AFRL-RH-WP-TR-2020-0146

BIOMARKERS AND TREATMENT OF HYPOBARIA—EXACERBATED TRAUMATIC BRAIN INJURY (TBI)

Alan I. Faden

University of Maryland Baltimore

OCTOBER 2020
Final Report

AIR FORCE RESEARCH LABORATORY
711TH HUMAN PERFORMANCE WING,
AIRMAN SYSTEMS DIRECTORATE,
WRIGHT-PATTERSON AIR FORCE BASE, OH 45433
AIR FORCE MATERIEL COMMAND
UNITED STATES AIR FORCE

NOTICE AND SIGNATURE PAGE

Using Government drawings, specifications, or other data included in this document for any purpose other than Government procurement does not in any way obligate the U.S. Government. The fact that the Government formulated or supplied the drawings, specifications, or other data does not license the holder or any other person or corporation; or convey any rights or permission to manufacture, use, or sell any patented invention that may relate to them.

This report was cleared for public release by the Air Force Research Laboratory Public Affairs Office and is available to the general public, including foreign nationals. Copies may be obtained from the Defense Technical Information Center (DTIC) (http://www.dtic.mil).

AFRL-RH-WP-TR-2020-0146 HAS BEEN REVIEWED AND IS APPROVED FOR PUBLICATION IN ACCORDANCE WITH ASSIGNED DISTRIBUTION STATEMENT.

BURKE.ALICIA.N.1125850452 Digitally signed by BURKE.ALICIA.N.1125850452 Date: 2022.02.18 10:30:13 -05'00'

ALICIA BURKE,
En Route Care Program Manager
Product Development Branch
Airman Biosciences Division

BEDFORD.THERESA.A.1236006359 Digitally signed by BEDFORD.THERESA.A.1236006359 Date: 2022.03.10 16:13:59 -05'00'

THERESA A. BEDFORD, Maj, USAF, NC, FNP-BC
Nurse Scientist, En Route Care
Product Development Branch
Airman Biosciences Division

This report is published in the interest of scientific and technical information exchange, and its publication does not constitute the Government's approval or disapproval of its ideas or findings.

REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. REPORT DATE *(DD-MM-YYYY)*	2. REPORT TYPE	3. DATES COVERED *(From – To)*
04-10-2020	Final Technical Report	05-04-2016 – 04-10-2020

4. TITLE AND SUBTITLE
Biomarkers and Treatment of Hypobaria-Exacerbated Traumatic Brain Injury (TBI)

5a. CONTRACT NUMBER
FA8650-16-2-6H02

5b. GRANT NUMBER

5c. PROGRAM ELEMENT NUMBER

6. AUTHOR(S)
Alan I. Faden, M.D.

5d. PROJECT NUMBER
15-017

5e. TASK NUMBER
02H

5f. WORK UNIT NUMBER

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
UNIVERSITY OF MARYLAND BALTIMORE
220 ARCH ST RM 02148
BALTIMORE MD 21201-1531

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Air Force Materiel Command
Air Force Research Laboratory
711th Human Performance Wing
Airman Systems Directorate
Airman Biosciences Division
Wright-Patterson AFB OH 45433

10. SPONSORING/MONITOR'S ACRONYM(S)

11. SPONSOR/MONITOR'S REPORT NUMBER(S)
AFRL-RH-WP-TR-2020-0146

12. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution Statement A: Approved for public release.

13. SUPPLEMENTARY NOTES
AFRL-2022-0448, cleared on 14 February 2022

14. ABSTRACT

Our studies have examined the molecular and cellular pathways activated following traumatic brain injury plus hypobaria exposure and their contribution to neuroinflammation leading to neuronal loss and neurological deficits.

We focused on key changes in extracellular vesicles, transcriptional activation of microRNA pathways and induction of pro-inflammatory genes. We also studied the effects of cell cycle inhibition using cyclin-dependent kinase (CDK) inhibitors on neuroinflammation, neuronal loss and neurological deficits after experimental traumatic brain injury+hypobaria.

Our data suggest that central and systemic (plasma) levels of key pro-inflammatory microRNAs are upregulated after TBI+hypobaria. Microparticles are also elevated in plasma after injury. Our results also suggest that TBI+hypobaria increases key pro-inflammatory genes in the cortex and hippocampus. In order to greatly increase our ability to quantitatively detect the microRNAs that play key roles in neuroinflammation responses we used the Nanostring approach, a state-of-the-art RNA analysis method. Our data have identified many microRNAs whose expression levels are modulated following TBI+hypobaria, both in the brain and plasma. These novel microRNAs should be the focus of future studies.

Our studies have also shown that administration of pharmacological inhibitors of CDKs attenuate neuroinflammation, neuronal loss and cognitive deficits after TBI+hypobaria suggesting that such drugs may serve to protect TBI patients requiring aeromedical evacuation.

15. SUBJECT TERMS
traumatic brain injury, lateral fluid percussion, cyclin-dependent kinase, Aeromedical Evacuation

16. SECURITY CLASSIFICATION OF:			17. LIMITATION OF ABSTRACT	18. NUMBER OF PAGES	19a. NAME OF RESPONSIBLE PERSON
a. REPORT	b. ABSTRACT	c. THIS PAGE			Alicia Burke
U	U	U	SAR	19	19b. TELEPHONE NUMBER *(include area code)* N/A

TABLE OF CONTENTS

LIST OF FIGURES

Figure **Page**

1.0 SUMMARY

We have examined central (cortex and hippocampus) and systemic (plasma) molecular pathways that regulate neuroinflammation following experimental traumatic brain injury (TBI) (lateral fluid percussion (LFP)) plus hypobaria in adult male rats. We also studied neuroinflammation, neurodegeneration and neurological (cognitive deficits) outcomes in the injured animals. We observed significant activation of specific central and systemic pro-inflammatory mechanisms as well as cognitive impairments in the rats exposed to TBI+hypobaria. Treatment with the cyclin-dependent kinase (CDK) inhibitors attenuated pro-inflammatory pathways, reduced neuroinflammation and neuronal loss and improved neurological outcomes after TBI+hypobaria.

2.0 INTRODUCTION

TBI is a serious global health problem and a major cause of mortality and long-term disability. Injured Active Duty Service Members (ADSMs) moved from theater are exposed to decreased atmospheric pressure (hypobaria) during Aeromedical Evacuation (AE), but effects of such pressure changes on the secondary injury after TBI have yet to be delineated.

