Prophylactic treatment with CN-105 improves functional outcomes in a murine model of closed head injury

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Abstract
The treatment of traumatic brain injury (TBI) in military populations is hindered by underreporting and underdiagnosis. Clinical symptoms and outcomes may be mitigated with an effective pre-injury prophylaxis. This study evaluates whether CN-105, a 5-amino acid apolipoprotein E (ApoE) mimetic peptide previously shown to modify the post-traumatic neuroinflammatory response, would maintain its neuroprotective effects if administered prior to closed-head injury in a clinically relevant murine model. CN-105 was synthesized by Polypeptide Inc. (San Diego, CA) and administered to C57-BL/6 mice intravenously (IV) and/or by intraperitoneal (IP) injection at various time points prior to injury while vehicle treated animals received IV and/or IP normal saline. Animals were randomized following injury and behavioral observations were conducted by investigators blinded to treatment. Vestibulomotor function was assessed using an automated Rotarod (Ugo Basile, Comerio, Italy), and hippocampal microglial activation was assessed using F4/80 immunohistochemical staining in treated and untreated mice 7 days post-TBI. Separate, in vivo assessments of the pharmacokinetics was performed in healthy CD-1. IV CN-105 administered prior to head injury improved vestibulomotor function compared to vehicle control-treated animals. CN-105 co-administered by IP and IV dosing 6 h prior to injury also improved vestibulomotor function up to 28 days following injury. Microglia counted in CN-105 treated specimens were significantly fewer ($P = 0.03$) than in vehicle specimens. CN-105 improves functional outcomes and reduces hippocampal microglial activation when administered prior to injury and could be adapted as a pre-injury prophylaxis for soldiers at high risk for TBI.

Keywords Traumatic brain injury · TBI · Neuroprotection · Prophylaxis · Neuroinflammation · Prehospital · Military · Outcome · Injury model

Abbreviations
A2AD Anti-access/area denial
ACURO Animal Care and Use Review Office
ANOVA Analysis of variance
ApoE Apolipoprotein E
CNS Central nervous system
DoD Department of Defense
LRP-1 Low density lipoprotein receptor-related protein 1
MRT Mean plasma residence time
IP Intraperitoneal
IPB Intelligence preparation of the battlefield
IV Intravenous
PBS Phosphate buffered saline
RT Room temperature
SD Standard deviation
$T_{1/2}$ Terminal elimination half-life
TBI Traumatic brain injury
$T_{\text{max}}$ Time to maximal plasma concentration
Background

Traumatic brain injury (TBI) has come to be known as the “signature injury” of the Global War on Terror (Hayward 2008; Chapman and Diaz-Arrastia 2014). Furthermore, TBI has become increasingly recognized as a complication of military training exercises such as combatives, obstacle courses, airborne operations, demolitions ranges, and heavy-weapon training (Chapman and Diaz-Arrastia 2014; Mckee and Robinson 2014; Ivins et al. 2003; Carr et al. 2015; Carr et al. 2016). According to the most recent data there have been 430,720 total TBIs of all severities reported across the Department of Defense (DoD) between 2000 and the third quarter of 2020 (DoD 2020), with projected health care costs related to the care of TBI of approximately $14 billion over the next 20 years (Pogoda et al. 2017). Unfortunately, there are no neuroprotective pharmacological therapies that have been demonstrated to improve long-term functional outcome following TBI, and the treatment options for patients with persistent TBI symptoms remain primarily supportive (Gruenbaum et al. 2010; Davidson et al. 2015). One genetic association tied to outcome after TBI is the apolipoprotein E (ApoE) polymorphism (Lynch et al. 2003; Laskowitz et al. 2007). ApoE is a 34 kD lipoprotein composed of 299 amino acids, and is the primary apolipoprotein produced in the brain. Its synthesis is upregulated after injury. ApoE has been demonstrated to reduce glial activation and inflammatory cytokine release in vitro and in vivo closed head injury models (Laskowitz et al. 2007). Although ApoE does not readily cross the blood brain barrier, we developed CN-105, a 5-amino acid peptide (Ac-VSRRR-NH2) derived from the polar face of the ApoE receptor binding region. This peptide readily crosses into the central nervous system (CNS), is well tolerated, and improves long-term histological and functional outcome in multiple preclinical models of acute brain injury, including subarachnoid hemorrhage (Liu et al. 2018), intracranial hemorrhage (Lei et al. 2016), stroke (Tu et al. 2017), blast injury (Yu et al. 2018), and closed head injury (Laskowitz et al. 2017). Moreover, CN-105 is an excellent candidate for clinical translation, and has demonstrated an excellent safety profile, as well as a linear and predictable pharmacokinetic profile in phase 1 escalating dose studies involving healthy volunteers (Guptill et al. 2017). We now test the proof-of-concept hypothesis that CN-105 retains its neuroprotective and anti-inflammatory effects when administered prior to injury in murine models of closed head injury.

