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14. ABSTRACT
Pressure waves due to explosions can damage the neurons of the eye and visual centers in the brain, leading to functional loss of vision. There are currently few treatments for such injuries that can be deployed rapidly in the field to mitigate such damage. Our research team is developing small molecule activators of TrkB, the cognate receptor for brain-derived neurotrophic factor (BDNF), which can be administered systemically and cross the blood brain/retina barrier (BBB). In the third year of the grant, we tested the effects of the TrkB receptor activator, HIOC, in a blast overpressure model of ocular trauma. HIOC produced a significant preservation of visual function, which in some experiments was nearly complete. We also found that HIOC partially prevented optic nerve degeneration, astrocytosis, and thinning of the retinal ganglion cell / nerve fibers following ocular blast injury. We also found that HIOC partially prevented the loss of visual function caused by blast directed at the head.

15. SUBJECT TERMS
trauma, neuroprotection, retina, optic nerve, TrkB, BDNF, brain, TBI

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Table of Contents

Page

1. INTRODUCTION

Pressure waves due to explosions can damage the neurons of the eye and visual centers in the brain, leading to functional loss of vision. There are currently few treatments for such injuries that can be deployed rapidly in the field to mitigate such damage. Our research team is developing small molecule activators of TrkB, the cognate receptor for brain-derived neurotrophic factor (BDNF). BDNF has been shown to have neuroprotective effects in a number of degeneration models, including optic nerve crush and bright light-induced retinal degeneration (Gauthier et al., 2005; Weber et al., 2010). However, BDNF must be injected intraocularly or into the brain to be effective, as it does not cross the blood brain/retina barrier (BBB), making it impractical to deploy in the field. In contrast, the compounds we are developing can be administered systemically and readily cross the BBB (Jang et al., 2010a,b,c). Following peripheral injection, the drugs activate TrkB receptors in the retina and the brain, and appear to show no systemic toxicity. In preliminary studies, we have shown that they protect against light-induced retinal degeneration (Shen et al., 2012). The goal of this project is to develop effective treatments for traumatic blast-related retinal and visual system damage that can be delivered on the battlefield. We hypothesize that small molecule activators of TrkB will be useful for this purpose. We proposed 3 specific aims to test this hypothesis, investigating the utility of TrkB activators to prevent retinal ganglion cell death following optic nerve crush, protect retinal cells from blast-induced injury to the eye, and protect central visual pathways from traumatic blast-induced injury.

2. KEYWORDS: trauma, neuroprotection, retina, optic nerve, TrkB, BDNF, brain, TBI

3. OVERALL PROJECT SUMMARY

The statement of work for year 4 was to complete the test of TrkB activators for treatment of blast-induced degeneration in visual pathways in the brain. These studies are still underway, but will be completed before the end of the no cost extension. We added new experiments to aim 2 to further characterize the protective effect of HIOC against ocular blast, as well as conducting experiments on effects of HIOC on visual function following TBI. Our studies demonstrate that HIOC, our lead TrkB activator, significantly reduces loss of visual function following blast injury to the eye and to the brain.

In year 1, experiments were initiated to establish assays for measuring retinal ganglion cell (RGC) loss after optic nerve crush. Three approaches were taken. One was to count Brn3a immunoreactive cells in retinal whole mounts. Brn3a is a specific marker for retinal ganglion cells (Nadal-Nicolas et al., 2009); it is expressed by approximately 90% of ganglion cells. The other approach was to count fluorescent RGCs of Thy1-CFP mice, which express CFP (cyan fluorescent protein) in retinal ganglion cells (Feng et al., 2000), or to measure fluorescence in retinal extracts of these mice. We initiated studies on effects of TrkB agonists on RGC loss following optic nerve crush, but found that systemic injection did not have a consistent neuroprotective effect.

In year 2, we investigated the effects of HIOC, delivered by various routes (i.p. or osmotic minipump), in combination with a variety anti-inflammatory and microglial-modulating drugs on optic nerve crush-induced retinal ganglion cell degeneration. None were effective. We concluded that such severe injury to the optic nerve might be beyond pharmacological intervention, at least with our tools. We built and calibrated the blast cannon, and initiated experiments to test the efficacy of TrkB activators on loss of visual function following blast-induced damage to the eye. In preliminary studies we showed potential efficacy of HIOC in preventing loss of visual function caused by blast-induced damage to the eye.

In year 3, we demonstrated that HIOC was effective in reducing vision loss and optic nerve axon degeneration following blast injury to the eye, that this effect was blocked by a TrkB antagonist, that the drug could be administered up to 3 hours after exposure to blast and still have a beneficial therapeutic effect. We also initiated studies to examine the effects of blast injury to the head on visual function and present the preliminary results of HIOC administered after blast on visual function. The results are detailed below.

YEAR 4

We conducted a dose-response study for the effect of HIOC on preservation of visual function following ocular blast. We demonstrated that the effect of HIOC on visual function preservation is due to its action on TrkB receptors. We found that HIOC reduced ocular blast-induced astrocytosis and thinning of the ganglion cell / nerve fiber layer. We showed that HIOC is more efficacious than two other BDNF receptor ligands, and examined the effect of repeated mild ocular blast on visual function. We also examined the effect of different blast pressures on head blast (TBI)-induced loss of visual function, showed that head blast causes cerebral microglial activation, and confirmed that HIOC reduces loss of visual function following TBI.

3A. Ocular Blast Injury

Effect HIOC on the loss of visual function following blast directed at the eye: dose–response study.

Mice were exposed to a single, ~48psi blast directed at the front of the eye. The mice were injected with vehicle or HIOC 30 mg/kg, 60 mg/kg, or 100 mg/kg immediately after blast and daily for the next 6 days. Contrast sensitivity and visual acuity were measured 1 month after blast. All doses of HIOC significantly reduced the blast-induced loss of contrast sensitivity, but 60 mg/kg showed the best response in this experiment (Figure 1A). A similar pattern was seen for improvement of visual acuity thresholds, except that no significant difference was seen between the 60 mg/kg and 100 mg/kg doses (Figure 1B).

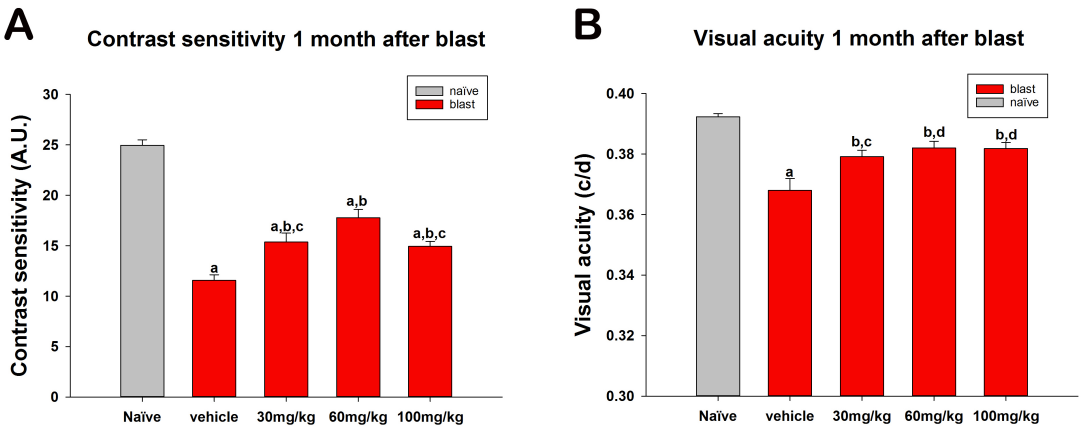

Figure 1. Effect of HIOC on contrast sensitivity and (B) visual acuity one month after ocular blast. *See text for details.* ***(A) Contrast Sensitivity:*** *a) p<0.001 vs Naïve, b) p<0.005 vs Vehicle, c) p<0.05 vs 60 mg/kg.* ***(B) Visual acuity:*** *a) p<0.001 vs Naïve, b) p<0.03 vs Naïve, c) p=0.22 vs vehicle, d) p=0.004 vs vehicle. N=6/group.*

Does HIOC preserve visual function by stimulating BDNF / TrkB receptors?

