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REVIEW

Therapeutic Development of Apolipoprotein E Mimetics for Acute Brain Injury: Augmenting Endogenous Responses to Reduce Secondary Injury

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Abstract

Over the last few decades, increasing evidence demonstrates that the neuroinflammatory response is a double-edged sword. Although overly robust inflammatory responses may exacerbate secondary tissue injury, inflammatory processes are ultimately necessary for recovery. Traditional drug discovery often relies on reductionist approaches to isolate and modulate specific intracellular pathways believed to be involved in disease pathology. However, endogenous brain proteins are often pleiotropic in order to regulate neuroinflammation and recovery mechanisms. Thus, a process of "backward translation" aims to harness the adaptive properties of endogenous proteins to promote earlier and greater recovery after acute brain injury. One such endogenous protein is apolipoprotein E (apoE), the primary apolipoprotein produced in the brain. Robust preclinical and clinical evidence demonstrates that endogenous apoE produced within the brain modulates the neuroinflammatory response of the acutely injured brain. Thus, one innovative approach to improve outcomes following acute brain injury is administration of exogenous apoE-mimetic drugs optimized to cross the blood–brain barrier. In particular, one promising apoE mimetic peptide, CN-105, has demonstrated efficacy across a wide variety of preclinical models of brain injury and safety and feasibility in early-phase clinical trials. Preclinical and clinical evidence for apoE's neuroprotective effects and downregulation of neuroinflammatory and the resulting translational therapeutic development strategy for an apoE-based therapeutic are reviewed.

Key Words Apolipoprotein E · Mimetic peptides · Neuroinflammation · Acute brain injury · Therapeutic development · Stroke

Neuroinflammation: the Brain's Endogenous Response to Injury

Acute central nervous system (CNS) injury resulting from cerebrovascular disease and trauma is associated with significant morbidity and mortality [1]. Decades of advances in fundamental knowledge of basic cellular and molecular mechanisms associated with primary and secondary acute neuronal injury have led to a host of potential therapeutic targets and compounds. However, none of these pharmacological interventions have translated into effective therapies with proven efficacy on either reduction in death or improved neurobehavioral recovery in human injury [2]. Supportive neurocritical care remains the mainstay of early management for patients with acute CNS injury, with minimal improvement in mortality rates over the last two decades [3, 4]. Thus, a clear and urgent unmet clinical need exists for development of innovative neuroprotective therapeutics for such patients. New development strategies are required if the translational "valley of death" is to be crossed.

Traditionally, new therapeutic strategies have utilized a reductionist approach, and focused on cell-specific, individual target receptors discovered from our increased mechanistic understanding of disease-specific pathophysiology. While the importance of cell- and disease-specific mechanisms is

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obvious, therapeutic development for CNS injury has been limited by the hunt for the "silver bullet." However, pleiotropic compounds might heighten the potential for successful translation by targeting several mechanisms across multiple cell types interconnected by a salient brain response. For example, neuroinflammation serves as a common denominator brain response that exacerbates secondary neuronal injury in a variety of acute and chronic neuropathologies. Moreover, in the setting of acute CNS injury, neuroinflammation plays an important role in mediating secondary tissue injury for days after initial insult [5, 6]; thus, the therapeutic window for such approaches may be wider than strategies solely targeting excitotoxicity. For these reasons, targeting neuroinflammatory responses for therapeutic development holds promise in the treatment of diverse forms of brain injury [7].

In the absence of infection, brain injury triggers immune reaction through microglial activation as a central factor in mediating neuroinflammatory responses, development of cerebral edema, and secondary neuronal death following acute CNS injury [8]. Activation of microglia and recruitment of peripheral mononuclear cells into the brain represent the cornerstone of the CNS neuroinflammatory response. Through initial release of damage-associated molecular pattern molecules, reactive oxygen species, and inflammatory cytokines, neuroinflammation has both adaptive and maladaptive effects on brain tissue survival and ultimate recovery [9]. This inflammatory cascade contributes to oxidative stress, secondary neuronal injury, blood–brain barrier breakdown, resulting cerebral edema, and tissue and cellular disruption. Microglial cells become activated within minutes of brain injury, and evidence supports a persistent chronic activation of microglia after initial injury [10, 11]. After their initial activation, microglial cells secrete proinflammatory cytokines and chemokines, such as interleukins and tumor necrosis factor, which contribute to blood–brain barrier breakdown, leukocyte margination, the development of cerebral edema, and, ultimately, cellular dysfunction and death [12, 13]. Although a variety of immunomodulatory strategies have been tried in the setting of acute CNS injury [14], no pharmacological interventions improve long-term neurobehavioral outcomes or mortality [2, 15]. Despite prior translational failures, future promise may lie in harnessing genetic influences known to modify acute CNS injury responses and recovery.