Methods

This study was carried out in accordance with the recommendations in the Guide for the Care and Use of Laboratory Animals of the National Institutes of Health. The protocol was approved by the Duke University Institutional...
Animal Care and Use Committee, Durham North Carolina as well as Department of Defense Animal Care and Use Review Office (ACURO).

**Closed head injury model**

The murine closed head injury model used in this study has been previously described and results in injury to selectively vulnerable neurons in the cortex and hippocampus associated with acute vestibulomotor deficits (Laskowitz et al. 2017). 10–12-week-old C57BL/6 male mice (Jackson Laboratories, Bar Harbor, ME) were used. The trachea was intubated after anesthesia induction with 4.6% isoflurane and the lungs were mechanically ventilated with 1.6% isoflurane in 30% O2/70% N2. Core body temperature was servo regulated at 37 °C via rectal probe. Ear bars were not used to avoid basilar skull fracture. The animal was secured in a stereotactic device, mechanically ventilated, the head was shaved, and a midline scalp incision was made to identify anatomical landmarks. A concave 3-mm metallic disk was adhered to the skull immediately caudal to the bregma. A 2.0-mm diameter pneumatic impactor (Air-Power Inc., High Point, NC) was used to deliver a single midline impact to the disk surface. The impactor was discharged at 6.8 ± 0.2 m/s with a head displacement of 3 mm. After impact, the animals were allowed to recover. Once spontaneous ventilation resumed the mice were extubated. Mice were allowed free access to food and water. Sham mice were treated identically but had no induced head injury. All mice were housed in the same facility.

**Drug administration**

CN-105 (Ac-VSRRR-amide) was synthesized by Polypeptide Inc. (San Diego, CA) to a purity of > 99%. During the first part of the experiment, CN-105 was administered intravenously at doses of 0.2 mg/kg or 0.05 mg/kg based on previously published data (Laskowitz et al. 2017) with animals randomized to receive treatments at 10, 20, 30 or 60 min prior to injury. Next, CN-105 was dissolved in sterile, 0.9% saline and administered intravenously (IV) through a tail vein at 0.5 mg/kg or by intraperitoneal (IP) injection at a dose of 1 mg/kg. To establish clinically relevant effects, and because it would be impossible to effectively scramble the 5 amino acid peptide containing 3 arginines, vehicle treated animals received 1 mg/kg IV and/or IP injection of 100 μl of normal saline at the same time points. Animals were randomly assigned to treatment groups by a coded study identification number after injury using Graphpad online randomizer.

**Testing of functional deficits**

Mice were randomly assigned to treatment groups immediately following injury and all behavioral evaluations were performed by investigators blinded to treatment. An automated Rotarod (Ugo Basile, Comerio, Italy) was used to assess vestibulomotor function. On the day prior to injury, mice (n = 10–15 mice per group) underwent one training trial at an accelerating rotational speed (4–40 rpm) for at least 200 s and then three additional test trials with the same accelerating rotational speed. The average time to fall from the rotating cylinder in the test trials was recorded as baseline latency. Mice were tested on consecutive days post-injury and received three consecutive daily trials with accelerating rotational speed (inter-trial interval = 15 min; n = 10–15 mice). The average latency to fall from the rod was recorded. Mice unable to grasp the rotating rod were given a latency value of 0 s.

