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TITLE: A Novel Nonsurgical Approach to Treat Post-Traumatic Hydrocephalus

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CONTRACTING ORGANIZATION: Research Foundation for The State University of New York
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13. SUPPLEMENTARY NOTES

14. ABSTRACT The main objective of this exploratory proposal is to develop and validate a novel pharmacological strategy for the management of hydrocephalus caused by traumatic brain injury (TBI). Our ***objective*** in this application is to prove/refute the central role played by efflux transporters (ABCB1 and/or ABCG2) in the development of PTH. Our ***central hypothesis*** is that macromolecules are eliminated out of the brain primarily by efflux transporters (ABCB1 and/or ABCG2) and a slower clearance results in PTH due to excess macromolecules remaining in the ventricular CSF.
Our experiments done so far are supportive of our central hypothesis that macromolecules are eliminated out of the brain by ABCB1. Our results show that ABCB1 knockout animals had a statistically significant increased ventricular volume and increased clearance times for macromolecules compared to normal animals using intraventricular hemorrhage hydrocephalus model. ABCB1 overexpressed animals had a statistically significant smaller ventricular volume and faster clearance times for macromolecules compared to normal animals using intraventricular hemorrhage hydrocephalus model. Further experiments to get the numbers to 12 in each group needs to be done. In addition, we have to conduct experiments to evaluate the role of other efflux transporters (ABCG2).

15. SUBJECT TERMS
Post-traumatic hydrocephalus, efflux transporters, intraventricular hemorrhage, ABCB1, ABCG2, Macromolecular transport, MRI

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1. Introduction:

The main objective of this exploratory proposal is to develop and validate a novel pharmacological strategy for the management of hydrocephalus caused by traumatic brain injury (TBI). Evidence indicates that posttraumatic hydrocephalus (PTH) is a result of osmotic fluid influx into the ventricles as a result of excess macromolecules due to IVH. Clearance of these macromolecules will resolve hydrocephalus and impaired clearance will sustain or promote hydrocephalus. Our ***objective*** in this application is to prove/refute the central role played by efflux transporters (ABCB1 and/or ABCG2) in the development of PTH. Our ***central hypothesis*** is that macromolecules are eliminated out of the brain primarily by efflux transporters (ABCB1 and/or ABCG2) and a slower clearance results in PTH due to excess macromolecules remaining in the ventricular CSF. Establishing the central role of efflux transporters in the genesis of PTH will be a significant advance in the pursuit of pharmacological treatment of this disorder. This proposal has two major Specific Aims:1) Utilize a genetic approach to clarify the involvement of ABCB1 and ABCG2 in the clearance of intraventricular hemorrhage products and 2) Utilize pharmacological manipulation of ABCB1 and ABCG2 to modify the severity of hydrocephalus after IVH.

2. Keywords

Post-traumatic hydrocephalus, efflux transporters, intraventricular hemorrhage, ABCB1, ABCG2, macromolecular transport, MRI

3. Accomplishments

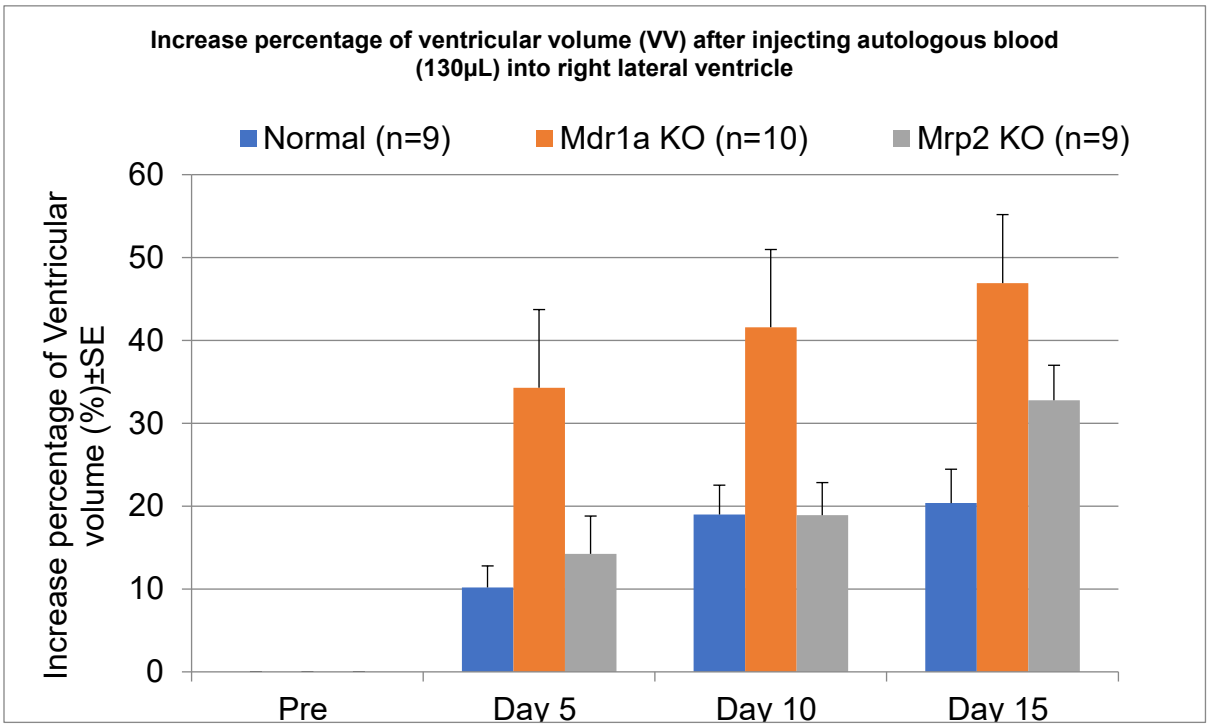
Major goals of the project and accomplishments under these goals:
Major task 1: Obtain local and USAMRMC approvals for animal and exempt human subject studies: (1-6 months)
Subtask 1: Submit the amendment institutional IACUC approval.
We were able to send the amendment of this IACUC application and get approval from the Wayne State University.
Subtask 2: Submit the documents for ACURO approvals
We were able to obtain the ACURO approvals to start the study on August 10, 2021.
Both the milestones listed on the SOW were achieved.