3.0 BACKGROUND

Our previous studies have established a model of AE after TBI in which LFP rats (at 6h post-trauma) are exposed to hypobaria (576 millimeters of mercury (mmHg) for 6 hours). This model simulates the hypobaric exposure during AE while maintaining oxygenation and allowed us to characterize hypobaric exacerbation of experimental TBI in a rat LFP model. Our results have shown that exposure to hypobaria worsens long-term cognitive function and neuroinflammation[1]. MicroRNAs (miR) are small (22 nucleotides) noncoding RNAs involved in the regulation of gene expression at the post-translational level, by modulating mRNA half-life or inhibiting their translation. Recent findings have suggested that microRNAs are novel and important regulators of neuroinflammation that can dynamically regulate microglia/macrophage activation in response to injury[2]. Some microRNAs such as the pro-inflammatory miR-155, are markedly up-regulated in pro-inflammatory M1 microglia[2]. Other microRNAs such as miR-124 appear to serve as key positive regulators of microglia quiescence or the M2 “resting state” and have anti-inflammatory effects. Our previous studies have also demonstrated that cell cycle inhibition using selective CDK inhibitors such as Roscovitine and CR8 significantly attenuate secondary injury pathways in multiple models of experimental traumatic brain injury[3-5]. Central administration of Roscovitine attenuated neuronal death and microglia activation and significantly decreased lesion volume and improved cognitive recovery in a rat LFP model[5]. Roscovitine also improved functional recovery, decreased lesion volume, and attenuated hippocampal and cortical neuronal cell loss and cortical microglial activation in a mouse controlled cortical impact (CCI) model[4]. Furthermore, delayed systemic administration of Roscovitine improved motor recovery and attenuated microglial activation after CCI[4]. Central administration of CR8, at 3 h after CCI, significantly attenuated sensorimotor and cognitive deficits, decreased lesion volume, and improved neuronal survival in the cortex and dentate gyrus[3]. CR8 (cyclin-dependent kinase (CDK) inhibitor also attenuated post-traumatic

neurodegeneration in the CA3 region of the hippocampus and thalamus at 21 days[3]. Moreover, CR8 administered using a delayed systemic significantly improved cognitive performance after CCI[3]. Systemic post-LFP administration of CR8 reduced cortical, hippocampal, and thalamic neuronal loss and attenuated microglial activation in a rat LFP model[6]. CR8 treatment attenuated sensorimotor and cognitive deficits, alleviated depressive-like symptoms, and decreased lesion volume[6].

The current studies have examined effects of TBI+hypobaria on extracellular vesicles release and activation of microRNA and pro-inflammatory genes. We also studied the therapeutic effects of CDK inhibitors on neurodegeneration, microglia activation and neurological deficits after LFP+hypobaria.

4.0 METHODS

Rat lateral fluid percussion (LFP, 2 atmospheres of pressure (atm)) and hypobaric exposure (568 mmHg for 6 hours) were performed as previously described[1]. Briefly, rats were anesthetized with isoflurane (4 percent (%) induction, 2% maintenance), and a 5-mm craniotomy was made over the left parietal cortex midway between the lambda and bregma. Using a LFP; Custom Design and Fabrication, VA) device, a 2 atm were used to produce the traumatic brain injury. Hypobaria was induced using a chamber equipped with internal oxygen, carbon dioxide, and pressure gauges and connected to a vacuum pump. Animals were placed into the chamber in their home cages with access to water and food to reduce stress from acclimation to the HB chamber. Multiple animals in various groups were randomly exposed simultaneously. The chamber was de-pressurized over 30 minutes (min) to reach 568 mmHg (=8000 feet (ft) altitude)—approximating the cabin pressure during military AE with cruising altitudes of 30,000–40,000 ft. To account for the mean oxygen saturation decrease of 5.5% experienced at this pressure, 28% oxygen (O_2) was continuously delivered to the chamber to maintain pO_2 at sea level despite the drop in atmospheric pressure. Chamber gases were continuously monitored to validate concentration of O_2 delivered, as well as to verify that carbon dioxide (CO_2) was not accumulating in the chamber. At 5.5 h of "flight," the chamber was re-pressurized over 30 min to 1 atm (765 mmHg), and the animals were then removed.

Controls were represented by naive animals that received no LFP. At the indicated timepoints, the experimental were euthanized and the brain and plasma samples were generated. The levels of mRNA for pro-inflammatory molecules and microRNAs were quantified by qPCR (real-time polymerase chain reaction. The nCounter miRNA assays by NanoString were used to quantitatively screen changes in several hundreds of microRNAs. Neurological testing included cognitive (Morris water maze-MWM) assesments[7]. Animals in the treatment groups received a dose of CR8 (5 mg/kg in saline, IP) or an equal volume of vehicle (saline)[7]. Statistical analysis included: One-tailed *t* test. One-way ANOVA and Tukey's multiple comparisons test for qPCR analysis; and One-way ANOVA and Bonferroni post-hoc test for MWM.

5.0 RESULTS

5.1 Effect of TBI+Hypobaria on plasma levels of microparticles

Local and systemic inflammatory responses are initiated early after TBI, and may play a key role in the secondary injury processes resulting in neuronal loss and neurological deficits. However,

the mechanisms responsible for the rapid expansion of neuroinflammation and its long-term progression have yet to be elucidated. We have recently investigate the role of microparticles (MP), a member of the extracellular vesicle family, in the exchange of pro-inflammatory molecules between brain immune cells, as well as their transfer to the systemic circulation, as key pathways of inflammation propagation following brain trauma[8]. We concluded that MP loaded with pro-inflammatory molecules initially released by microglia following trauma can activate additional microglia that may contribute to progressive neuroinflammatory response in the injured brain, as well as stimulate systemic immune responses. Due to their ability to independently initiate inflammatory responses, MP derived from activated microglia may provide a potential therapeutic target for other neurological disorders in which neuroinflammation may be a contributing factor[8].

In the present study, we have examined the time-course of systemic (plasma) changes in MP following TBI+hypobaria. Using flow cytometry techniques, we have quantified the number of total MP in the plasma, identified using the Annexin marker. We also determined the sub-populations of MPs derived from myeloid cells (CD45+), erythrocytes (CD54+) and platelets (CD61+). Our observations suggest that while early (24h and 7 days) after TBI all MP populations are significantly decreased, their levels increase at 28 days after trauma (Figure (Fig.).1; one-tailed *t* *t*est).