APOE: Prototype of Genetic Association with Neuroinflammation

One of the most robust genetic associations with outcome after acute CNS injury is apolipoprotein E (APOE—gene; apoE—protein). Cumulative evidence suggests that APOE4 is associated with poor outcome after acute CNS injury [16, 17], although several conflicting reports exist on APOE's relationship with prognosis after subarachnoid hemorrhage [18, 19] and intracerebral hemorrhage [20, 21].

ApoE is the primary apolipoprotein produced within the brain, where its glial secretion is upregulated after injury. ApoE has three common human protein isoforms (designated apoE2, apoE3, and apoE4) which differ by single cysteine to arginine interchanges at residues 112 and 158 [22]. In addition to its role in cholesterol transport, biologically relevant concentrations of apoE modify glial activation and inflammatory cytokine release [23, 24]. In fact, an established literature exists suggesting lipoproteins play a significant role in immune function and modulating inflammatory responses. For example, HDL-associated apoA-I modifies innate immune responses against foreign pathogens [25] and is essential for lipopolysaccharide (LPS) neutralization to protect against pathogenic LPS effects in preclinical models [26]. In addition, HDL plays a key role in downregulating systemic inflammation in preclinical models for several disease processes including sepsis, atherosclerosis, and lung injury [27–29]. Similarly, an extensive body of work has demonstrated that apoE plays an essential role in modulating host defenses and innate immune responses against foreign pathogens, both by modifying monocyte responses and by modulating T cell–mediated immune responses [30, 31]. A number of preclinical studies showing abnormal immune responses in apoE-deficient animals further substantiate a significant role for apoE in modulating the immune response and blood–brain barrier integrity [32–34].

Subsequent studies demonstrated that apoE may exert isoform-specific modulation of the inflammatory response of the injured CNS in the clinical setting [35]. In particular, the APOE4 polymorphism has been associated with increased systemic inflammatory responses [36] and delirium in the intensive care setting [37]. Further, presence of an APOE4 allele has been associated with poor neurobehavioral outcome in a number of acute clinical brain injuries, including increased cerebral edema after intracerebral hemorrhage [35], and poor functional outcome after subarachnoid hemorrhage [18], and traumatic brain injury [38]. These preliminary clinical observations are reinforced by murine models of brain injury demonstrating worse neurobehavioral outcomes and increased neuroinflammatory responses in the presence of the APOE4 allele [33, 39–42]. One plausible explanation for these isoform-specific effects is that apoE modulates glial activation and neuroinflammatory cascades in an isoform-specific fashion. Indeed, presence of the apoE4 isoform is associated with enhanced glial activation and secondary neuronal injury as compared to apoE3 in both *in vitro* and *in vivo* paradigms of acute brain injury [42, 43].

The exact mechanism(s) of apoE's influences in brain injury and recovery are not completely defined, although evidence suggests its interaction with the low-density lipoprotein receptor–related protein 1 (LRP-1), also known as alpha-2-

macroglobulin receptor, apolipoprotein E receptor, or cluster of differentiation 91 (Fig. 1) [44]. The LRP-1 receptor, present on both glia and neurons, may be necessary to mediate anti-inflammatory and neuroprotective effects of apoE [45]. This hypothesis is consistent with the fact that peptides containing the apoE receptor-binding region appear to bind LRP-1 and modulate N-methyl-aspartic acid (NMDA) receptor activity in neurons [46–49]. These same peptides also downregulate the inflammatory phenotype of microglia, an effect that is not observed in LRP1-deficient microglia [50]. Although apoE's polymorphic regions determining E2, E3, and E4 isoforms (cysteine to argine interchanges at positions 112 and 158) lie outside the receptor-binding region (residues 130–149), these polymorphisms clearly affect receptor binding. Notably, apoE3, but not apoE4, mediates LRP-1 dependent clearance of Aβ [51], and reports have suggested impaired binding of apoE4 to LRP-1 *in vivo* [52]. Importantly, although LRP-1 is the presumed target receptor of apoE binding, it is only one of a family of low-density lipoprotein receptors that have significant overlap with regard to ligand binding.