**Pharmacokinetic methods**

An in vivo assessment of the pharmacokinetics of CN-105 following 1 mg/kg IV or intraperitoneal (IP) administration in healthy fed adult male CD-1 mice was conducted by Charles River (Worcester, MA). This was derived from dosing used in previously conducted phase I trials (Guptill et al. 2017). Briefly, CN-105 was aseptically resuspended in 0.9% sodium chloride and filtered through a 0.22-micron PVDF syringe filter prior to IV or IP dosing. Terminal blood was collected at 0.083, 0.25, 0.5, 1, 2, 4, and 8 h following IV or IP administration (n = 3 per dose group) in K2EDTA tubes and stored on wet ice until processed to plasma by centrifugation at 3500 rpm for 10 min at 5 °C within 30 min of collection. Plasma was transferred to fresh tubes containing Halt protease inhibitor cocktail (ThermoFisher Scientific) at a final concentration of 1%, vortexed, frozen and stored at − 80 °C. Plasma CN-105 levels were determined by liquid chromatography (Agilent 1290)-tandem mass spectrometry (API6500 +). The analytical range was 5–5000 ng/ml (R2=0.995). Pharmacokinetic parameters were determined by non-linear compartmental analysis using WinNonlin (Certara, Princeton, NJ, USA).

**Brain harvesting and sectioning**

Mice were perfused 7 days post-TBI with 25 ml phosphate buffered saline (PBS) and fixed overnight in 4% paraformaldehyde in PBS. Brains were left in sucrose 30% for cryoprotection prior to being sectioned in 40 μm sections by freezing microtome. The slices were stored at − 20 °C in freezing solution (sodium phosphate 0.05 mol/L, pH 7.4, ethylene glycol 30%, sucrose 15%). Mid-sagittal sectioning
was performed after sacrifice, with one hemisphere used for histology and the other for biochemical analysis. The hemispheres were harvested immediately after perfusion with saline to allow drop fixation of one hemisphere and snap freezing of the contralateral hemisphere for protein extraction.

**F4/80 immunohistochemistry**

F4/80 has been used in prior CN-105 experiments to evaluate microgliosis and reflects neuroinflammatory response after injury. Although it does not directly reflect activation state, it stains all cells of monocyte lineage to include hematogenous monocytes recruited to the injured region as well as endogenous microglia. Briefly, 1 of every 8 sections was permeabilized in saponin 1%, for 1 h at room temperature (RT). The sections were blocked for 30 min in tris buffered saline (TBS) plus 10% goat serum. F4/80 antibody (Invitrogen cat # PA5 21399, 1:30,000 in TBS plus 1% Goat serum) was incubated overnight at 4 °C. Goat anti-mouse biotinylated antibody (vector cat # BA-9400, 1:3000 in TBS plus 1% goat serum) was incubated for 2 h at RT. Secondary antibody was labeled using Vectastin Elite ABC-HRP Kit (Vector cat # PK 6100) for 1 h at RT. The peroxidase activity was detected using DAB substrate kit (vector cat SK 4100) for 5 min at RT.

**Unbiased stereology**

Unbiased stereology to assess microglial activation (density of F4/80 stained cells) was performed as previously described (Laskowitz et al. 2017). Briefly, microglia cells were counted using the Stereo Investigator program version 2019.1.3, (MBF Bioscience Inc). A total of 6 sagittal sections 0.36–2.40 mm lateral position were assessed. The Optical fractionator probe was run using the following parameters: Interval among sections 320 µm, sampling grid 400 µm x 400 µm, counting frame 200 µm x 200 µm. Slice thickness 40 µm, mounted slice thickness 10 µm. In these sections of the brain, the top and the bottom areas of the hippocampus are not connected. Only the top area of the hippocampus was counted. The counting area for the cortex was defined by (i) surface of the brain, (ii) the corpus callosum, and (iii) two straight lines from the corpus callosum to the surface of the brain (the anterior and the posterior borders). The anterior border was located on top of the union between fornix and hippocampus and the posterior border was located at the end of the corpus callosum.