We previously showed that HIOC stimulates TrkB, resulting in its phosphorylation and activation of downstream signaling (Shen et al., 2012), but it is unknown if the efficacy of the drug in preventing blast-induced vision loss occurs through this mechanism. Towards the end of Q3 FY2015, we initiated a study to explore the mechanism. ANA12 is a selective TrkB antagonist that binds to the receptor and inhibits downstream signaling (Cazarola et al., 2011). We tested the ability of ANA12 to block the protective action of HIOC. The results suggested that ANA12 blocked the beneficial effect of HIOC on visual function. However, the blast-induced decrease in visual function was less than typically observed in previous experiments, and the beneficial effect of HIOC was smaller than usual. We therefore repeated the experiment. Mice were exposed to a single ~48 psi blast and administered HIOC (40 mg/kg ip) or vehicle15 min later. Daily injections continued for 6 days. Mice were pretreated with ANA12 (0.5 mg/kg ip) or its vehicle 2.5 hours before each HIOC / vehicle injection. One month after exposure to blast, contrast sensitivity and visual acuity were reduced in the vehicle-treated mice (Figure 2; $p<0.001$). Treatment with HIOC (plus the vehicle for ANA12) reduced the loss of contrast sensitivity ($p<0.001$). Administration of ANA12 alone had no effect on the blast-induced loss of contrast sensitivity, but completely blocked the effect of HIOC. The results indicate that HIOC mitigates blast-induced vision loss by activating TrkB.

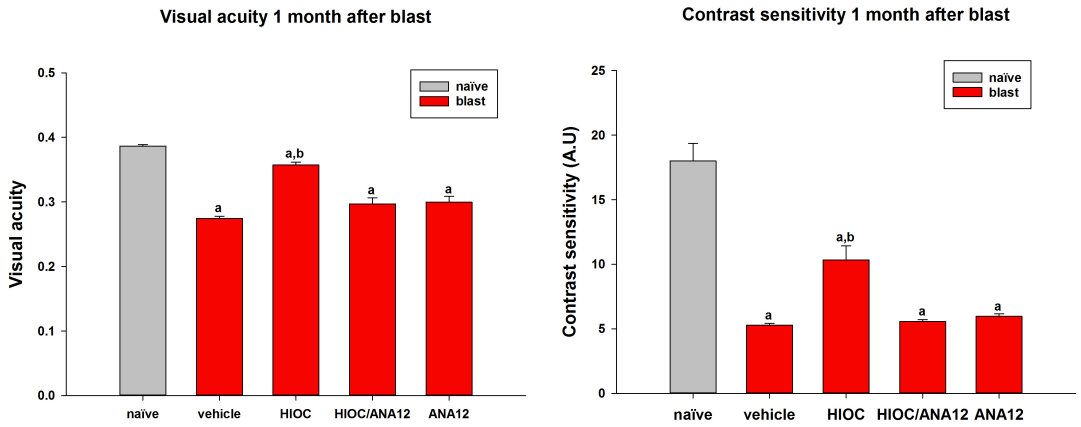

Figure 2. Effect of ANA12 on the mitigation of blast-induced vision loss by HIOC*. See text for details. N=6 / group. a) p<0.001 vs Naïve; b) p<0.001 vs Vehicle, ANA12, and HIOC/ANA12.*

Optic nerve axon counts from this experiment showed that blast injury caused ~35% loss of optic nerve axons in vehicle-treated mice measured 5 weeks after blast (Figure 3; p<0.013). HIOC nearly completely prevented this axon loss. ANA12 appeared to partially block the effect of HIOC, as there was no significant difference between the axon numbers of mice receiving ANA12 alone and ANA12 plus HIOC. However, there was no significant difference between HIOC alone and ANA12 plus HIOC.

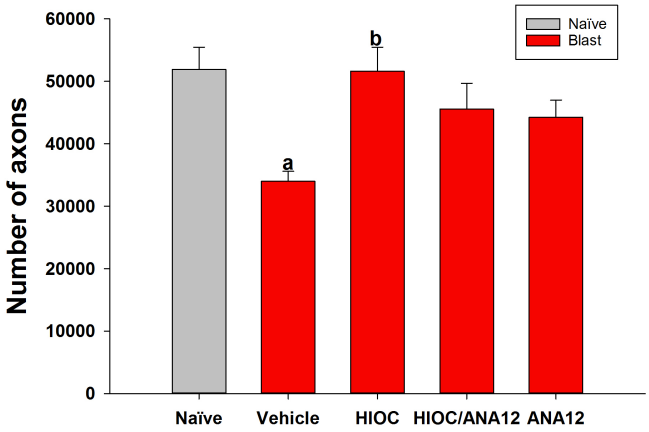

Figure 3. Effect of ANA12 on the mitigation of blast-induced optic nerve axon loss by HIOC*. See text for details. N=5-6 / group. a) p<0.013 vs Naïve; b) p<0.014 vs Vehicle.*

Effect of HIOC on ocular blast-induced GFAP expression in the retinal ganglion cell layer.

Glial fibrillary acidic protein (GFAP) is a marker of astrocytes, and is expressed in Müller glial cells when the outer retina is damaged. We previously reported that 48 psi blast directed at the front of the eye caused an upregulation of glial fibbrillary acidic protein (GFAP) in astocytes in the nerve fiber and ganglion cell layer, but not in Müller glial cells. In this study, we exammined the effect of HIOC (40 mg/kg, i.p.) on this response. Mice were subjected to a single blast at 48 psi. HIOC or vehicle was injected 15 min after blast, and once daily for the next 6 days. Eyes were dissected 7 days after blast. As shown in the representative images in Figure 4, exposure to blast caused an increase of GFAP expression, indicative of gliosis in the nerve fiber layer and ganglion cell layer. Consistent with our previous results, there was no increase in GFAP in Müller cells. In 5 of 6 mice, HIOC produced an obvious reduction GFAP expression in the eyes exposed to blast. The results suggest that blast induces reactive astrocytosis, and that treatment with HIOC reduces this effect. This may contribute to the preservation of visual function by HIOC.

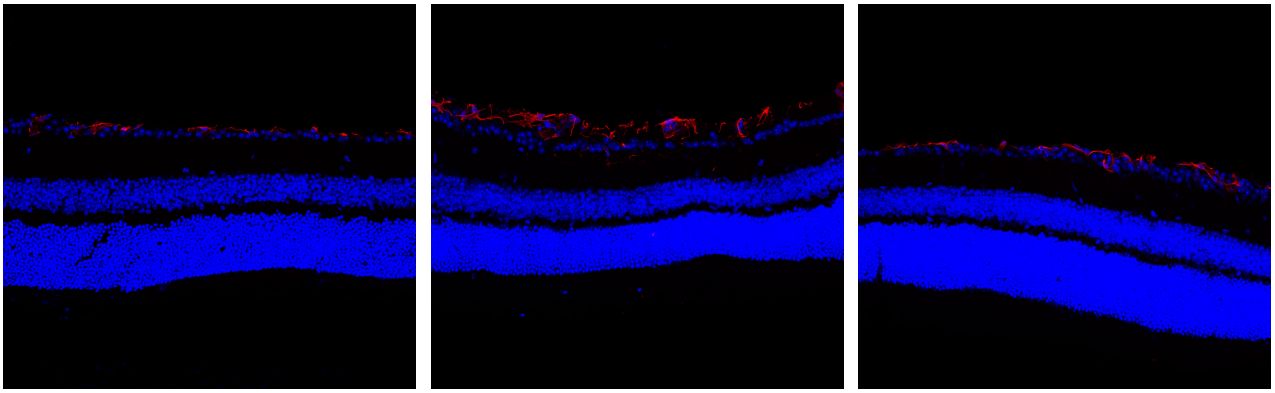

Figure 4. HIOC reduces the induction of gliosis caused by ocular blast.
Representative images of GFAP labeling (red) in mice exposed to 48 psi blast, treated with vehicle or HIOC (40 mg/kg, i/p.). Six mice were examined in each condition. Blue labeling is the nuclear stain DAPI.