Clinical relevance of the collective evidence of apoE's response to brain injury in preclinical modeling is suggested by use of APOE-targeted replacement mice expressing the human protein isoforms. Mice expressing human apoE4 have increased systemic and brain inflammatory responses following challenge with bacterial endotoxin [33]. Seminal observations have demonstrated that biologically relevant concentrations of apoE modify glial activation in cell culture [23, 24], an observation that was later extended to demonstrate isoform specificity [53]. These isoform-specific effects of apoE on inflammation are highly likely to be clinically relevant, as a pilot clinical study in patients with intracerebral hemorrhage demonstrated increased cerebral edema and worsened outcome associated with the APOE4 allele [35]. This finding was backward translated demonstrating humanized apoE4 association with increased cerebral edema and worse neurobehavioral outcome in preclinical models [49]. Moreover, targeted replacement animals expressing the apoE4 isoform have evidence of increased glial activation subsequent inflammation and worse behavioral outcomes than their apoE3

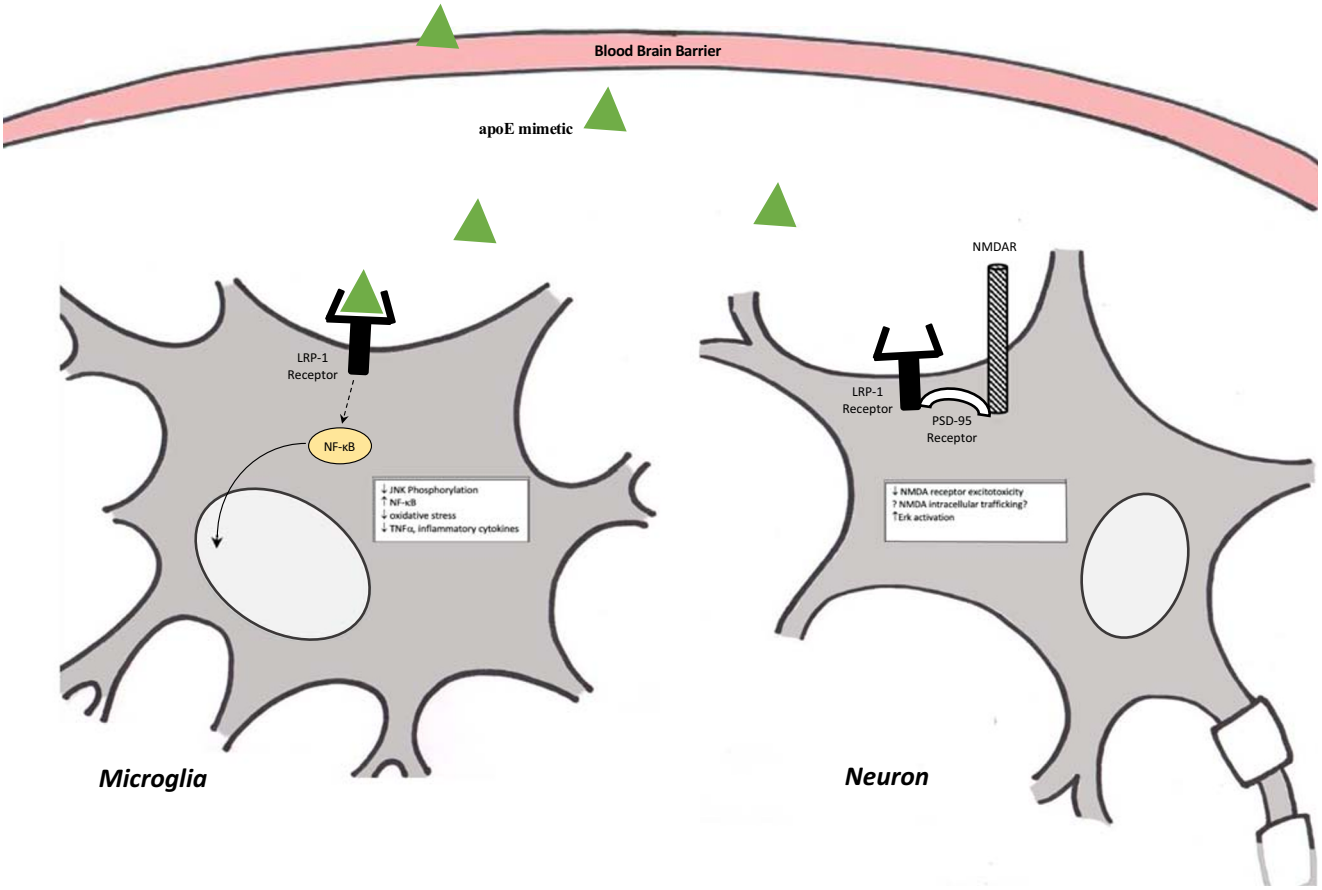

Fig. 1 Apolipoprotein E (apoE)-mimetic peptides, such as the pentapeptide CN-105, are derived from apoE holoprotein receptor-binding region and have the ability to cross the blood–brain barrier. These peptides exhibit anti-inflammatory and neuroprotective responses after acute CNS injury by binding lipoprotein receptor-related protein 1 (LRP-1) on both microglia and neurons. Note: Erk, extracellular signal-regulated kinase; JNK, c-Jun N-terminal kinase; NF-KB, nuclear factor kappa-light-chain-enhancer of activated B cells; NMDA, N-methyl-D-aspartic acid; PSD-95, postsynaptic density protein 95; TNF, tumor necrosis factor

counterparts in murine models of traumatic brain injury [42, 54] and subarachnoid hemorrhage [39, 55]. Despite the bulk of evidence of isoform-specific differences, whether apoE4 plays an active role in exacerbating maladaptive neuroinflammatory responses, i.e., "direct negative effect" or whether apoE4 interferes with the beneficial neuroprotective effects of apoE3, is unknown [43].

Finally, in addition to modulating neuroinflammatory responses, apoE and apoE-mimetic peptides exert direct neuronal protection from NMDA-mediated excitotoxicity [49, 56–58]. Several mechanisms have been proposed to explain the effects of apoE on NMDA function, including modulating function via the intracellular scaffolding protein PSD-95 and modulating intracellular trafficking [59, 60].