Estimated population via user defined section thickness was calculated using the following formula:

\[
N = \sum Q^T \cdot \frac{t}{h} \cdot \frac{1}{asf} \cdot \frac{1}{ssf}
\]

\(Q^T\) is the particles counted; \(t\) is the section mounted thickness; \(h\) is the counting frame height; \(asf\) is the area sampling fraction (counting frame/grid size); \(ssf\) is the section sampling fraction.

**Statistical analysis**

Serial tests of functional performance, including Rotarod and MWM performance, were compared with a two factor repeated measures analysis of variance (ANOVA) with time as the repeated variable. When F-value was significant for group effect, pairwise comparison was performed using post hoc Scheffe test for correcting multiple comparisons. To test the hypothesis that the behavioral improvement associated with treatment was associated with a reduction in the number of hippocampal number of F4/80 positive cells, a one-tailed T test was used. Parametric values are expressed as mean ± standard deviation (SD). Significance was assumed if \(P < 0.05\).

**Results**

**Improvement in vestibulomotor function after treatment with CN-105**

We first designed a series of experiments to assess whether CN-105 could reduce functional deficits as defined by Rotorod latency when administered intravenously prior to injury. Previously, we demonstrated neuroprotection when CN-105 was administered post-treatment, and this was used as a positive control. Dosing during this phase was based on previously established effective thresholds, with 0.05 mg/kg being the lowest effective dose (Laskowitz et al. 2017). At 10- and 20-min prior to injury, IV administration of 0.2 mg/kg CN-105 \((n = 14)\) was associated with dura-

\(P = 0.007\) and 0.006 at 10- and 20-min pretreatment, respectively; Fig. 1A). Functional benefit was also observed when CN-105 0.05 mg/kg IV was administered 30 min prior to injury \((n = 14, P = 0.003)\), although there was no significant benefit when administration occurred 60 min prior to injury (Fig. 1B).

Based on these results, we modified the dosing paradigm to include co-administration of CN-105 by IP (0.5 mg/kg) as well as IV (0.1 mg/kg) dosing at 3 or 6 h prior to injury. These dose ranges were determined based on similar dosing regimens used in prior clinical models (Laskowitz et al. 2017; Tu et al. 2017; Lei et al. 2016;
Guptill et al. 2017). As shown in Fig. 2, this dosing regimen was associated with an improved trajectory of vestibulomotor recovery that was durable when longitudinal testing was performed out to 28 days following injury \((n = 14 \text{ in each group, } P < 0.001 \text{ at 3 h and } P = 0.005 \text{ at 6 h})\).

**Pharmacokinetics of CN-105**

While our data support prophylactic use, the known half-life of CN-105 in mice is 30 min \([24]\). To address this limitation, we conducted a series of pharmacokinetic experiments to
explore the possibility that intraperitoneal injection of drug would extend the functional half-life of systemic CN-105, which is metabolized by serum proteases (Guptill et al. 2017). Figure 3 shows the mean CN-105 plasma concentrations as a function of time in uninjured CD-1 mice following IV or IP administration of 1 mg/kg CN-105 in 0.9% sterile sodium chloride. Following IV infusion, CN-105 plasma concentration declined in a polyphasic manner. Following IP administration, a short distribution phase was seen followed by a polyphasic reduction in CN-105 plasma concentration that closely mirrored that seen following IV infusion. Individual plasma concentration versus time profiles of CN-105 following IV versus IP administration were analyzed by non-compartmental analysis to determine the PK parameters (Table 1). The bioavailability of CN-105 after IP dosing was 93%. Although the CN-105 terminal elimination half-life \( T_{1/2} \) was similar following IP versus IV administration \( T_{1/2} \sim 1.1 \) to \( 1.3 \) h, the \( T_{\text{max}} \) was \( \sim \) threefold longer, and the mean residence time was \( \sim \) 1.5-fold longer following IP administration. Of note, the pharmacokinetics profile of intravenous CN-105 in the CD-1 strain was identical to that previously reported in C57-B16 (Lei et al. 2016).