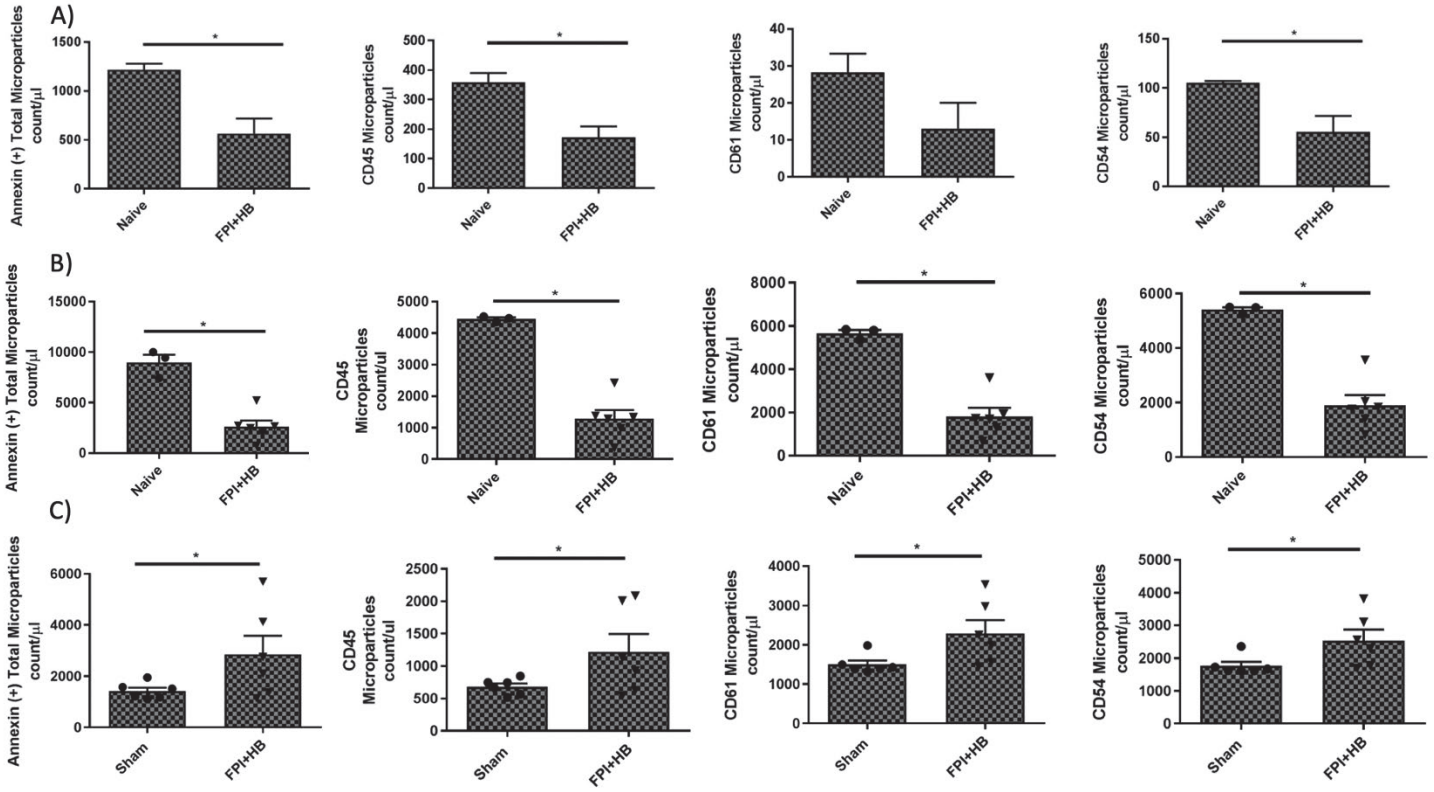

Figure 1. Change in plasma MP levels after TBI+hypobaria. * p<0.05 compared to naive

5.2 TBI+Hypobaria induces the expression of pro-inflammatory and cell death genes

We examined the changes in expression of various pro-inflammatory genes in the injured cortex after TBI+Hypobaria. We detected significant elevation of CD74, IL-1β, TNFα, IL-10, Nox2

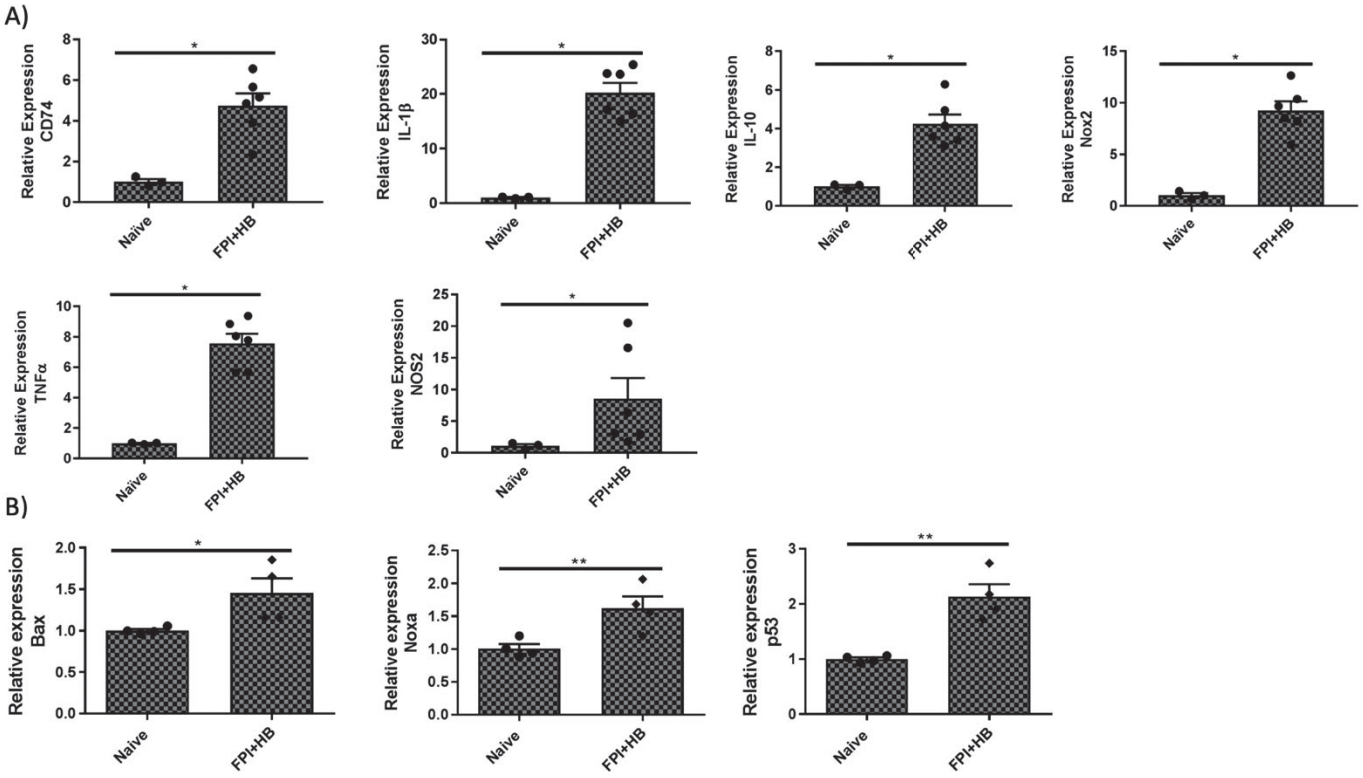

Figure 2. Activation of pro-inflammatory
(*A) and cell death genes (B) in the injured cortex at 24h after TBI+Hypobaria. * p<0.05; **p<0.01 compared to naive.*

and NOS2 (Nitric Oxide Synthase 2) suggesting a robust activation of neuroinflammation at 24h after trauma (Fig.2A; one tail *t t*est). We also detected activation of cell death genes including Bax, Noxa and p53(Fig.2B; one-tailed *t t*est).

The activation of neuroinflammation continues at 7 days post-trauma. We detected significant elevation in the expression of pro-inflammatory genes including TNFα, IL-1β, NOS2 and TGFβ in the injured cortex 7 days following TBI+Hypobaria (Fig.3; one-tailed *t t*est).

