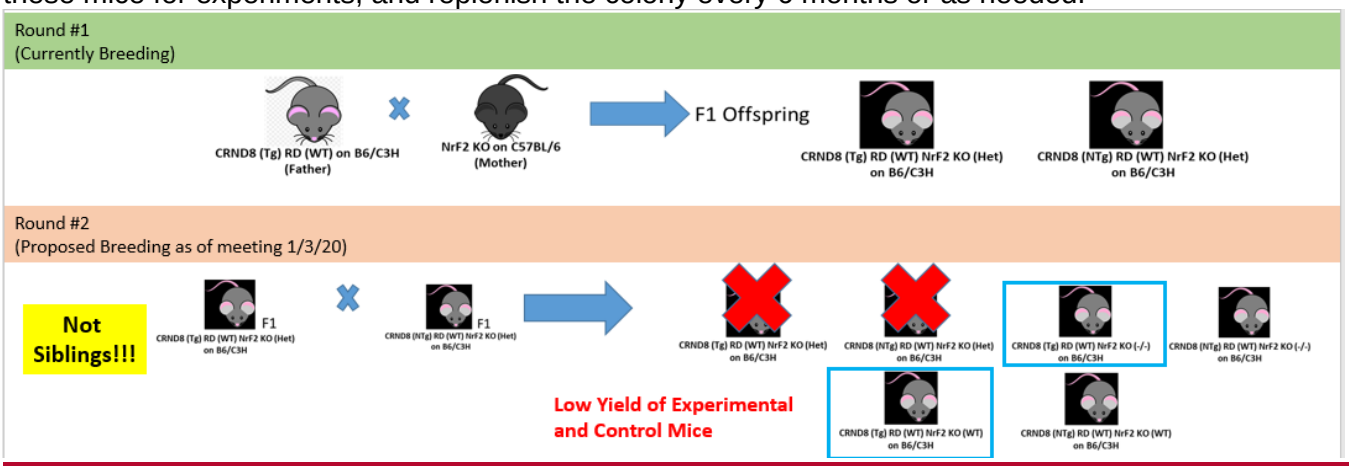
Due to COVID-19, we had to sacrifice all mice before we were able to perform the behavioral studies.

The brains have been harvested, and we are in the process of slicing them. Since we used undergrads, this has been slowed down because of COVID19; it will resume as soon as students are coming back.

We also did CCI in males and Females CRND8 and non-transgenic (n=~13 per group), and we are now letting them age. Though because of the COVID-19 UF Lab Shutdown, we have to sac all mice; we perfused and saved the ones we could before the institutional shutdown.

AIM 2
Major Task 2: Repeat the optimal conditions in the Nrf2-/- mice

We had to sacrifice the CRND8 x Nrf2-/- colony due to poor breeding outcomes. However, the brains from these animals have been harvested and will be used by staff to practice slicing before slicing the experimental brains from AIM 1 in order to ensure that the experimental brains will be sliced properly and can be stained and quantified. We discovered that we will have better breeding outcomes in the F1 generation if the CRND8 gene comes from the male mouse. We are restarting the CRND8 x Nrf2-/- colony using 5 CRND8 male mice and 5 Nrf2-/- female mice. We will recreate the F1 generation using better breeding methods, genotype the mice, use these mice for experiments, and replenish the colony every 6 months or as needed.



As a plan for this, we have also already started the proper breeding strategy and colonies to have the mice needed.

We generated from test/preliminary data to look at the W.T. and Nrf2-/- outcomes following CCI. Happy to report that these Nrf2-/- do survive following the TBI.

For the Nrf2-/-, we treated 5 mice with vehicle, 5 mice with DMF, and 5 mice with MMF.

No behavioral data due to COVID-19.

The brains have been harvested, and we are in the process of slicing them. Since we used undergrads, this has been slowed down because of COVID19. All undergrads are still not allowed to work in the lab. Thus, we have recruited 1 OPS Technician who was previously in the lab.