**CN-105 inhibits microgliosis**

Next, we tested the hypothesis that CN-105 reduced microglial activation by quantifying F4/80 immunoreactivity in mouse brains harvested 7 days post-TBI. Five vehicle treated mouse brains and five mouse brains treated with CN-105 three hours prior to TBI were evaluated, and microglia were counted in both hippocampus and cortical sections of both vehicle and CN-105 treated animals. We found a significant reduction in hippocampal F4/80 positive cells in treated vs untreated animals (5933.85/mm\(^3\) vs. 4068.86/mm\(^3\) in the vehicle treatment vs. control mice, \( P = 0.64 \); Fig. 4B). These results are consistent with the hypothesis that the reduction in post-injury deficits associated with treatment may be due to a reduction in microgliosis and neuroinflammation.

### Table 1 CN-105 pharmacokinetic parameters following intravenous (IV) versus intraperitoneal (IP) administration in CD-1 mice

| PK Parameter | IV   | IP   |
|--------------|------|------|
| \( T_{\text{max}} \) (h) | 0.083 | 0.25 |
| \( C_{\text{max}} \) (ng/ml) | 3997 | 2230 |
| \( \text{AUC}_{0-\infty} \) (ng h/ml) | 1568 | 1455 |
| \( T_{1/2} \) (h) | 1.33 | 1.10 |
| CL (ml/h kg) | 662 | 830 |
| MRT (h) | 0.35 | 0.53 |
| \( V_{z,\text{obs}} \) (ml/kg) | 1270 | 1164 |
| \( F \) (%) | 100 | 93 |

\( T_{\text{max}} \), time to reach maximal plasma concentration, \( C_{\text{max}} \), maximal plasma concentration, \( \text{AUC}_{0-\infty} \), area under the plasma-concentration time curve for entire dosing, \( T_{1/2} \), elimination half-life, CL body clearance, MRT mean residence time, \( V_{z,\text{obs}} \), observed volume of distribution in terminal phase, \( F \), fraction bioavailability

Discussion

Traumatic brain injury is a heterogeneous disorder that is the result of both primary and secondary injury mechanisms. It can vary in severity and is frequently associated with high morbidity in all forms that negatively impacts the health and readiness of individual soldiers. As a result, considerable attention has been given to understanding the complex array of secondary injury mechanisms to develop neuroprotective therapies. Although many interventions have been evaluated for this purpose, over 30 phase III prospective clinical trials evaluating various therapies have failed to reach their primary endpoints (Narayan et al. 2002; Schouten 2007; Maas et al. 2010; Chakraborty et al. 2015; Davenport 2016). Furthermore, many of these therapies have been evaluated as post-injury interventions. While this may serve civilian TBI patients, where 60–75% of patients present shortly after injury (Setnik and Bazarian 2007; Sosin and Sniezek 1991), evaluation and documentation rates following acute mild TBI in military populations has historically been low, particularly in special operations units (Fortier et al. 2014).

A number of factors unique to military service have been identified as impediments to care, including various in-theater assessment barriers, exposure to concurrent injuries that are often more severe, symptoms that are common to alternative diagnoses or attributed to the stress of military service, and co-existing mental health comorbidities. Underreporting in the military has also impacted TBI diagnosis and care. Suggested hypotheses to explain this underreporting have included concerns among affected soldiers that reporting symptoms may result in removal from their units, colleagues and responsibilities, fear of delays return to home and family following deployment, beliefs that existing symptoms are minor and will resolve on their own, fear of stigmatization and a reduced ability to recognize TBI symptoms until return to less structured garrison and civilian lifestyles (Agimi et al. 2018; Brenner et al. 2009; French et al. 2014; Milliken et al. 2007).