Effect of HIOC on blast-induced Iba1-labeled microglia.

Mice were treated as described above. The samples are still being imaged, but preliminary observations suggest that HIOC reduces blast-induced microglial activation (see Figure 5).

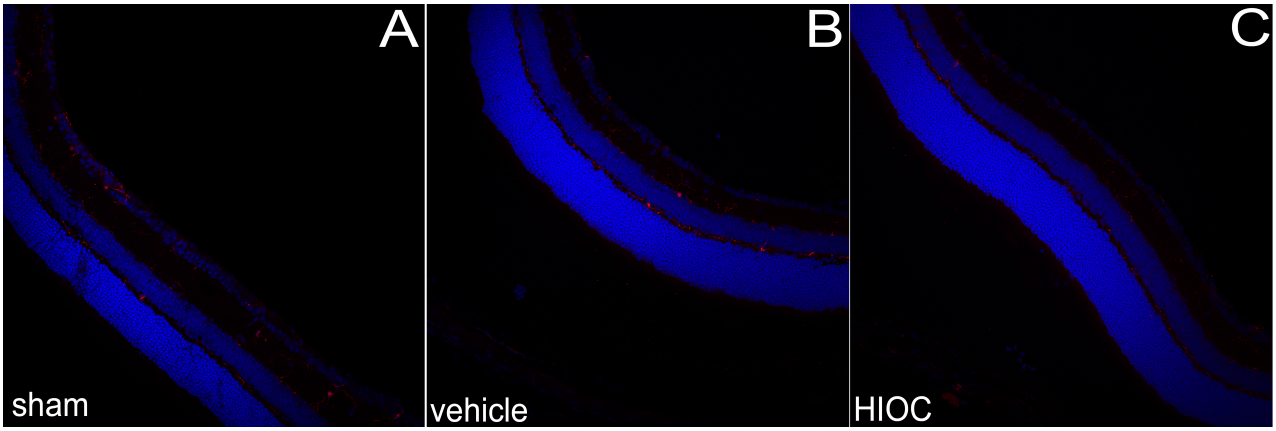

Figure 5. Effect of HIOC on ocular blast-induced microglial activation in the retina.
Representative images of Iba 1 labeling (red) in mice exposed to 48 psi blast, treated with vehicle or HIOC (40 mg/kg, i/p.). Six mice were examined in each condition. Blue labeling is the nuclear stain DAPI.

SD-OCT measurements of retina following blast directed at the eye.

Spectral-domain optical coherance tomography (SD-OCT) was used to assess effects of blast on retinal layers 1 day and 7 days after 48 psi blast, in mice treated with vehicle or HIOC as described above. One day after blast, no significant changes in total retinal thickness, photoreceptor layer thickness, or ganglion cell / nerve fiber layer thickness were observed (data not shown). However, 7 days after blast there was a statistically significant reduction in ganglion cell / nerve fiber layer thickness in the vehicle-treated, blast-exposed mice (Fig. 6; $p<0.001$). Other retinal layers were unaltered. Treatment with HIOC (40 mg/kg) significantly reduced the loss of ganglion cell / nerve fiber layer thickness caused by ocular blast (Fig. 6; $p<0.01$).

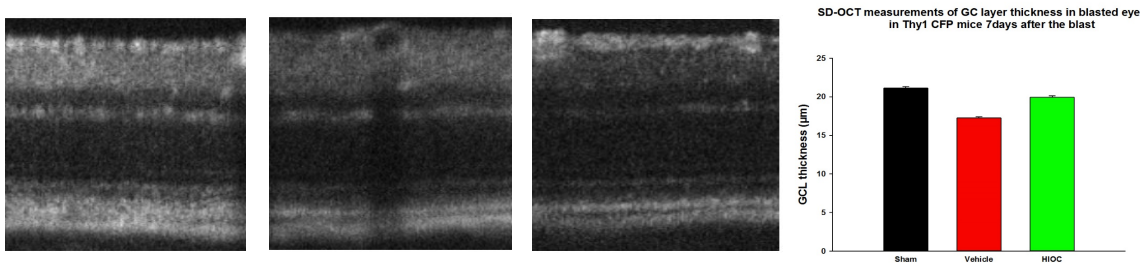

Figure 6. Ocular blast reduces ganglion cell / nerve fiber layer thickness: protection by HIOC. N=6 per group.

Effect LM22A-4 and LM11A-31 on the loss of visual function following blast directed at the eye.

We examined the ability of another TrkB agonist, LM22A-4 (Massa et al., J. Clin. Invest. 120:1774-85, 2010), and a p75NTR antagonist, LM11A-31 (Massa et al., 2006) to mitigate the loss of visual function following ocular blast damage. Mice were exposed to a single, ~48psi blast directed at the front of the eye. The mice were injected with vehicle, LM22A-4 (50 mg/kg, ip), LM11A-31 (30 mg/kg, ip), or both drugs together 15 minutes after blast and daily for the next 6 days. Contrast sensitivity and visual acuity were measured 1 month after blast. Both drugs and the combination caused a small improvement in contrast sensitivity compared to the vehicle-treated mice (Figure 7; $p < 0.05$). However, the magnitude of the protection was much less than that typically observed with HIOC. This may be due to pharmacokinetic issues or other unknown variables. No significant improvement of visual acuity was observed. We conclude that HIOC is more efficacious in preventing blast-induced vision loss than the LM compounds.

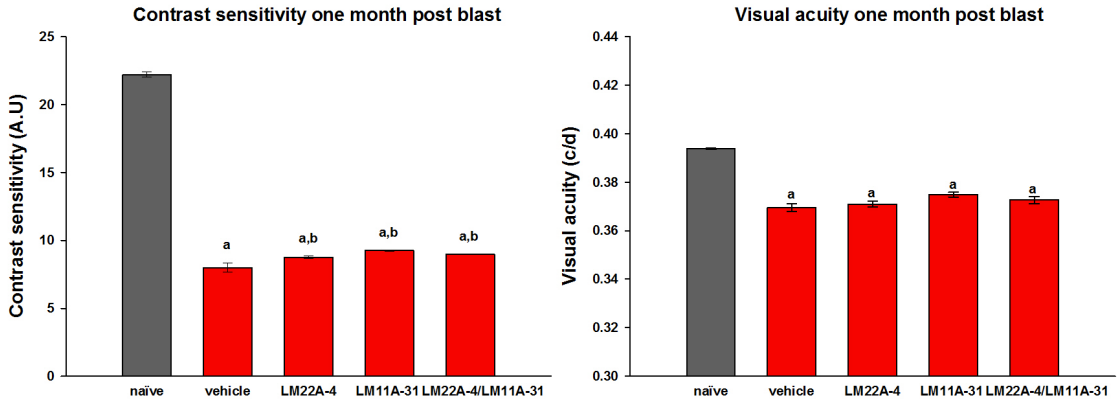

Figure 7. Effect of LM22A-4 and LM11A-31 on visual acuity and contrast sensitivity one month after ocular blast. *See text for details. a) $p<0.001$, b) $p<0.05$, N=6/group.*

Effect of repeated mild blast overpressure on contrast sensitivity and visual acuity.