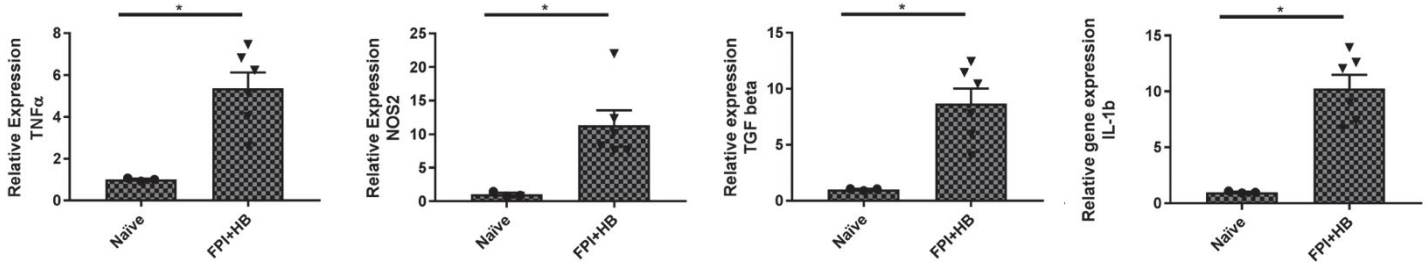

Figure 3. Activation of pro-inflammatory genes in the injured cortex at 7 days after TBI+Hypobaria. * p<0.05 compared to naïve

5.3 TBI+Hypobaria induces the expression of pro-inflammatory microRNAs in the injured brain

We have examined the expression of microRNAs known to have potent immuno-modulatory roles such as miR-155 and miR-146 believed to promote neuroinflammation and miR-124 that may attenuate neuroinflammation[8]. miR-223, has complex effects modulating inflammation that have yet to fully elucidated[9,10]. We have found that miR-155 and miR-146 are significantly elevated in the injured cortex 7 days after TBI+Hypobaria (Fig.4; one tail *t* test).

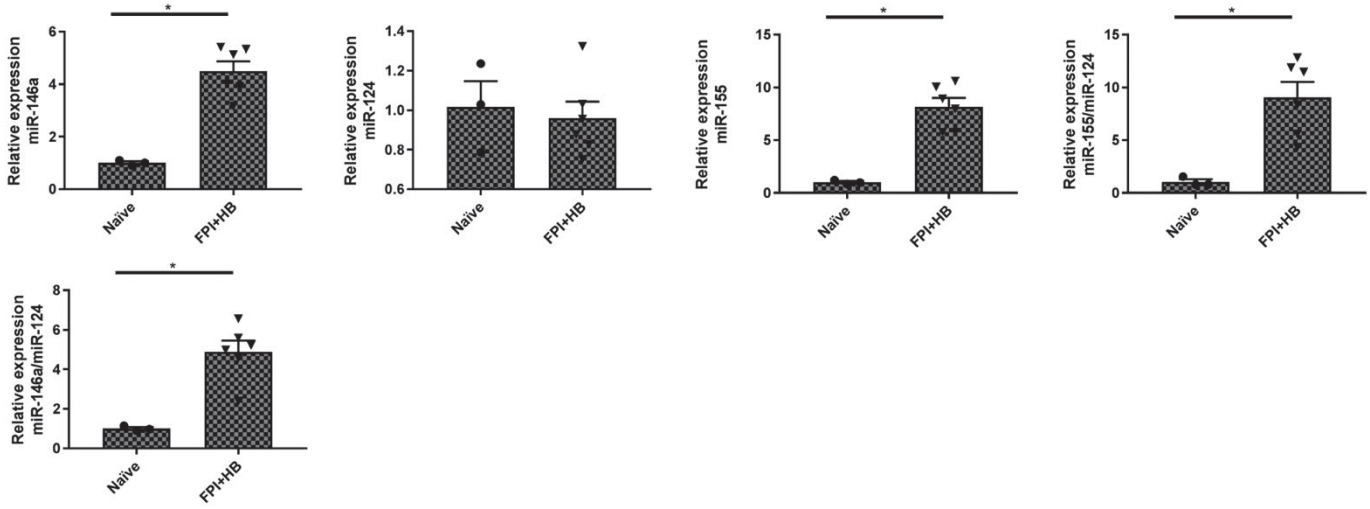

Figure 4. Activation of pro-inflammatory microRNAs in the injured cortex at 7 days after TBI+Hypobaria. * p<0.05 compared to naive

We also confirmed that significant elevation in miR-155, miR-146 and miR-124 is detected in the injured hippocampus at 7 days after TBI+Hypobaria (Fig.5; one-tailed *t t*est).

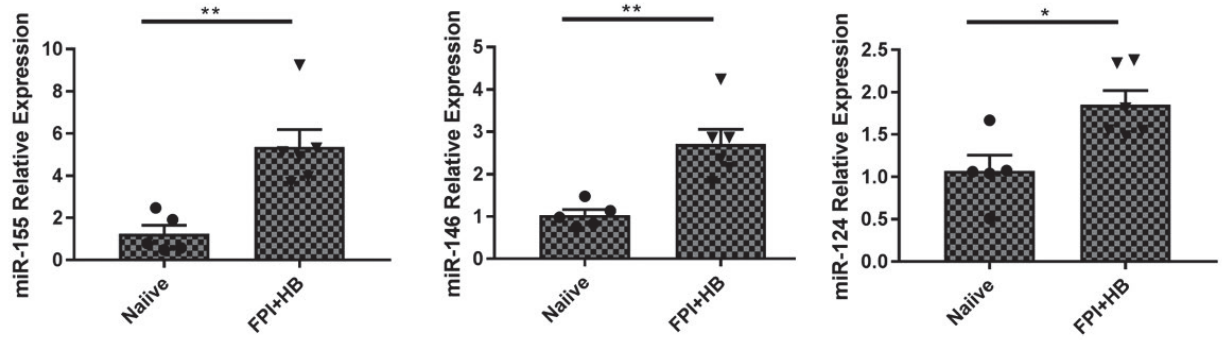

Figure 5. Activation of pro-inflammatory microRNAs in the injured hippocampus at 7 days after TBI+Hypobaria. * p<0.05; **p<0.01 compared to naive

MiR-223 is also elevated in both injured cortex and hippocampus at 7 days after TBI+Hypobaria (Fig.6; one-tailed *t t*est).

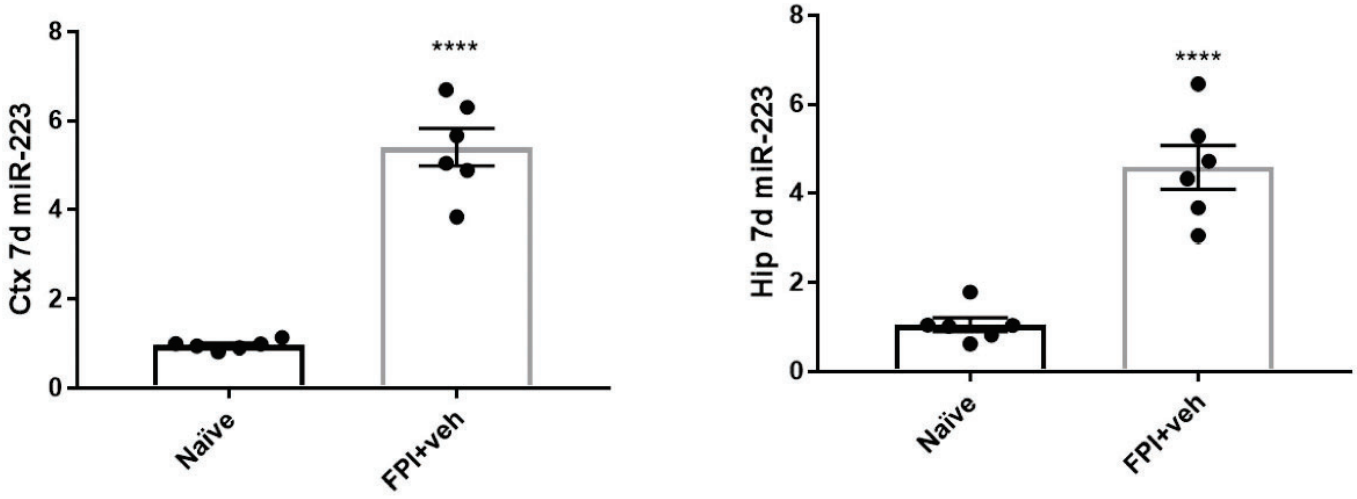

Figure 6. miR-223 is elevated in the injured cortex and hippocampus at 7 days after TBI+Hypobaria. **** p<0.0001 compared to naive.