Prophylactic neuroprotection for high-risk training and operations could address some of these issues. Part of the military decision-making process involves analyzing mission variables and their effect on military operations. This
includes adversary strength, composition, disposition, weapons, and most likely courses of action that allows planners to anticipate injury patterns. Although TBI can never be completely anticipated, it is possible to identify operations where TBIs would be a high-risk injury (HQ DOTA 2020). Further, certain operations, such as airborne operations, have a high prevalence of TBI. One study of US parachute operations over a 20-month period reported closed head injuries as being the second most common injury (18.4%) (Craig and Morgan 1997). Pretreatment may be a desirable option and risk mitigation measure for an airborne commander.

The development of ApoE mimetic neuroprotective therapies was based on initial observations demonstrating that endogenous ApoE played an adaptive role following brain injury by reducing neuroinflammation and secondary neuronal injury (Laskowitz et al. 2007, 2006; Zhou et al. 2008). Although the intact ApoE lipoprotein is too large to cross the blood brain barrier and thus cannot serve as a viable therapeutic (Linton et al. 1991), ApoE peptides can be created from the ApoE receptor binding region, which is believed to mediate its anti-inflammatory and neuroprotective effects via interactions with the glial LRP-1 receptor (Croy et al. 2004; Laskowitz et al. 2001). Of note, apoE mimetic peptides derived from the receptor binding region such as CN-105 share the ability of the native apolipoprotein demonstrated to reduce glial inflammatory cytokine release (Tu et al. 2017) and mitigate glutamate mediated excitotoxicity (Aono et al. 2002, 2003) in cell culture, as well as reduce neuronal degeneration and neuroinflammation in preclinical models of brain injury (Laskowitz et al. 2010, 2017; Lei et al. 2016). Importantly, there is convergent evidence that ApoE-based neuroprotectants improve outcomes in a variety of preclinical animal models including closed head injury, parenchymal and subarachnoid hemorrhage, and ischemia (Supplemental Table 2).

CN-105 was rationally developed to optimize potency and CNS penetration by linearizing the polar surface of the helical receptor binding region of ApoE. This region

Fig. 4 A A reduction in F4/80+ hippocampal microgliosis following treatment with CN-105 as compared to vehicle. At higher magnification, ramified microglial morphology is more evident in untreated mice. Formal unbiased stereology revealed a reduction in number of hippocampal F4/80 microglia as a function of treatment (*P < 0.05). B A trend toward reduced cortical microgliosis in animals treated with CN-105 compared to vehicle was observed, but did not achieve statistical significance.
bonds LRP-1 and modulates inflammatory and excitotoxic responses. CN-105 has been demonstrated to reduce glial activation in vitro, and in vivo to improve histological and functional outcomes in a number of preclinical models of brain injury. Moreover, CN-105 is stable and can be stored in lyophilized form or in solution. Importantly, phase 1 single and multiple dose escalation studies have demonstrated linear and predictable pharmacokinetic profile, and a favorable toxicity profile both in the Phase 1 and ongoing Phase 2 trials (Guptill et al. 2017). Our current observations demonstrating prophylactic efficacy of CN-105 in reducing post-TBI vestibulomotor deficit was likely a function of adequate blood levels at the time of injury. Of note, the measured half-life in humans ~ 3.5 h, is considerably longer than in rodent models (< 1.5 h) and increases the feasibility of prophylactic administration in the military setting (Laskowitz et al. 2017; Guptill et al. 2017). These data indicate that prophylactic dosing of CN-105 may be effective in improving functional outcomes and microglial activation in the hippocampus when administered prior to traumatic brain injury. Considering that balance skills have been associated with increased hippocampal volumes (Rogge et al. 2017), the reduction in microglial activation may have contributed to the improved vestibulomotor function in CN-105 treated mice. Our rodent model of closed head injury was designed to recapitulate the clinical sequelae of moderate to severe TBI, including both vestibulomotor and learning deficits. Both of these deficits occur concurrently, are reproducibly associated with each other to the extent of deficit, and are mitigated by the effects of CN-105 (Laskowitz et al. 2017). However, the short half-life associated with intravenous dosing in a murine model may limit the prophylactic window required to achieve therapeutic tissue concentrations at the time of injury. While vestibulomotor deficits are associated with injury in the motor cortex, cerebellum, or pathways associated with these structures, and hippocampal pathology is associated with deficits in learning, memory, and cognition, there is histological variability in the cortex adjacent to the impact, whereas the hippocampus is more remote, has stereological coordinates, and anatomic structure, and is easier to quantify histological injury and microgliosis by formal, unbiased stereology. This may explain why a significant difference in cortical microglial activation was not seen in CN-105 treated mice.