Soldiers in the field are often exposed to multiple blasts. In an initial study to assess the effect of repeated blast overpressure, mice were exposed to a three blasts of ~40 psi directed at the right eye at 1 week intervals. Visual acuity and contrast sensitivity were measured seperately for right and left eyes one week after the third blast (Figure 8). Repeated blast did not result in any mortality. However, it caused significant reductions ($p<0.001$) in contrast sensitity mediated by the eyes exposed to blast, but not on that mediated by their contralateral eyes. We will continue to assess visual function in these mice up to 1 month after the last blast and then examine optic nerve axon loss.

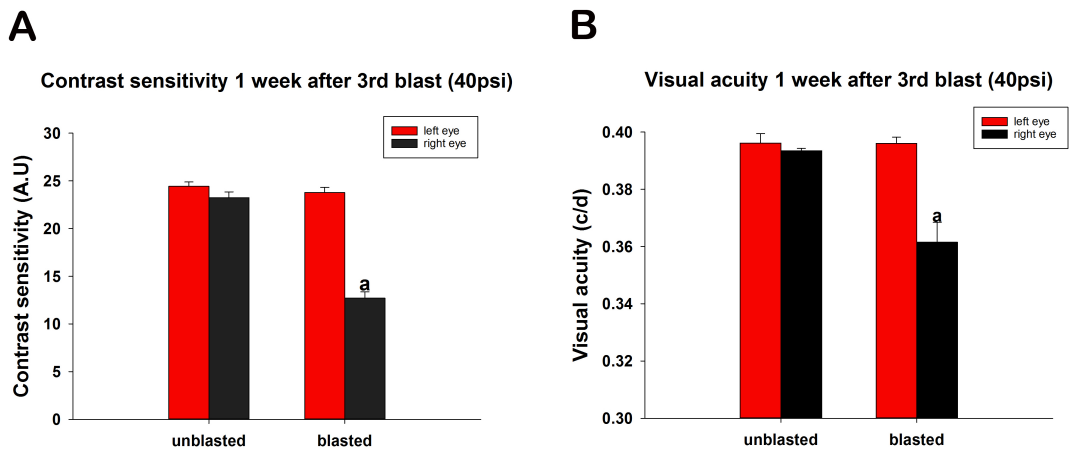

Figure 8. Effect of repeated blast on visual function.
Mice were exposed to three 40 psi blasts directed at the front of the right eye at 1 week intervals. A naïve, unblasted control group was included for comparison. Visual acuity and contrast sensitivity were tested one week after the third blast. ***A) Contrast sensitivity****: repeated blast to the right eye had no effect on contrast sensitivity of the contralateral, left eye (p=0.375 left eye unblasted vs left eye blasted; N=6/group), but greatly decreased contrast sensitivity mediated by the right eye ([a]p<0.001 vs all other groups; n=6/group).* ***B) Visual acuity:*** *repeated blast to the right eye had no effect on visual acuity of the contralateral, left eye (p=0.452 left eye unblasted mice vs left eye blasted mice; N=6/group), but greatly decreased contrast sensitivity mediated by the right eye ([a]p<0.001 vs all other groups; n=6/group).*

After visual function had been assessed, one week after the third blast, mice were sacrificed and optic nerves removed, fixed, and sectioned (5μm). Repeated blast showed a trend for a reduced number of axons in the right optic nerve, but the effect was not statistically significant (Fig. 9), despite the significant reduction in visual function (Fig. 8). Retinal ganglion cells were also counted, but also did not show a statistically significant effect of blast (data not shown). We conclude that either more blasts or higher pressures will be needed to see a consistent effect on optic nerve and ganglion cell degeneration. Alternatively, additional time after the last blast may be needed to observe degeneration.

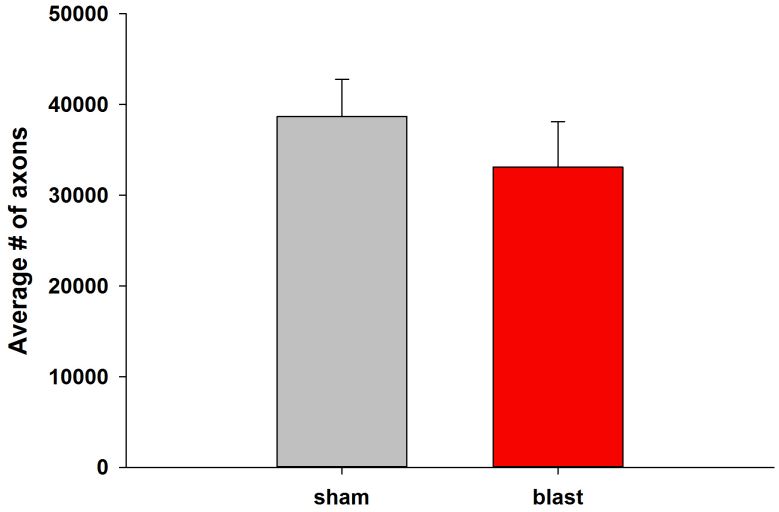

Figure 9. Effect of repeated blast on optic nerve axon number. *Mice were treated as described above. Optic nerve axons were counted 1 week after the third blast. N=6 per group.*

3B. Traumatic Brain Injury

Effect of different blast pressures directed at the head on visual function. To further characterize the effects of blast directed at the head, we compared the effects of 50 psi and 70 psi blast pressure. Mice were exposed to single blast directed at the right side of the head. Visual function was tested one and two weeks after blast exposure. Contrast sensitivity was reduced more at 2 weeks (Fig. 11) than at 1 week (Fig. 10) for both pressures. For visual acuity, no significant reduction was observed at 1 week after exposure, but

significant reductions were observed at 2 weeks. These observations are indicative of a progressive loss of visual function following head blast. With both blast pressures, the loss of contrast sensitivity detected through the right eye was reduced more than that detected through the left eye when measured two weeks after blast exposure ($p<0.001$), and the effect of 70psi blast pressure was significantly greater than that of 50 psi ($p<0.001$; Fig. 11). A similar pattern was observed for loss of visual acuity at 2 weeks, where acuity detected through the right eye was significantly worse than through the left eye following 70 psi blast ($p<0.001$), but not following 50 psi blast (Fig. 11). Similar to contrast sensitivity, 70 psi caused a greater loss of visual acuity compared to that observed with 50 psi ($p<0.001$ right eye; $p<0.05$ left eye). In view of these results we have chosen to use 70 psi blast in all future experiments because of the more robust loss of visual function compared to 50 psi.

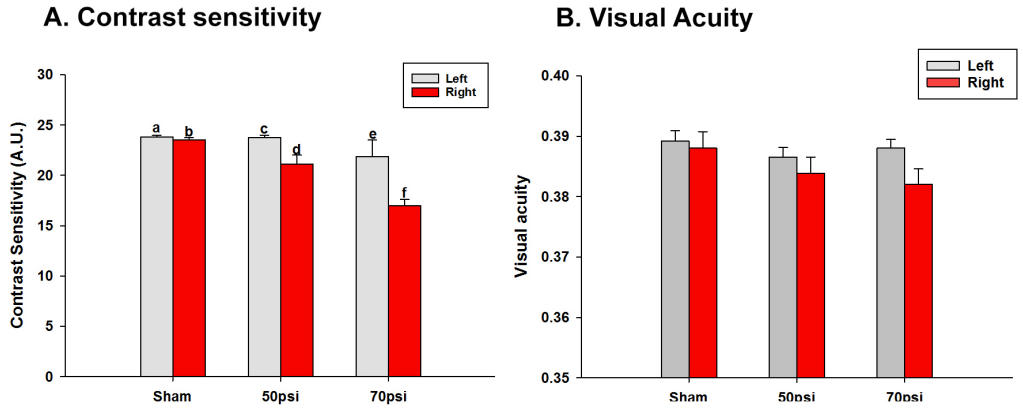

Figure 10. Comparison of different blast pressures measured 1 week after exposure. *Mice were treated as described above.* ***Contrast sensitivity:*** *a vs b, c, and e, not significant; b vs d, $p<0.05$; b vs f, $p<0.001$; d vs f, $p<0.005$; c vs d, $p<0.05$; e vs f, $p<0.001$.* ***Visual acuity:*** *no significant differences. N=6 mice per condition.*