5.4 miR-155 and miR-223 are elevated in the plasma at 7 days after TBI+Hypobaria

Our data demonstrate that plasma levels of key inflammatory microRNAs including miR-155 and miR-223are elevated at 7 days after TBI (Fig.7; one-tailed *t t*est). The level of miR-124 did not change.

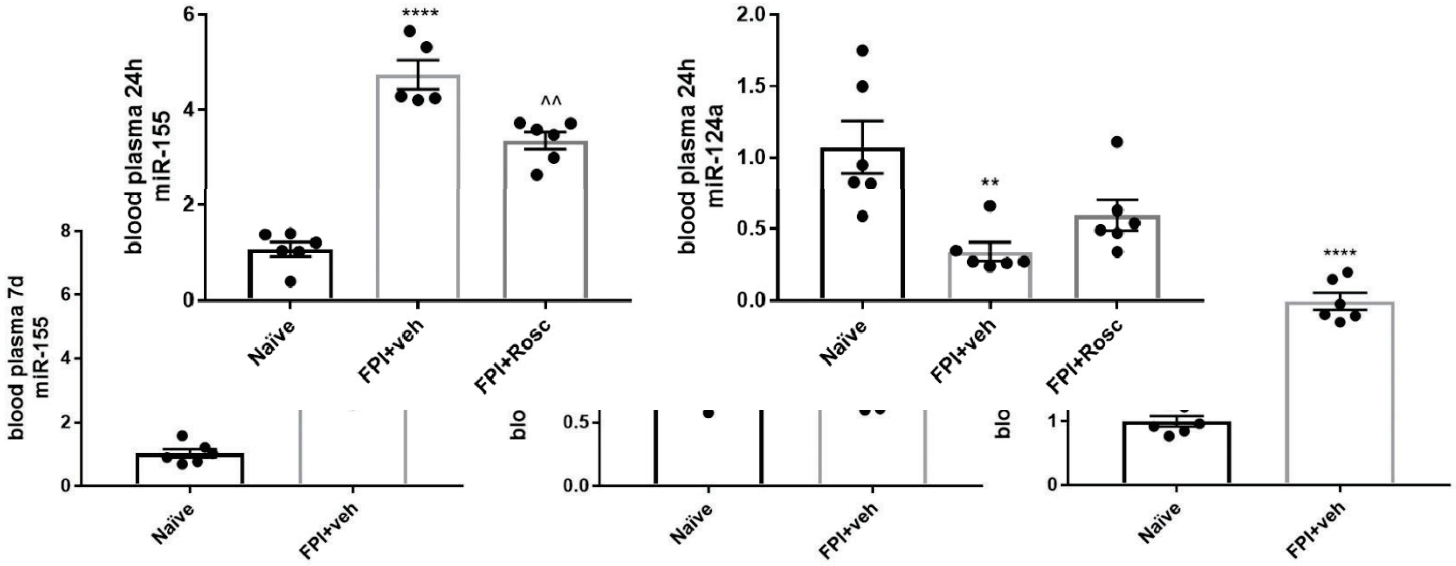

Figure 7. miR-155 and miR-223 are elevated in the plasma at 7 days after TBI+Hypobaria. **** p<0.0001 compared to naive

5.5 Roscovitine attenuates the elevation of key pro-inflammatory genes and microRNAs in the injured brain and/or plasma after TBI+Hypobaria

We examined the effects of a systemic administration of Roscovitine, a CDK inhibitor on central and systemic inflammation markers after TBI+Hypobaria. We determined that administration of Roscovitine significantly attenuated the increased levels of pro-inflammatory markers IL-1β and CD74 in the cortex at 24h after TBI+Hypobaria (Fig.8; one-way ANOVA, Tukey post-hoc test) and reduced the increased levels of miR-223 in the cortex (trend) and hippocampus at 24h after injury ((Fig.9; one-way ANOVA,Tukey post-hoc test). Roscovitine also attenuated the miR-155 increased levels in the plasma at 24h after TBI+Hypobaria; there was a trend for Roscovitine to attenuate the decreased levels of miR-124 in the plasma at 24h after injury (Fig.10; one-way ANOVA, Tukey post-hoc test).

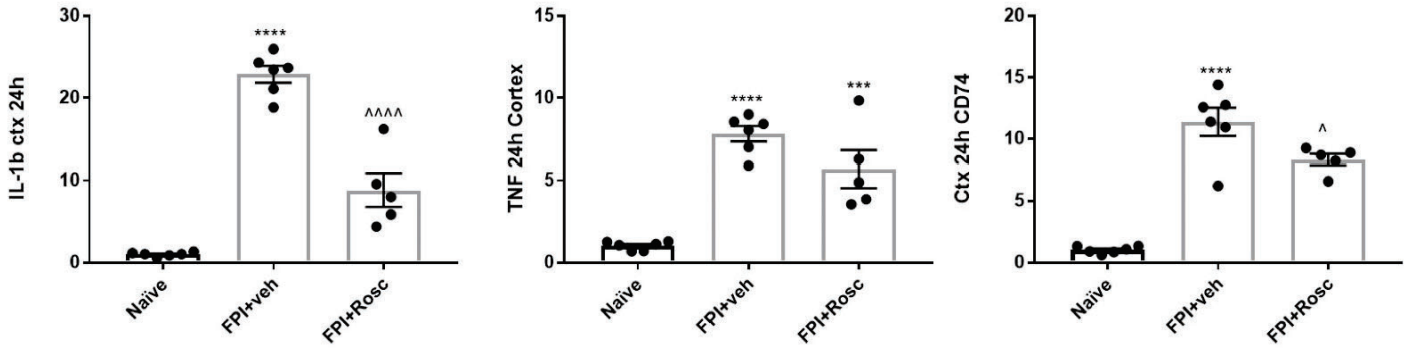

Figure 8. Roscovitine attenuates the TBI+Hypobaria-induced elevation of IL-1β and CD74 in the cortex at 24h post-injury
***** p<0.0001 compared to naïve; *** p<0.0005 compared to naïve; ^^^^ p<0.0001 compared to FPI+veh; ^ p<0.05 compared to FPI+veh.*