Research-Specific Tasks:
SA1: Utilize a genetic approach to clarify the involvement of ABCB1 and ABCG2 in the clearance of IVH products.
Subtask 1: Contact animal company to estimate the time when specific Mdr1a, Mrp2, Mdr1a plus BCRP and ABCG2 knockout rats are available. (seventh month)
We have found out that it will take 6 weeks of Envigo (Animal Company) for animal ready to deliver all the above genetically modified rats once we place the order.

Subtask 2: Prepare material and contact Wayne State University DLAR for animal work and setup 7T MR schedule. (seventh month)
We are able to book the 7T MRI schedule online for at least three days of the week starting from April 2022 and we plan to schedule them once the order is ready to ship.

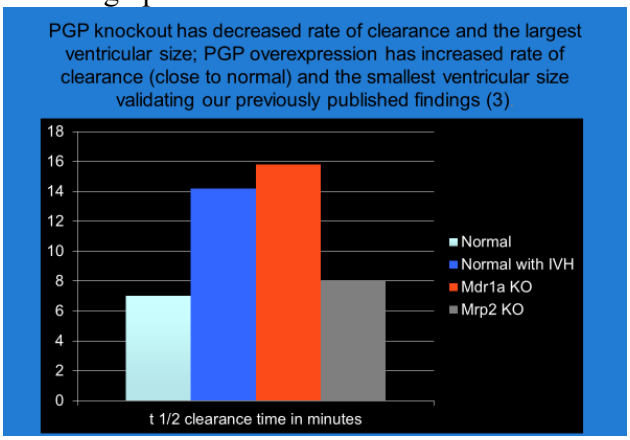
Subtask 3: Carry on experiment 1 and 2. Inducing hydrocephalus in animals and performing MRI scan at different time points (Pre-, 5 days, 10 days and 15 days post hydrocephalus induction) (n=72) (7-12 months)
Experiment 1: Determine the role of ABCB1/PGP in the clearance of IVH products:
Although we could not do any experiments following the DOD ACURO approval, we have performed some experiments utilizing funds outside of this DOD grant but relevant to this particular experiment PRIOR to receiving

the DOD grant funding. Intraventricular hemorrhage was induced using autologous blood in normal rats (n=8), Mdr1a knockout rats (n=10) and Mrp2 knockout rats (n=9). MRI scans were performed at different time points (Pre-, 5 days, 10 days and 15 days post hydrocephalus induction). We calculated the volumes of the ventricles based on the semiquantitative method using three dimensional MRI scan data in different animals at different time points. The chart below shows the results with the preinjection baseline ventricular volume for each animal recorded as zero to represent only the increase in percentage of ventricular volume after intervention.



	T-Test Pre	T-Test D5	T-Test D10	T-Test D15
Normal vs Mdr1a KO		0.032755114	0.0445302	0.0129235
Normal vs Mrp2 KO		0.454777471	0.98781169	0.05983696
Mdr1a KO vs Mrp2 KO		0.082501017	0.0469458	0.15306341

The numbers in red are significant although the total numbers for the experiments proposed in the DOD grant is not complete (we had proposed n=12 in each group). Further, we determined the clearance rate for iron tagged dextran from the ventricles in different groups of animals at the end of the experiments as noted in our proposal. The results are noted in the graph below.



From the results obtained so far, the following conclusions can be drawn.

1. Intraventricular hemorrhage model results in progressive hydrocephalus in all the animals with an increase in ventricular volume between 20 and 50%.
2. P-glycoprotein knock out (Mdr1a KO) animals had a statistically significant ($p<0.05$) increased ventricular volume compared to normal animals. The clearance times were also increased compared to normal animals without intraventricular hemorrhage (data from prior experiments).
3. P-glycoprotein overexpressed (Mrp2 KO) animals had a statistically significant ($p<0.05$) smaller ventricular volume compared to the p-glycoprotein knock out animals on day 10 but not on day 15. The clearance times for these overexpressed animals were closer to normal animals without intraventricular hemorrhage (data from prior experiments).