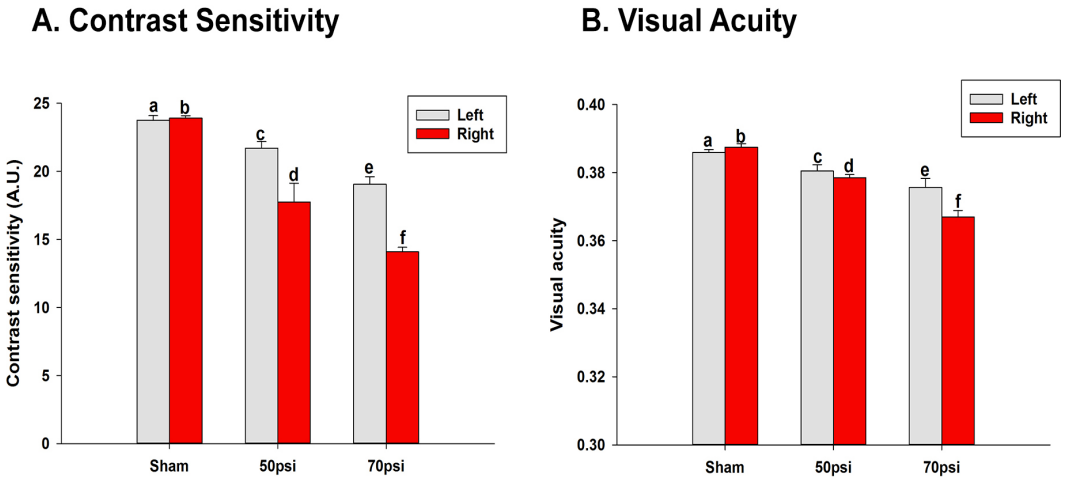

Figure 11. Comparison of different blast pressures measured 2 weeks after exposure. *Mice were treated as described above.* ***Contrast sensitivity:*** *a vs b, not significant; a vs c; $p<0.05$; a vs e, $p<0.001$; b vs d and f, $p<0.001$; d vs f, $p<0.001$; c vs d, $p<0.001$; e vs f, $p<0.001$.* ***Visual acuity:*** *a vs b, not significant; a vs c, not significant; a vs e, $p<0.001$; b vs d and f, $p<0.001$; d vs f, $p<0.001$; c vs d, not significant; e vs f, $p<0.001$. N=6 mice per condition.*

<u>Effect of blast overpressure directed at the side of the head on contrast sensitivity and visual acuity.</u>

We have initiated tests of the efficacy of HIOC in treating the loss of visual function from blast overpressure directed at the side of the head (Figure 12). Mice were exposed to a single blast of ~70 psi directed on the right

side of the head. Visual acuity and contrast sensitivity were measured seperately for right and left eyes. Blast directed at the right side of the head resulted in decrease in contrast sensitivity and visual acuity mediated through both eyes ($p<0.001$), indicative of bilateral brain damage. However, the loss of visual function was greater ipsilateral to the blast ($p<0.001$). HIOC, administered for the first week after blast, significantly reduced the loss of contrast sensitivity and visual acuity when measured one month after the blast ($p<0.001$).

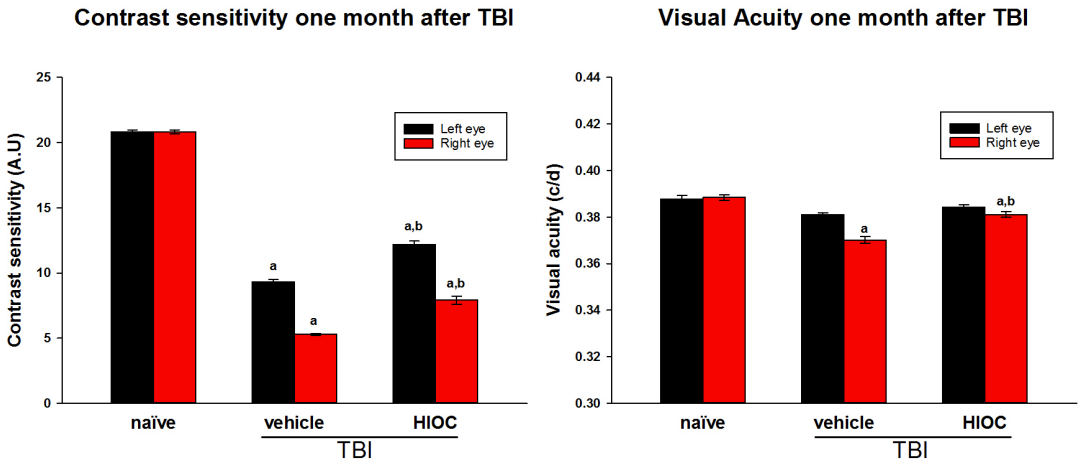

Figure 12. Effect of HIOC on the loss of visual function from head trauma.
Mice were exposed to a single ~70psi blast directed at the right side of the head. HIOC (40mg/kg i.p.) or vehicle was administered 15 minutes after exposure to blast, and daily for the next six days. A naïve control group was included for comparison. Visual acuity and contrast sensitivity were tested one month after exposure to blast. Head trauma significantly decreased contrast sensitivity and visual acuity in both eyes ($^{a}p<0.001$ vs Naïve; n=6/group), but the decrement was geater in vision mediated by the right eye ($^{b}p<0.001$ vs Vehicle left; n=6/group). HIOC partially prevented this loss of visual function ($^{c}p<0.001$ vs Vehicle; n=6/group).

<u>Effect of blast overpressure directed at the side of the head on cerebral microglia</u>
Mice were exposed to a single ~70 psi blast to the side of the head (TBI). Brain sections, prepared 21 days after blast exposure, were immunostained for Iba1 to label microglia. Based on this initial experiment, there is microglial activation in the cortex of the blasted side of the brain (Figure 13).

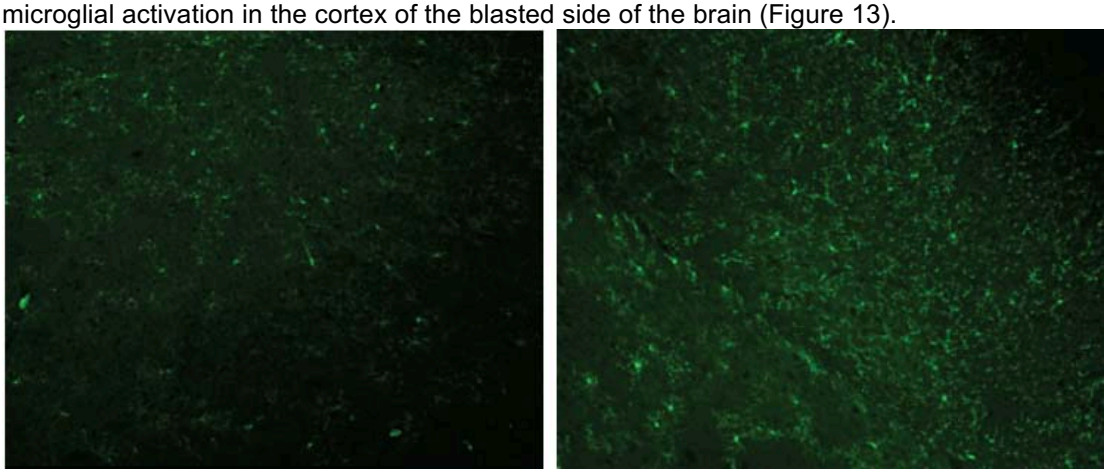

Figure 13. Representative Iba1 immunostained brain section from naïve mice and mice exposed to blast directed at the side of the head (TBI).