Development of apoE-Based Therapies: Reverse Engineering a Neuroprotective Therapy

Given the neuroprotective effects of apoE in the setting of acute CNS injury, one therapeutic strategy might be to administer exogenous apoE protein. However, the intact apoE holoprotein does not readily cross the blood–brain barrier (BBB), and is, thus, unsuitable for peripheral administration [61]. An innovative approach to improve outcomes following acute CNS injury is the administration of apoE-mimetic drugs designed to penetrate into the CNS compartment. To address this problem, we originally created a series of apoE-mimetic peptides derived from the apoE receptor-binding region (residues 130–150) [53, 62]. These peptides had robust efficacy in cell culture and improved neurobehavioral and histological endpoints in preclinical models of CNS inflammation and acute CNS injuries (see Table 1), including intracerebral and subarachnoid hemorrhage [31, 38, 39, 52, 63] and both closed skull and cortical contusion models of brain trauma [63–67]. Most recently, a second generation of mimetic peptides were produced to improve CNS penetration after intravenous administration. CN-105, a 5-amino acid peptide derived from the binding face of the apoE receptor-binding region, is such a second-generation apoE-mimetic. CN-105 is associated with increased potency and CNS penetration [63–65] and maintains the anti-inflammatory and neuroprotective functionality of the intact apoE protein. We have recently demonstrated that systemic administration of CN-105 is well tolerated, and associated with a reduction in cerebral edema and improved neurobehavioral outcomes after intrastriatal collagenase injection model to induce intracerebral hemorrhage [68], as well as models of other related acute CNS pathologies such as subarachnoid hemorrhage [69–72], focal ischemia/reperfusion [73–76], and traumatic brain injury [77–79]. Finally, significant pharmacokinetics-pharmacodynamics dissociation for CN-105 effects in the brain. Although serum half-life of CN-105 is short (30 min in rodents), durable pharmacodynamic effect occur when CN-105 is administered after CNS injury, as evidenced by long-term improvement in neurological outcomes [78].