AIM 3
Major Task 3: Based on the result from Aim 2, select the first cre mice to breed with the Nrf2fl mice (and compare results with matched controls)

As we plan for this, we are actively continuing the breeding of Nrf2flox mice with the various cre mice. We started with the cre neuronal, cre astrocytic, and cre microglial.

As these are inducible knockouts (which reduced compensatory mechanisms as compared to neonates cre-flox). We have now set up the optimal protocols for the tamoxifen treatment to induce the recombination.

We also started the characterization for the efficiency of the recombination (this is mostly done by PCR; we have now established standard protocols).

We are pursuing the breeding strategy as planned, and the F1 generation has already been crossed and weaned. We now need to genotype for setting up the breeders for experimental mice; this is being slowed down because of COVID19. Since we used undergrads, this has been slowed down because of COVID19. All

undergrads are still not allowed to work in the lab. Thus, we have recruited 1 OPS Technician who was previously in the lab.

- 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
Subtask 2: Treat animals with DMF/MMF after TBI, do behavioral, harvest brains, and brain slicing
Subtask 3: Analyze lesion volume and behavioral
Subtask 4: Perform various stainings/assays, do quantifications, and complete analyses
Subtask 5: Monitor toxicity at the different doses
Continue all these subtasks

AIM 2
Major Task 2: Repeat the optimal conditions in the Nrf2-/- mice
Continue the breeding strategy to have the mice needed. We have now recruited 1 tech to restart fully.

AIM 3
Major Task 3: Based on the result from Aim 2, select the first cre mice to breed with the Nrf2fl mice (and compare results with matched controls)
Continue to breed and complete the characterization of these unique cre-flox mice. Such that once needed, then we have the mice ready for the planned protocol. Though because of the COVID-19 UF Lab Shutdown, we had to sac many animals. We have now recruited 1 tech to restart entirely.

4. Impact
- 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
Nothing to Report.

6. Products
Nothing to Report.

7. Participants & Other Collaborating Organizations
- What individuals have worked on the project?

Name:	*Sylvain Doré, PhD, FAHA*
Project Role:	*P.I.*
Researcher Identifier (e.g. ORCID ID):	0000-0003-3771-5109
Nearest person month	1
Contribution to Project:	*S.D. has been managing the project and coordinated the breeding, etc.*
Funding Support:	*Nothing to Report*

Name:	*Yona Levites, PhD*
Project Role:	*Co-I*
Researcher Identifier (e.g. ORCID ID):	0000-0001-6925-4525
Nearest person month	1
Contribution to Project:	Y.L. is assisting with the mouse injections, and the use of the CRND8 mice
Funding Support:	*Nothing to Report*

Name:	*Abdullah S. Ahmad, PhD*
Project Role:	*PhD Scientist*
Researcher Identifier (e.g. ORCID ID):	0000-0002-8368-2443
Nearest person month	1
Contribution to Project:	ASA is assisting with the TBI protocols, He was recruited for promotion by another institution as of Sept. 7th. We are now posted the jobs and have identified a senior postdoc with appropriate experience.
Funding Support:	*Nothing to Report*

Name:	*Kristy Dillon*
Project Role:	*Biological Scientist*
Researcher Identifier (e.g. ORCID ID):	*Nothing to Report*
Nearest person month	1
Contribution to Project:	K.D. is providing tech support for the mice
Funding Support:	*Nothing to Report*

Name:	*Madison Fangman*
Project Role:	*Lab Technician*
Researcher Identifier (e.g. ORCID ID):	*Nothing to Report*
Nearest person month	1
Contribution to Project:	M.F. is providing lab support for the various protocols.
Funding Support:	*Nothing to Report*

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

ASA was recruited for promotion by another institution as of Sept. 7th. We are now posting a Senior Level Position, and have identified a senior postdoc with appropriate experience. We have also been recruiting 1 other additional full-time technician to assist in catching up with the proposed aims; the tech was previously trained in the lab with more than 1.5-2yrs of experience.

- What other organizations were involved as partners?

Nothing to Report.

8. Special Reporting Requirements

Nothing to Report.

9. Appendices

Quadchart