4. KEY RESEARCH ACCOMPLISHMENTS

- HIOC reduces the loss of visual function following blast injury to the eye or the head.
- The protective effect of HIOC is dose dependent

- HIOC reduces the induction of astrocyctosis in the ganglion cell and nerve fiber layers from ocular blast.
- HIOC reduces the thinning of the ganglion cell / nerve fiber layers caused by ocular blast.
- The neuroprotective effect of HIOC involves activation of BDNF / TrkB receptors.

5. CONCLUSIONS

Our results indicate that HIOC preserves visual function and optic nerve axons following blast injury to the eye. HIOC reduces loss of visual function following blast injury to the head. The mechanism of action of HIOC involves activation of BDNF / TrkB receptors. HIOC may be useful for preventing vision loss in soldiers exposed to blast overpressure.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTAIONS

a. Publications

1. Lay press: none

2. Peer-reviewed scientific journals: none

3. Invited articles:
Nothing to report

b. Abstracts and Presentations

Iuvone PM, Lyuboslavsky, P Sidhu C, He L, Boatright JH, Geisert EE. Protection from blast-induced vision loss by the N-acetylserotonin derivative HIOC through a BDNF/TrkB receptor mechanism. Association for Research in Vision and Ophthalmolgy, eAbstract 737-B0370, May 2016.

Iuvone PM, Dhakal S, Lyuboslavsky P, He L, Struebing FL, Boatright JH Geisert EE. HIOC, a TrkB receptor activator, for the treatment of blast-induced vision loss. XXII Biennial Meeting of the International Society for Eye Research, September 2016.

Iuvone PM, Dhakal S, Lyuboslavsky P, He L, Struebing FL, Boatright JH Geisert EE. Closed-globe trauma to the eye causes loss of visual function and optic nerve degeneration: Protection by the N-acetyltryptamine derivative HIOC through a BDNF/TrkB receptor mechanism. XVII International Symposium on Retinal Degeneration, September 2016.

7. INVENTIONS, PATENTS AND LICENSES

Nothing to report

8. REPORTABLE OUTCOMES

Nothing to report

9. OTHER ACHIEVEMENTS

Organized and spoke at a symposium at the XXII Biennial Meeting of the International Society for Eye Research, “TBI (traumatic brain injury): visual dysfunction and treatment.” Abstracts attached.

10. REFERENCES

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Appendices

Protection from blast-induced vision loss by the N-acetylserotonin derivative HIOC through a BDNF/TrkB receptor mechanism

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Posterboard #: B0370

Abstract Number: 737 - B0370

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Disclosure Block:P. Michael Iuvone, None; Polina Lyuboslavsky, None; Curran Sidhu, None; Li He, None; Jeffrey H. Boatright, None; Eldon E. Geisert, None

Purpose:N-Acetylserotonin activates BDNF/TrkB receptors but its biological half-life following systemic injection is short and not therapeutically beneficial. The present study examines the neuroprotective effects of HIOC, a structural analog of N-acetylserotonin with a longer half-life, in preserving visual function after blast injury to the eye.

Methods:Mice were exposed to a single ~48psi blast directed at the eye using the method of Hines-Beard et al. (Exp Eye Res 2012;99:63-70). They were injected with vehicle or HIOC (40mg/kg, intraperitineally) 30 min before, or 0.25hr, 1hr, 3hr, or 24hr after exposure to blast. Injections continued daily for 6 days. Contrast sensitivity and visual acuity were measured 1 week, 1 month, and 4 months after exposure to blast by optokinetic tracking. Optic nerve axon counts were made 4 months after blast exposure in mice treated initially 15 min after blast. To test the role of BDNF/TrkB receptors, mice were treated with ANA-12, a selective TrkB antagonist (Cazorla et al., J Clin Invest 2011;121:1846-1857), 2.5 hr before each HIOC or vehicle injection.

Results:One week after blast, contrast sensitivity ($p<0.001$), but not visual acuity, was significantly reduced in vehicle treated mice compared to naïve controls that were not exposed to blast. At 1 and 4 months after blast, both contrast sensitivity ($p<0.001$) and visual acuity ($p<0.001$) were reduced compared to naïve controls. In mice initially treated with HIOC 30 min before or 0.25hr, 1hr, or 3hr after blast, contrast sensitivity and visual acuity were significantly better than vehicle-treated mice ($p<0.001$), and not significantly different than naïve controls. If the initial treatment with HIOC was delayed by 24hr after blast, the protective effect on visual function was not observed. Four months after exposure to blast, axon numbers in the optic nerve were significantly reduced in vehicle-treated mice ($p<0.001$), but not in HIOC treated mice. Pretreatment with ANA-12 completely blocked the protective effect of HIOC against blast-induced vision loss.

Conclusions:HIOC preserves vision in mice exposed to blast if the initial treatment is within a critical period (<3hr). Treatment with HIOC for 1 week preserves visual function for at least 4 months. The effect of HIOC is mediated by activation of BDNF/TrkB receptors.

Layman Abstract (optional): Provide a 50-200 word description of your work that non-scientists can understand. Describe the big picture and the implications of your findings, not the study itself and the associated details.:

XVII International Symposium on Retinal Degeneration

Closed-globe trauma to the eye causes loss of visual function and optic nerve axon degeneration: Protection by the N-acetylserotonin derivative HIOC through a BDNF/TrkB receptor mechanism

P. Michael Iuvone, Susov Dhakal, Polina N. Lyuboslavksy, Li He, Felix Struebing, Jeffrey H. Boatright, and Eldon E. Geisert

Purpose

To characterize the effect of blast-induced ocular trauma on retinal and optic nerve degeneration, and on visual function loss. To determine if HIOC (N-[2-(5-hydroxy-1H-indol-3-yl)ethyl]-2-oxopiperidine-3-carboxamide), a potent, small molecule activator of the BDNF/TrkB receptor, prevents loss of visual function and optic nerve axons.

Methods

Mice were exposed to a single ~48psi blast directed at the front of the eye using the method of Hines-Beard et al. (Exp Eye Res 2012;99:36-41). Contrast sensitivity and visual acuity were measured by optokinetic tracking. Dark-adapted ERG recordings were made by standard techniques. Microglial activation and gliosis were examined by Iba1 and GFAP immunocytochemistry. Retinal ganglion cell counts were made on whole-mounted retinas of Thy1-CFP mice. Axons were counted in plastic sections of optic nerves using a tablet-based program (Templeton et al., Exp Eye Res. 2014;128:102-8). Mice were injected intraperitoneally with vehicle or HIOC before or after exposure to blast. Injections continued daily for 6 days. To test the role of BDNF/TrkB receptors, mice were treated with ANA-12, a selective TrkB antagonist (Cazorla et al., J Clin Invest 2011;121:1846-1857), 2.5 hrs before each HIOC or vehicle injection.

Results

One week after exposure to blast, retinal ganglion cells (RGCs) appeared swollen, GFAP immunoreactivity was increased in the nerve fiber layer, Iba1immunoreactive microglial cells increased, and Thy1 mRNA levels declined. Dark-adapted ERG a- and b-wave amplitudes were not significantly affected. One week post exposure, blast significantly reduced contrast sensitivity ($p<0.001$), but not visual acuity compared to sham controls that were not exposed to blast. At 1 and 4 months after blast, both contrast sensitivity ($p<0.001$) and visual acuity ($p<0.001$) were reduced compared to sham controls. There were small decreases in the number of Thy1-GFP positive RGCs, and, at 4 months after blast, a significant decrease in optic nerve axon numbers ($p<0.01$). HIOC dose-dependently reduced the blast-induced declines in contrast sensitivity and visual acuity. In mice initially treated with HIOC (40 mg/kg, ip) 30 min before or .25hr, 1hr, or 3hr after blast, contrast sensitivity and visual acuity were significantly better than in vehicle-treated mice ($p<0.001$), and not significantly different than sham controls. If the initial treatment with HIOC was delayed by 24hr after blast, the protective effect on visual function was not observed. Four months after exposure to blast, axon numbers in the optic nerve were significantly reduced in vehicle-treated mice ($p<0.001$), but not in HIOC treated mice. Pretreatment with ANA-12 completely blocked the protective effect of HIOC against blast-induced vision loss.