Clinical Development: Phase 1 Study

Based on promising preclinical results and safety data, CN-105 was selected for drug development to treat patients with acute CNS injuries. Following Investigational New Drug (IND) enabling studies, a first-in-human single ascending dose (SAD) and multiple dose placebo-controlled clinical trial was performed to define the safety and pharmacokinetics (PK) of CN-105 in healthy human adults (NCT02670824) [80], In the SAD portion of this first-in-human study, 8 participants were randomized to CN-105 or saline control (6 active, 2 control) at 0.01, 0.03, 0.1, 0.3, and 1.0 mg/kg administered over 30 min. In the multiple dose placebo-controlled clinical trial, additional subjects (6 active, 2 control) were randomized to receive 1.0 mg/kg at 6-h intervals over 72-h period. All 48 randomized subjects completed the study.

No significant safety issues were identified with either dosing regimen, and pharmacokinetic analysis revealed linearity without significant drug accumulation. The median half-life in the terminal elimination phase of CN-105 following a single or repeated dosing regimen was approximately 3.6 h, and did not appreciably change as a function of dosing. There was minimal drug accumulation after repeated 6-h intravenous doses, and steady state was achieved in the first 24 h. CN-105 was confirmed to have a linear and predictable pharmacokinetic profile in humans with minimal accumulation after repeat dosing. Importantly, in both the SAD and multiple dosing paradigms, administration of CN-105 was safe and well tolerated with no serious drug-related adverse events reported. Based on the results of this phase 1 study, a dose of 1 mg/kg, administered intravenously at 6-h intervals for 72 h, was selected for use in first-in-disease state phase 2 studies.

Early Phase 2: the CATCH Study

To prepare for more definitive studies in acute brain injury, our strategy was to first test the safety and feasibility of administering CN-105 in patients with intracerebral hemorrhage, as this indication presents important features of traumatic pathology, and conferred several tactical advantages. Spontaneous (hypertensive) intracerebral hemorrhage represents a tractable initial clinical study; as it is a relatively homogeneous disease process, patients commonly present early after symptom onset, and are often accompanied by family members, thus facilitating informed consent. This initial study also allowed us to test the safety and

Table 1 Evidence of CN-105 efficacy in preclinical models

Injury	Species	Histological/biochemical outcome measures	Functional outcome measures	References
Stroke-MCAO	Rat	Reduction in infarct volume (35 days)	Improved vestibulomotor function, locomotor function	Tukhovskaya et al., 2009
Stroke-MCAO	Mouse	Reduced microglial activation, infarct volume, improved survival	Improved vestibulomotor function	Tu et al., 2017
Hypoxia	Rat	Less tissue loss	Reduced mortality	McAdoo et al., 2005
Stroke-MCAO	Mouse	Reduced infarct volume and radiographic infarct progression	Improved vestibulomotor function	Wang et al. 2013
ICH	Mouse	Reduction in inflammatory cytokines, cerebral edema	Improved vestibulomotor function	James et al. 2009
ICH*	Mouse	Reduction in microgliosis, edema, neuronal injury, inflammatory signaling (p38; NFKB)	Improved vestibulomotor function and memory	Lei et al., 2016
SAH*	Mouse	Reduction in vasospasm,	Improved vestibulomotor	Liu et al., 2018
SAH	Mouse	Reduction in vasospasm, edema, mortality	Improved functional exam, vestibulomotor function	Gao et al.,2006
SAH	Mouse	Reduction in vasospasm	Improved vestibulomotor fun	Mesis et al.,2006
SAH	Mouse	Reduced microgliosis and apoptosis, enhanced Akt activation and suppressed caspase-3, cytokine production	Alleviated neurological deficits	Wu et al.,2016
SAH	Mouse	Reduced BBB disruption, edema and neuron apoptosis, increased cerebral glucose uptake	Improved neurological functions	Pang et al., 2017
SAH	Mouse	Suppressed JAK/STAT3 signaling, reduced M1 microglia activation	Attenuation of oxidative stress and inflammation	Pang et al.,2018
TBI (closed head)	Mouse	Reduction in oxidative stress (aconitase), neuronal degeneration, cytokine release	Improved vestibulomotor function and memory	Lynch et al.,2005
TBI (closed head)	Mouse	Reduction in degenerating neurons, microgliosis	Improved vestibulomotor function and memory	Laskowitz etal., 2007
TBI (cortical contusion)	Rat	Smaller lesion volume, reduction in astrocytosis	Improved motor outcomes	Hoane et al.,2007
TBI (closed head injury)	Mouse	Reduction in degenerating neurons, microgliosis, TNF	Improved vestibulomotor function	Wang et al.,2007
TBI (cortical contusion)	Rat	Reduction in degenerating neurons	Improved sensorimotor function, reference, and working memory	Hoane et al.,2009
TBI (closed head)	Mouse	Suppressed activation of MMP-9, reduced breakdown of blood–brain barrier, reduced TBI lesion volume and vasogenic edema	Decreased functional deficits compared with saline-treated TBI animals	Cao et al.,2016
TBI (closed head)	Mouse	Reduction in neuronal degeneration, microgliosis, and subset of inflammatory genes	Improved vestibulomotor function, improved memory	Laskowitz etal., 2017