Although CN-105 represents an excellent candidate for clinical translation in the setting of traumatic brain injury, there are several potential limitations, which should be addressed. The results of this study, while encouraging, represent a proof of concept that are not directly applicable to the clinical setting. As a peptide, CN-105 has minimal oral bioavailability, and current clinical trials utilize intravenous administration (Laskowitz et al. 2007, 2017; Tu et al., 2017; Lei et al. 2016). Although this does not represent a challenge for administration following injury, it would not be optimal for repeated prophylactic administration. To this end, minimally or non-invasive routes of delivery, such as intranasal, subcutaneous, or transdermal administration are under investigation. Depot injections are also being considered as a potential answer to problem posed by the agent’s short half-life. It is important to note that although CN-105 was designed to mimic the receptor binding region of ApoE, the mechanism(s) by which CN-105 exerts its neuroprotective effects remains incompletely defined. Receptor binding activity and any affinity compared to full-length ApoE are not known, although interaction with the LRP-1 receptor present on neurons and glia have been demonstrated with ApoE and ApoE mimetic peptides (Croy et al. 2004; Misra et al. 2001; Yang et al. 2016; Aono et al. 2003). LRP-1 is present on neurons and glia and has been demonstrated to induce neuroprotective and anti-inflammatory effects (Laskowitz et al. 2001, 2007; Lynch et al. 2005; Aono et al. 2003). A better understanding of the physicochemical nature of this interaction may allow the rational development of small molecule therapies. Finally, although we demonstrate as proof of principle that prophylactic administration of CN-105 improves recovery and functional outcome after TBI as long as adequate blood/tissue concentrations are achieved, intraperitoneal administration is not feasible in the clinical setting. Moreover, rodent models are not ideal for studies of human clinical pharmacokinetics or disease intervention. Nevertheless, the positive effects on vestibulomotor function are encouraging. With safety and tolerability in humans previously established (Guptill et al. 2017), future studies should aim to determine if the neuroprotective effects of CN-105 seen in murine TBI trials can translate into outcome benefits in human models.

**Conclusions**

Our results demonstrate that administration of CN-105 prior to an induced murine closed head injury produced a durable improvement in vestibulomotor function. Further, there was a statistically significant reduction in microgliosis counted in CN-105 treated hippocampal sections compared to vehicle treated hippocampi. These findings suggest that based on the longer half-life of the drug observed in humans, CN-105 may be an effective prophylactic strategy for improving functional outcomes in soldiers at high risk for head injury in both training and combat environments.
**Supplementary Information** The online version contains supplemental material available at [https://doi.org/10.1007/s00221-022-06417-4](https://doi.org/10.1007/s00221-022-06417-4).

**Author contributions** DVW played primary role in data analysis, writing of manuscript. HW performed animal experiments and played a role in editing. BKJ played a role in data interpretation and editing. VC performed immunohistochemistry and editing of the manuscript. MM reviewed and edited the manuscript final draft. DTL played a role in experimental design, data analysis, writing and drafting.

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**Data availability statement** The datasets generated during and/or analyzed during the current study are available from the corresponding author on reasonable request.

**Declarations**

**Conflict of interest** The views expressed are solely those of the authors and do not reflect the official policy or position of the US Army, US Navy, US Air Force, the Department of Defense, or the US Government. Dr. Laskowitz is an officer and has equity in Aegis CN, LLC which supplied the study drug. Dr. Wang serves as a consultant for Aegis CN, LLC. Aegis CN, LLC had no editorial control over the study design, its execution, or the writing of this manuscript. Duke University has equity and an intellectual property stake in CN-105.

**Ethical approval and consent to participate** All experiments were approved by and conducted in accordance with the Duke University Institutional Animal Care and Use Committee.

**Consent for publication** Not applicable.

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