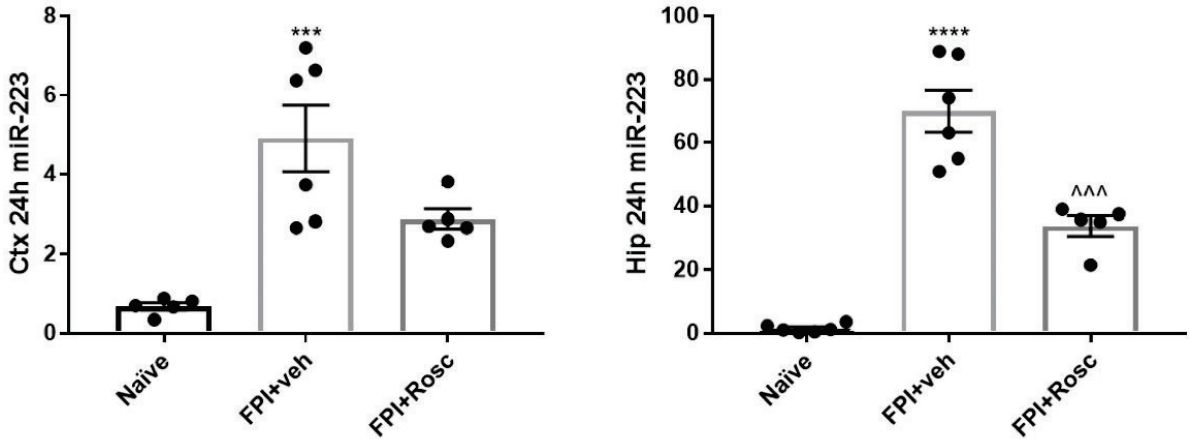

Figure 9. Roscovitine reduces the TBI+Hypobaria-induced elevation of miR-223 in the cortex and hippocampus at 24h post-injury
**** p<0.0005 compared to naïve; **** p<0.0001 compared to naïve; ^^^ p<0.0005 compared to FPI+veh*

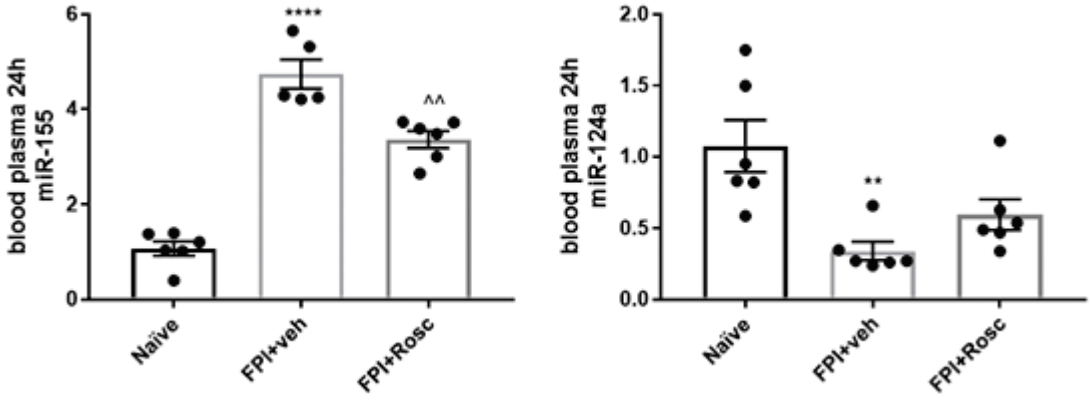

Figure 10. Roscovitine attenuates the TBI+Hypobaria-induced elevation of miR-155 in the plasma at 24h post-injury
***** p<0.0001 compared to naïve; ** p< 0.005 compared to naïve; ^^ p<0.005 compared to FPI+veh*

5.6 CR8 attenuates microglia activation, neurological deficits and neurodegeneration after TBI+Hypobaria

We examined the therapeutic effects of CR8, a potent and selective CDK inhibitor on neuroinflammation/neuronal loss secondary injury processes after TBI+Hypobaria. As recently described[7], we concluded that systemic CR8 attenuates microglia activation after injury (Fig.11; one-tailed *t* test).

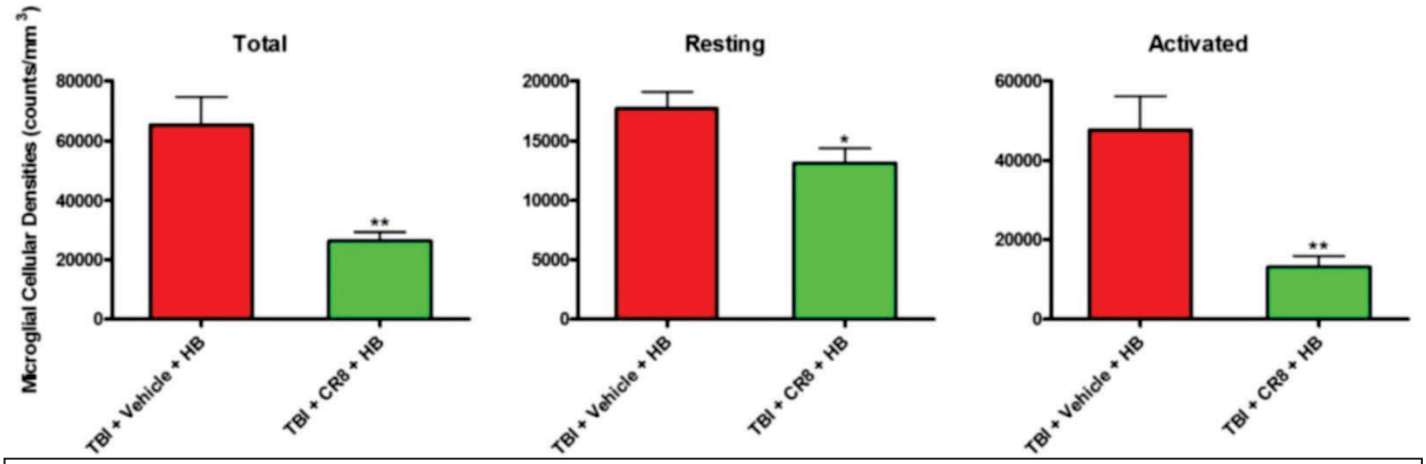

Figure 11. Microglial densities in the injured cortex were determined using unbiased stereological quantifications

*CR8 treatment significantly reduced the total number of microglia and the number of resting and activated microglia in comparison to the TBI + Veh + HB group. N = 6 (TBI/Veh/HB), 6 (TBI/HB/CR8). *p < 0.05, TBI/HB/CR8 vs. TBI/Veh/HB: **p < 0.01. TBI/HB/CR8 vs. TBI/Veh/HB*

CR8 also attenuated lesion volume (Fig.12; one-tailed *t* test) and reduced neuronal loss (Fig.13; one-tailed *t* test) after TBI+Hypobaria.