Conclusion

Ocular blast injury resulted in retinal and optic nerve changes consistent with inflammatory reactions and retinal degeneration, primarily in the inner retina. HIOC preserves vision in mice exposed to blast if the initial treatment is within a critical period. Treatment with HIOC for 1 week preserves visual function for at least 4 months. The effect of HIOC is mediated by activation of BDNF/TrkB receptors. Blast-induced ocular trauma may serve as a useful model to screen for treatments for traumatic optic neuropathy.

Grants: DoD CDMRP Grants W81XWH-12-1-0436, W81XWH1210255; Research to Prevent Blindness; The Abraham and Phyllis Katz Foundation.

Abstract Preview - Step 3/4

- print version -

Topic: 9. Ocular Pharmacology and Therapeutics

Title: HIOC, a TrkB receptor activator, for the treatment of blast-induced vision loss

Author(s): Iuvone P.M., Dhakal S., Lyuboslavsky P., He L., Struebing F.L., Boatright J.H., Geisert E.E.

Institute(s): Emory University, Atlanta, United States

Text: Trauma from explosions or other blunt force injury frequently results in loss of visual function. We hypothesize that drugs that activate the brain-derived neurotrophic factor (BDNF) receptor, TrkB, will slow the progressive loss of visual function following blast injury to the eye. Blast overpressure directed at the mouse eye results in microglial activation in the retina and astrocytosis in the retinal ganglion cell and nerve fiber layers. This is followed by loss of retinal ganglion cells and optic nerve axons, and a decrease in visual contrast sensitivity and acuity thresholds. N-[2-(5-hydroxy-1H-indol-3-yl)ethyl]-2-oxopiperidine-3-carboxamide (HIOC) is a small molecule, potent activator of TrkB receptors in the mammalian nervous system. It crosses the blood brain barrier and blood retinal barrier following systemic administration. Treatment with HIOC for 1 week following blast injury mitigates the loss of visual function for at least four months. HIOC treatment reduces the loss of retinal ganglion cells and optic nerve axons, and partially prevents the decrease of contrast sensitivity and visual acuity. HIOC is effective when first administered within three hours after blast exposure. The effect of HIOC is blocked by ANA12, an antagonist of the TrkB receptor, supporting the role of the BDNF receptor in its mechanism of action. We conclude that HIOC and similar activators of the TrkB receptor represent useful therapeutic approaches for the treatment of trauma-induced vision loss.

Travel Grant: I will not apply for Travel Grant

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Effects of Blue Light on the Circadian System and Eye Physiology

GIANLUCA TOSINI
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Light emitting diodes (LEDs) are now utilized to provide illumination in industrial and commercial environments. Furthermore LEDs are also employed in TV, computers, smart phones and tablets. Although the light emitted by most of LEDs appears white, LEDs have a peak of emission in the blue light range (400-490 nm). Accumulating experimental evidence indicate that exposure to blue light can affect many physiological functions and can be used to treat circadian and sleep dysfunctions. However blue light can also induce photoreceptor damage. Hence, it is important that the spectral output of LED-based light sources should be considered in order to minimize the danger that may be associated with blue light exposure. In this talk I will summarize the current knowledge on the effects of blue light in the regulation of physiological functions and the possible effects of blue light exposure on ocular health.

Circadian Clocks within the Retina Synchronize to Light: Dark Cycles Using OPN5

ETHAN BUHR[1], Richard Lang[2], Russell Van Gelder[1]
[1]University of Washington, Department of Ophthalmology, Seattle, United States, [2]Cincinnati Children's Hospital Medical Center, Center for Chronobiology, Cincinnati, United States

Cell populations within the mammalian retina have the ability to entrain local circadian rhythms of gene expression to light:dark cycles independently of behavioral or SCN phase. In previous work, we demonstrated that OPN5 is necessary for this photoentrainment in cultured mouse retinas. To study this in vivo, mice without OPN5 (*Opn5*$^{-/-}$) and wild-type littermates were behaviorally entrained to light:dark cycles for at least 2 weeks. Retinas and livers were harvested at 3 hour intervals across a 24 hour cycle. RNA transcript levels of the clock genes *Per1* and *Per2* were analyzed by quantitative RT-PCR. The transcripts of *Per1* and *Per2* in wild-type retina displayed predictable differences across the 24 hour day as has been previously reported. However, these transcripts showed variable levels from retina to retina among *Opn5*$^{-/-}$ mice, and the circadian component of *Per1* and *Per2* transcript levels was not observed in the averaged values of *Opn5*$^{-/-}$ retinas. The rhythms of transcript abundance of *Per1* and *Per2* were not different between the livers of wild-type and *Opn5*$^{-/-}$ mice. OPN5 is expressed in the ganglion cell layer in cells distinct from OPN4-expressing ganglion cells. In conclusion, *Opn5*$^{-/-}$ retinas are deficient in local photoentrainment both *in vivo* and *in vitro* while visual and behavioral photoreception through rods, cones, and melanopsin remains intact.

OPT10 - TBI (traumatic brain injury): Visual dysfunction and treatment

Visual Aspects of TBI

RICHARD BLANCH[1,2,3]
[1]University of Birmingham, Neurotrauma Research Group, Birmingham, United Kingdom, [2]Royal Centre for Defence Medicine, Academic Department of Military Surgery and Trauma, Birmingham, United Kingdom, [3]Birmingham and Midland Eye Centre, Birmingham, United Kingdom

Traumatic optic neuropathy (TON) describes optic nerve damage and retinal ganglion cell (RGC) death after trauma, causing irreversible visual loss. TON can be direct - where the optic nerve is crushed or cut or - more commonly - indirect, where brain or eye injury is associated with secondary RGC degeneration. Lost central nervous system (CNS) neurons, including RGC, are not replaced. Eye injuries affect both military and civilian populations and TON develops in ~5% of patients after closed head injury, representing 20% of ocular injuries in military personnel.
Traumatic optic neuropathy can be induced in a number of different ways in animal models - directly by crush injury and indirectly by blunt ocular trauma and primary blast injury.
The cell death protease caspase-2 has features of both initiator and executioner caspases and active enzyme is detectable by western blotting and immunohistochemistry in rat RGC after injury by optic nerve crush and blunt ocular trauma, when its inhibition is RGC neuroprotective. Caspase-2-dependent RGC apoptosis has been prevented by both pharmacological inhibitors and siRNA. Chemical modification of siRNA confers endonuclease resistance and improves tissue retention, making siRNA knockdown a translatable neuroprotective treatment.
RGC and related glial stress responses after injury and axotomy will be discussed including apoptotic and alternative RGC death signalling pathways such as necrosis and necroptosis, which are also active after blunt ocular trauma, with the potential for neuroprotective interventions.

Mechanisms and Therapy in Air Blast Induced Eye Trauma

TONIA REX[1], Courtney Bricker-Anthony[1], Brendan Lunn[2], Lauren D'Surney[2], Minhee Jo[1], Alexandra Bernardo-Colon[1]

[1]Vanderbilt University Medical Center, Nashville, United States, [2]University of Tennessee Health Science Center, Memphis, United States

Purpose: To understand molecular mechanisms underlying blast-induced vision loss and test potential therapies.
Methods: Adult mice were exposed to a single 26psi over-pressure air-wave directed at the left eye. Retinal oxidative stress, neuroinflammation, cell death, and vision were assessed out to 1-month after injury. A subset of mice was treated with erythropoietin, a cytokine currently in Phase III trials for the treatment of neurotrauma.
Results: Blast trauma causes an increase in oxidative stress in the first week followed by cell death and axon degeneration. Erythropoietin exacerbated oxidative stress and cell death early on through increased oxygen and iron delivery to the retina. Prolonged or delayed treatment with erythropoietin was protective due, at least in part, to increased expression of antioxidant enzymes.
Conclusions: Closed-globe eye trauma induces an increase in reactive oxygen species that may contribute to neuronal death. Intraocular treatment with erythropoietin may be more effective in treating blast-induced retinal damage by avoiding an elevation in the hematocrit.