feasibility of administering CN-105 in a vulnerable patient population with acute brain injury which is often associated with polypharmacy. Importantly, this indication also allowed categorization of CN-105 as an orphan drug. The initial trial open label trial was designed to establish safety and feasibility. Thus, we initiated the Proof of Concept Study to Evaluate CN-105 in ICH Patients (CATCH) trial as a multi-site, open label, safety and feasibility trial (Fig. 2; NCT03168581). In addition to establishing safety and feasibility, CATCH was designed to establish molecular and radiographic markers of target engagement and surrogate markers of efficacy (length of ICU stay, development of intracranial hypertension, mortality, mRS at 30 and 90 days; Fig. 2). To evaluate target engagement, we included exploratory endpoints to assess the anti-inflammatory activity that we observed in preclinical models. These include radiographic progression of relative and absolute perihematomal as defined by protocolized imaging studies at days 1, 2, 3, and 5 and serum biomarkers of glial activation, neuronal injury, and inflammation, which were evaluated on a daily basis for the first week of treatment. We have enrolled 38 patients across 6 sites with no concerning safety signals. Based on these encouraging interim results, we have recently initiated a multicenter, randomized, double-blind, placebo-controlled clinical trial for patients with acute intracerebral hemorrhage in Singapore, the S-CATCH trial (NCT03711903).

Finally, participants in both clinical trials receive APOE genotyping to explore potential pharmacogenomic interactions of CN-105 on surrogate and clinical outcomes. Designed to characterize key methodologic variables, such as suitable timing of drug administration following injury, appropriate surrogate outcomes, and optimal duration of follow-up, taken together these early safety and feasibility phase studies will ultimately inform more definitive efficacy phase clinical trials.