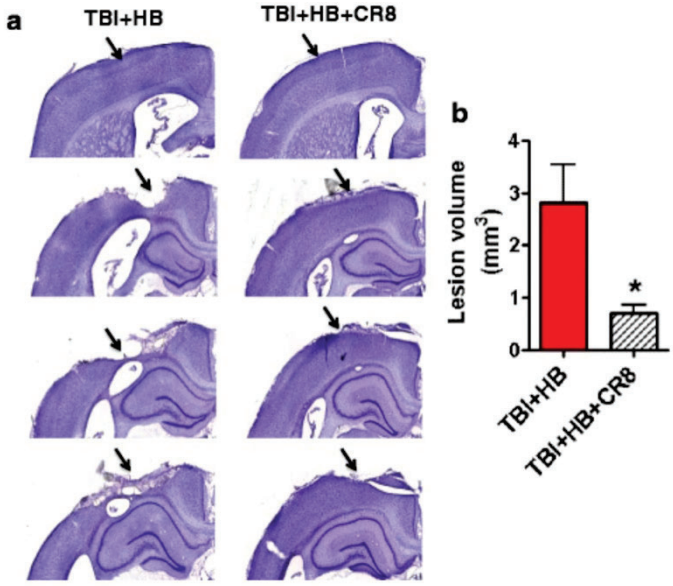

Figure 12. CR8 treatment reduces lesion volume induced by hypobaria exposure following TBI. Stereological assessment of lesion volume was performed at 30 days post-injury

*a Representative images from each group are shown. Lesion cavities are marked by arrows. b There was a significant reduction in lesion volume in the TBI + CR8 + HB group when compared with the TBI + Veh + HB group. N = 5/group. *p < 0.05, TBI + CR8 + HB vs. TBI + Veh + HB*

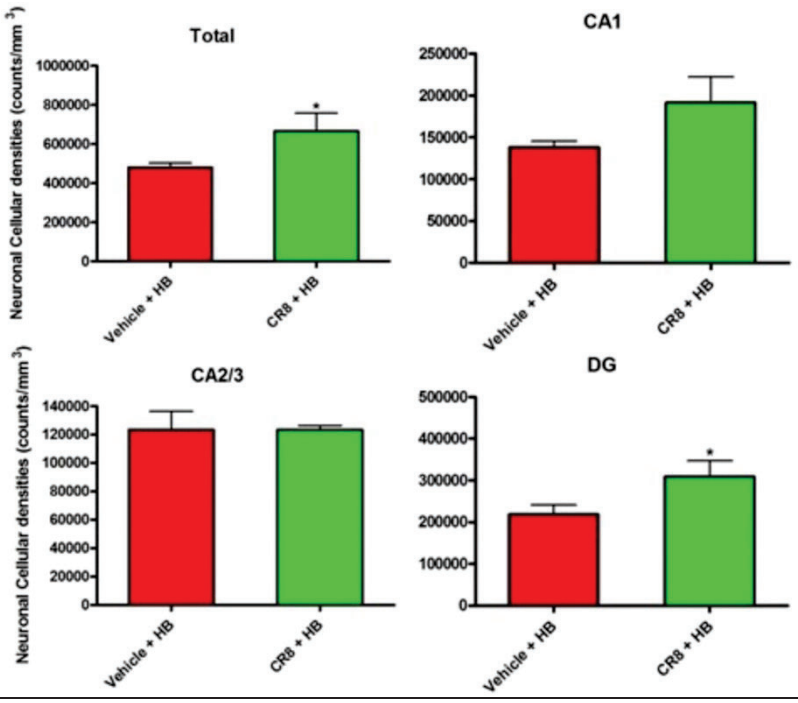

Figure 13. Effect of CR8 treatment following TBI plus hypobaria on neuronal cell loss in the hippocampus

*Total neuronal cell numbers in the hippocampus ipsilateral to the site of injury were evaluated at 30 days post-injury. Unbiased stereological quantifications show that treatment with CR8 increased total neuronal density in the hippocampus and in the DG subregion compared with the TBI + Veh + HB group. N = 5 (TBI/Veh + HB), 5 (TBI/CR8/HB). * p < 0.05 TBI + CR8 + HB vs. TBI + Veh + HB*

Importantly, administration of CR8 significantly attenuated cognitive deficits after TBI+Hypobaria as demonstrated by improvements in tasks such as Morris water maze and Novel object recognition (Fig.14).

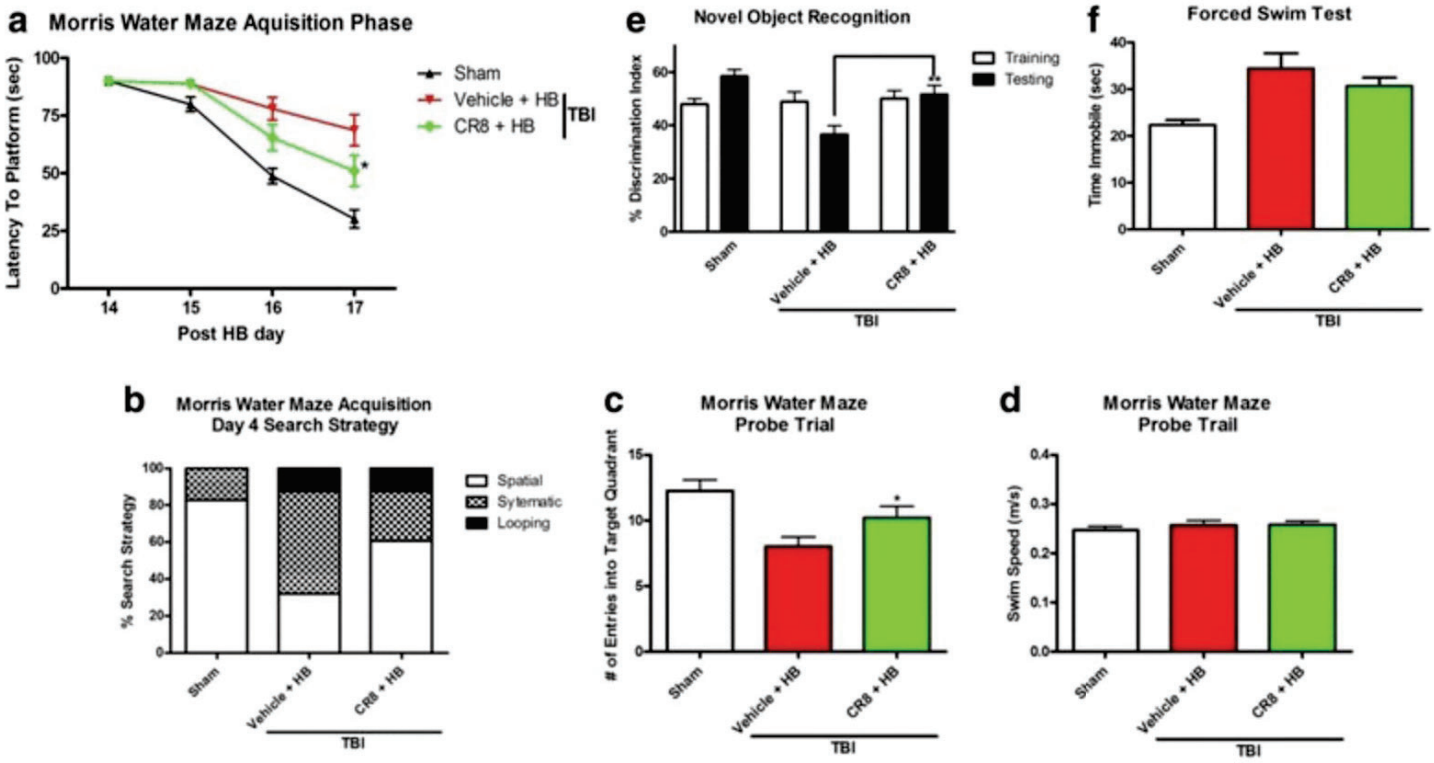

Figure 14. Cell cycle inhibition by CR8 improves functional outcomes following TBI plus hypobaria. a–d Cognitive assessment of CR8 treatment using the Morris water maze (MWM)