HIOC, a TrkB Receptor Activator, for the Treatment of Blast-induced Vision Loss

P. MICHAEL IUVONE, Susov Dhakal, Polina Lyuboslavsky, Li He, Felix L. Struebing, Jeffrey H. Boatright, Eldon E. Geisert

Emory University, Atlanta, United States

Trauma from explosions or other blunt force injury frequently results in loss of visual function. We hypothesize that drugs that activate the brain-derived neurotrophic factor (BDNF) receptor, TrkB, will slow the progressive loss of visual function following blast injury to the eye. Blast overpressure directed at the mouse eye results in microglial activation in the retina and astrocytosis in the retinal ganglion cell and nerve fiber layers. This is followed by loss of retinal ganglion cells and optic nerve axons, and a decrease in visual contrast sensitivity and acuity thresholds. N-[2-(5-hydroxy-1H-indol-3-yl)ethyl]-2-oxopiperidine-3-carboxamide (HIOC) is a small molecule, potent activator of TrkB receptors in the mammalian nervous system. It crosses the blood brain barrier and blood retinal barrier following systemic administration. Treatment with HIOC for 1 week following blast injury mitigates the loss of visual function for at least four months. HIOC treatment reduces the loss of retinal ganglion cells and optic nerve axons, and partially prevents the decrease of contrast sensitivity and visual acuity. HIOC is effective when first administered within three hours after blast exposure. The effect of HIOC is blocked by ANA12, an antagonist of the TrkB receptor, supporting the role of the BDNF receptor in its mechanism of action. We conclude that HIOC and similar activators of the TrkB receptor represent useful therapeutic approaches for the treatment of trauma-induced vision loss.

Activation of the Innate Immune System Following Blast Injury to the Eye

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Our group at Emory is characterizing the effects of an 48psi blast injury to the eye. The present study examines the activation of the innate immune system and the chronic infiltration of T-cells into the compromised retina. Blast injury was produced using a modified paintball gun (Hines-Beard et al. Exp. Eye Res 2012, 99:36-41) in the BXD recombinant inbred (RI) strain set. Expression datasets were generated 5 days after blast and compared with normal retinal microarray datasets respectively constructed from 56 mouse strains. The dataset is presented on GeneNetwork (genenetwork.org). The innate immune network and microglia are activated following blast with significant upregulation of C4b ($p=0.01$), Cx3cr1 ($p=0.067$), and Il-10 ($p< 0.001$). In addition, there is a clear indication that T-cells are invading the retina by the upregulation of CD4, FoxP3 and CD8 (known markers of lymphocyte subtypes). To investigate the possibility that lymphocytes were invading the retina, we examined a blast eye 10, 21 and 30 days following the initial injury. Surprisingly, we found a significant number of invading CD4 and CD8 positive T-cells. We examined the ligands for these receptors and found that message for Csf and Fasl were correlated with both networks. This led us to hypothesize that the activation of the innate immune network as seen 2 days after optic nerve crush goes on to involve the acquired immune system resulting in the infiltration of lymphocytes into the neuronal tissue. Our bioinformatic analysis of the DoD Blast Injury dataset indicates that the cytokines CSF and the membrane protein FASL are likely

links between the activation of the innate immune system and the infiltration of lymphocytes into the retina. Future studies will focus on targeting this inflammatory cascade to counter long-term detrimental consequences.
This work was supported by grants: R01EY017841, P30EY06360, DoD CDMRP Grant W81XWH1210255, DoD CDMRP Grant W81XWH-12-1-0436

OPT11 - Dry eye diagnosis

Staining of Ocular Surface: Optimizing Diagnosis and Interpretation. Basis of Successful Surgery and Adaptive Therapy of Dry Eye

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Dry eye disease (DED) is a condition of the ocular surface condition that is increasingly recognized to affect not only the patients' well-being but also the surgical outcome of various procedures. Although corneal fluorescein staining (CFS) is the key indicator of ocular surface health, changes in the conjunctiva and sub conjunctival tissue are important actors in DED. Dry eye induced tissue stress and inflammation is linked to pre-fibrotic and fibrotic processes, tissue reactions that do may also determine the future success of ocular surgery. The parameters defining optimized CFS performance and understanding will be presented. The importance of conjunctival staining will be outlined, providing clues for adaptive therapy; both in normal eyes with DED alone as well as in eyes having undergone surgery. This will be demonstrated with cases of patients having undergone glaucoma surgeries with bleb formation. Although CFS is a hallmark of ocular surface health and its used to judge improvement of ocular surface condition during treatments, conjunctival staining apparently persist longer when improvement of DED is considered. Even when the intensity of CFS has normalized during treatment, the conjunctival pathology is not necessarily synchronized with this improvement. In patients undergone surgery intensified staining of the area of surgery or areas thereof does persist a long time after surgery. Accurate staining technique in judgement of CFS is essential. However, the detected delay in decrease of conjunctival staining in DED improvement and after surgery does deserve more attention. This emphasizes the need for proper dry eye care to be adapted to the specific form of dry eye pathology in each patient. Especially in glaucoma patients the structure of the blebs, and their sensitivity to mechanical disturbance does indeed emphasize the urgent need for appropriate dry eye treatment. Such optimized treatment is of major interest as possible scarring of the filtration site is a known major threat for the persistence of the surgical success. The correct staining of the entire ocular surface is very important in DED. Neglect of conjunctival staining and single focus on CFS may lead to miss-interpretation of the ocular surface condition and premature change of therapy, causing possible relapse of therapy success and possibly contributing to failure of surgery.

Inflammation in the Diagnosis of Dry Eye Disease

CHRISTOPHE BAUDOUIN
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Ocular surface disorders (OSD) constitute a series of complex diseases involving the lacrimal gland and the tear film, meibomian glands and eyelids, and all cellular components of the cornea and the conjunctiva. Whatever the mechanisms initially involved causing dry eye, i.e., allergic, infectious, toxic or environmental aggressions, blepharitis, autoimmunity or steroid hormone imbalance, they stimulate a series of pathological pathways, the main two being inflammation and apoptosis. Tear film instability or hyposecretion can be considered as the central key point of DED. Either will cause local or diffuse hyperosmolarity of the tear film and therefore of superficial epithelial cells of cornea and/or conjunctiva, stimulating epithelial cells and resident inflammatory cells. Cell damage that will result at levels of cornea and conjunctiva, by mean of apoptosis, direct mechanical and/or osmotic stress, will stimulate the reflex neurosensory arc, stimulating lacrimal gland and neurogenic inflammation, with inflammatory cytokine release, MMP activation and inflammatory involvement of the conjunctival epithelium. Goblet cell loss is thus directly related to chronic inflammation and surface cell apoptosis subsequent to cell hyperosmolarity and chronic damage, resulting in further tear film instability/imbalance and actually leading to a vicious circle, characteristic of severe dry eye disease. Inflammation can be assessed by the way of biological, namely proteomics or impression cytology-based expression of biomarkers like HLADR, or morphological techniques, like corneal confocal microscopy. Either primary, and directly cause of tear film impairment, or secondary to corneoconjunctival damage, inflammation has therefore become a major therapeutic target. Various anti-inflammatory strategies based on steroids, topical cyclosporin A, oral doxycycline or other immunomodulating agents have been developed or are under investigations.