The results obtained so far are supportive of our central hypothesis that macromolecules are eliminated out of the brain primarily by efflux transporters (ABCB1 and/or ABCG2) and a slower clearance results in PTH due to excess macromolecules remaining in the ventricular CSF.

Opportunities for training and professional development:

Nothing to report.

How were the results disseminated to communities of interest?

Nothing to report as yet. We plan to disseminate the results by means of presentation at scientific conferences and publication of the results in scientific journals.

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

We are in the process of getting ready to do the experiments by acquiring the animals noted above and scheduling the MRI scans for our experiments at Wayne State University. We should be able to accomplish our tasks this next year as long as there are no interruptions from further COVID pandemic related closures.

4. Impact

What was the impact on the development of the principal discipline of the project?

Hydrocephalus from brain injury is a poorly understood disorder that causes significant cognitive and physical damage in patients of all ages. Hydrocephalus is commonly treated with surgery to implant a tube (called the shunt) into the brain cavities (ventricles) and divert the fluid from the brain into the abdomen (peritoneal cavity where all the intestines reside). The research that we are conducting is to understand why blood from the brain injury results in hydrocephalus. Our hypothesis is that blood products are cleared from the brain by special transporters on the cells called efflux transporters (ABCB1 and/or ABCG2 which are the names of the efflux transporters). We are investigating this possibility by using rats that have genetically poorly functioning efflux transporters and rats that have genetically over functioning efflux transporters. We create hydrocephalus in these animals by injecting blood into the brain ventricles in three groups of animals. Rats with poorly functioning efflux transporters had severe hydrocephalus and rats with over functioning efflux transporters had milder degree of hydrocephalus compared to normal rats. This suggests that efflux transporters are primarily responsible for clearance of blood from the brain. This is truly exciting and groundbreaking if the rest of the experiments that we planned to do this coming year yield similar results. Efflux transporters can be made to function better with medications and can pave the way for non-surgical treatment of hydrocephalus.

What was the impact on other disciplines?

Macromolecular transport is involved in a variety of neurological disorders including storage disorders, Alzheimer's disease and metastatic cancer. In addition, hydrocephalus has been reported in long term spaceflight. It is possible that understanding the role of macromolecular transport in and out of the brain can lead to better understanding and treatment of other neurological disorders of the brain.

What was the impact on technology transfer?
Nothing to report.

What was the impact on society beyond science and technology?
Understanding the underlying mechanisms that lead to hydrocephalus can pave the way for treating this disorder using medications instead of multiple shunt surgeries. Current shunt systems do not have a warning system when they are not working and this can lead patients with shunts and their families through distress as they live under constant fear of failed shunt needing emergent surgery. A medication treatment can make the quality of life better for patients with shunted hydrocephalus.

5. Changes/Problems

Changes in approach and reasons for change: None

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

Due to the COVID-19 pandemic, our ability to do experiments were hampered. There was temporary closures of the labs at SUNY Upstate Medical University as well as the MRI scanner at Wayne State University. These problems have since been resolved and we plan to go ahead with our experiments in an expeditious manner according to the plans.

Significant changes in use or care of human subjects, vertebrate animals, biohazards, and/or select agents.
None.

Significant changes in use or care of human subjects: Not relevant to this proposal. No human subjects involved.

Significant changes in use or care of vertebrate animals: None

Significant changes in use of biohazards and/or select agents: None

6. Products

Publications, conference papers and presentations: None

Website(s) or other Internet site(s): None

Technologies or techniques: None

Inventions, patent applications, and/or licenses: None

Other products: None

7. Participants & Other Collaborating Organizations

Name	Satish Krishnamurthy MD, MCh
Project Role	Project Director/ Primary Investigator
Researcher Identifier (e.g., ORCID ID):	0000-0001-7808-7407
Nearest person month worked	2

Contribution to the project	Management and coordination of getting the animal protocol approvals, submission to the DOD for ACURO approval, management of the animal procurement and MRI time slots etc as listed in the SOW.
Funding support	None from this grant. Salary from SUNY Upstate Medical University

Name	Jie Li MD
Project Role	Research Scientist
Researcher Identifier (e.g., ORCID ID):	
Nearest person month worked	6
Contribution to the project	Writing and coordinating to obtain the animal protocol approvals, helping with submission to the DOD for ACURO approval, management of the animal procurement and MRI time slots etc as listed in the SOW.
Funding support	Funded from this grant 62% of his salary since July 1st, 2021 Rest of his salary from SUNY Upstate Department of Neurosurgery funds

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: Wayne State University
Location of Organization: 818 W. Hancock Street, Detroit, MI Michigan 48201
Partner's contribution to the project:

Facilities:

Project staff use the DLAR facilities for animal housing, lab to perform experiments and MRI scanner (7T MRI) for performing MRI scans on the animals.

Collaboration:

Dr E Mark Haacke is a consultant on this project and he is going to be helping us with the interpretation of the information generated from the MRI scans of the animals.

8. Special Reporting Requirements

None

9. Appendices

None