The TBI + CR8 + HB group showed significant improvements in spatial learning deficits in comparison to the TBI + vehicle + HB group following prolonged hypobaria exposure at 6 h after TBI. The swimming patterns during all trials on the fourth day of the acquisition phase were analyzed to assess the search strategies utilized by the animals to locate the hidden platform. A chi-square analysis was used to compare strategies between groups and was found to be significant ($p < 0.0001$, $\chi 2 = 57.79$, $df = 4$). Animals in the vehicle + HB group were less efficient in their search strategy while attempting to locate the hidden platform. CR8 treatment increased the percentage of trials in which a spatial search strategy was utilized. Spatial memory was assessed using the MWM probe trial on day 18 after HB by examining the number of entries into the target quadrant. CR8 treatment increased the number of target quadrant entries in comparison to the TBI + Veh + HB group indicating a reduction in retention memory deficits in the probe trial. Swim speeds did not differ across groups ($p = 0.4382$). e Nonspatial memory was assessed using the novel object recognition test on post-HB day 21. Animals showed an equal preference for the two identical objects during the training phase. CR8 treatment significantly increased the discrimination index in comparison to the TBI + Veh + HB group indicating an improvement in nonspatial memory. f Depressive-like behaviors were assessed using the forced swim test. CR8 treatment did not significantly reduce the depressive-like behavior caused by TBI plus HB. N = 16 (sham), 14 (TBI + Veh + HB), 15 (TBI + CR8 + HB). $*p < 0.05$, $**p < 0.01$, TBI + CR8 + HB vs. TBI + Veh + HB

6.0 DISCUSSION

Our works highlights the remarkable complexity of the secondary injury processes triggered by traumatic brain injury + hypobaria. In particular, we demonstrate the robust and persistent elevation of many pro-inflammatory markers both centrally (brain) and systemically (plasma). Microparticles numbers decrease early after injury but are upregulated in the plasma at 28 days

post-trauma. We demonstrate the robust and persistent elevation of many pro-inflammatory genes in the cortex at both 24h and 7d post-injury time points; cell death modulators are induced at 24h post-injury. We also provide support for the hypothesis that microRNAs may play important roles as regulators of both central and systemic immune responses. Thus, pro-inflammatory microRNAs are upregulated both in the cortex and hippocampus and importantly in the plasma at 7 days after TBI+Hypobaria. An important challenge is to improve the knowledge of the role of many of the examined microRNAs whose function is likely much more complex than currently understood. Nonetheless, changes in circulating (plasma) microRNA levels may serve as sensitive biomarkers of brain trauma processes and as indicators of therapeutic responses. Systemic administration of the CDK inhibitor Roscovitine attenuated the injury-induced up-regulation of pro-inflammatory genes in the cortex and hippocampus. Roscovitine also reduced the brain and plasma elevation of pro-inflammatory microRNAs. Thus, Roscovine may be able to modulate both central neuroinflammation and systemic immune activation in response to TBI+Hypobaria. As we have recently described[7], the potent and selective CDK inhibitor CR8, attenuates microglia activation, reduces neurodegeneration and improves neurological outcomes after TBI+Hypobaria. These data further validate the therapeutic potential of CDK inhibitors in this injury model.

Note: An IACUC reviewed this protocol and all animal subjects in this effort were used and cared for in compliance with federal regulations governing the protection of animals and research, and followed/practiced standards consistent with the Guide and/or AAALACi.

7.0 CONCLUSIONS

Future studies should continue the analysis of the data generated by these studies. Especially important is the mining of the data generated by the NanoString analysis of hundreds of microRNA changes centrally (brain) and systemically (plasma) after TBI+hypobaria. We are optimistic that better understanding of neuroinflammation mechanisms and injury biomarkers may result from these investigations.
Cell cycle inhibition using CDK inhibitors in the Roscovitine/CR8 family may develop into a useful therapeutic agent for TBI+hypobaria due to their attenuation of multiple secondary injury mechanisms.

8.0 REFERENCES

1 Skovira, J. W. *et al.* Simulated Aeromedical Evacuation Exacerbates Experimental Brain Injury. *J Neurotrauma* **33**, 1292-1302, doi:10.1089/neu.2015.4189 (2016).

2 Henry, R. J. *et al.* Inhibition of miR-155 Limits Neuroinflammation and Improves Functional Recovery After Experimental Traumatic Brain Injury in Mice. *Neurotherapeutics*, doi:10.1007/s13311-018-0665-9 (2018).

3 Kabadi, S. V. *et al.* CR8, a selective and potent CDK inhibitor, provides neuroprotection in experimental traumatic brain injury. *Neurotherapeutics* **9**, 405-421, doi:10.1007/s13311-011-0095-4 (2012).

4 Kabadi, S. V. *et al.* Selective CDK inhibitor limits neuroinflammation and progressive neurodegeneration after brain trauma. *J Cereb Blood Flow Metab* **32**, 137-149, doi:10.1038/jcbfm.2011.117 (2012).

5 Hilton, G. D., Stoica, B. A., Byrnes, K. R. & Faden, A. I. Roscovitine reduces neuronal loss, glial activation, and neurologic deficits after brain trauma. *J Cereb Blood Flow Metab* **28**, 1845-1859, doi:10.1038/jcbfm.2008.75 (2008).

6 Kabadi, S. V., Stoica, B. A., Loane, D. J., Luo, T. & Faden, A. I. CR8, a novel inhibitor of CDK, limits microglial activation, astrocytosis, neuronal loss, and neurologic dysfunction after experimental traumatic brain injury. *J Cereb Blood Flow Metab* **34**, 502-513, doi:10.1038/jcbfm.2013.228 (2014).

7 Skovira, J. W. *et al.* Cell cycle inhibition reduces inflammatory responses, neuronal loss, and cognitive deficits induced by hypobaria exposure following traumatic brain injury. *J Neuroinflammation* **13**, 299, doi:10.1186/s12974-016-0769-2 (2016).

8 Kumar, A. *et al.* Microglial-derived microparticles mediate neuroinflammation after traumatic brain injury. *Journal of Neuroinflammation* **14**, 47, doi:10.1186/s12974-017-0819-4 (2017).

9 Galloway, D. A. *et al.* miR-223 promotes regenerative myeloid cell phenotype and function in the demyelinated central nervous system. *Glia*, doi:10.1002/glia.23576 (2018).

10 Chen, L. *et al.* MicroRNA-223 Promotes Type I Interferon Production in Antiviral Innate Immunity by Targeting Forkhead Box Protein O3 (FOXO3). *J Biol Chem* **291**, 14706-14716, doi:10.1074/jbc.M115.700252 (2016).

LIST OF ABBREVIATIONS AND ACRONYMS

AE	Aeromedical Evacuation
atm	Atmospheres of Pressure
CCI	controlled cortical impact
CDK	cyclin-dependent kinase
CO2	Carbon Dioxide
CR8	Cyclin-dependent kinase (CDK) inhibitor
DoD	Department of Defense
Fig	Figure
ft	Feet
LFP	Lateral fluid percussion
Min	Minutes
miR	microRNA
mmHg	Millimeters of Mercury
MWM	Morris water maze
NOS2	Nitric Oxide Synthase 2
%	percent
O2	Oxygen
pO_2	Partial Pressure of Oxygen
qPCR	Real-time polymerase chain reaction
TBI	Traumatic brain injury
USAF	United States Air Force