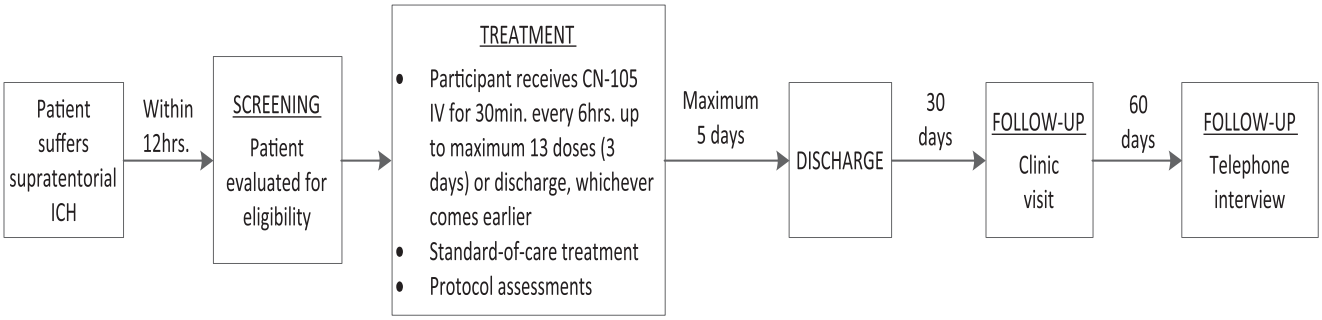

Fig. 2 The proof of Concept study to evAluate CN-105 in inTraCerebral Hemmorrhage patients (CATCH) trial is a multi-site, open label, safety and feasibility trial (NCT03168581). Patients presenting within 12 h after supratentorial primary intracerebral hemorrhage are eligible for enrollment into the CATCH study. Participants are administered CN-105 at a dose of 1 mg/kg every 6 h for 3 days. Pharmacokinetics, serum protein, and radiographic biomarkers and clinical endpoints at 30 and 90 days after intracerebral hemorrhage were assessed

Postoperative Neuroprotection: the MARBLE Study

CN-105's strong anti-inflammatory and neuroprotective mechanism of action represents a potential treatment for multiple forms of acute brain injury (blunt trauma, stroke, intracerebral and subarachnoid hemorrhage, and blast trauma), as well as more subacute processes associated with glial activation, neuroinflammation, and secondary neuronal injury. For example, perioperative neurocognitive disorders (PNDs) are common postoperative complications in older adults associated with increased 1-year mortality and long-term cognitive decline. One risk factor for worsened long-term postoperative cognitive trajectory is APOE4, which may elevate Alzheimer's disease risk partly by increasing neuroinflammation, also a theorized mechanism for PND. To address the potential for CN-105 to reduce CNS inflammatory mechanism following surgery, a phase 2 randomized placebo-controlled trial of CN-105 in postoperative patients has been funded by the Alzheimer's Drug Discovery Foundation. The Modulating ApoE Signaling to Reduce Brain Inflammation, deLirium and postopErative Cognitive Dysfunction (MARBLE; NCT 03802396) study was designed as a single center, randomized, tiered, dose escalation study evaluating perioperative neurocognitive disorders in patients undergoing prolonged non-cardiac surgery. In addition to providing information on dosing, serial lumbar puncture will be performed to evaluate the effect of CN-105 on cerebrospinal fluid markers of injury and inflammation and their correlation with a neurocognitive battery. The primary aim is safety, measured by adverse event rates in CN-105 versus placebo-treated patients. Secondary aims include assessing the feasibility of perioperative CN-105 administration and its efficacy for reducing postoperative neuroinflammation and PND risk and severity. Patients will receive either intravenous CN-105 at 0.1, 0.5, or 1.0 mg/kg or placebo immediately before and every 6 h after surgery, for up to 3 days or 13 doses maximum. CN-105 efficacy will be assessed upon trial completion by comparing cognitive test results, delirium rates, and blood/CSF markers of neuroinflammation between the drug and placebo groups. Analysis will be stratified by CN-105 dose and by patient APOE genotype to account for pharmacogenomic effects. This study is currently recruiting patients.

Conclusions

Effective pharmacological interventions to improve functional outcomes after acute brain injury remain a compelling unmet medical need. Although maladaptive neuroinflammatory responses have long been recognized to contribute to secondary tissue injury, the development of apoE-based therapies represents the first reverse translation of an endogenous brain protein that is known to exert anti-inflammatory and neuroprotective effects. A wide literature supports that contention that apoE-based therapies may improve outcomes in preclinical models of acute and chronic brain injury. In particular, CN-105, a pentapeptide derived from the polar receptor-binding face of the apoE receptor has demonstrated efficacy across a wide variety of preclinical acute CNS injury models, has demonstrated safety in phase 1 study, and is now being tested in several phase 2 studies to evaluate efficacy after acute intracerebral hemorrhage and postoperative neuroprotection.

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Compliance with Ethical Standards

Disclosures DTL is an officer and has equity in Aegis-CN, which sponsored the clinical studies of CN-105. Duke University has equity and an intellectual property stake in CN-105 and might benefit if proven effective and successful commercially. HW serves as a consultant for AegisCN. The CATCH trial was subsidized by orphan drug grant FDA FD-R-5387. MLJ serves as Principal Investigator for the CATCH trial and NIH/NINDS 1 R41 NS108821-01. Preclinical work was also supported by the Department of Defense grant CDMRP#W81XWH-16-C-0142.

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