Alpha-7 nicotinic acetylcholine receptor agonists in intracerebral hemorrhage: an evaluation of the current evidence for a novel therapeutic agent

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Intracerebral hemorrhage (ICH) is the most deadly and least treatable subtype of stroke, and at the present time there are no evidence-based therapeutic interventions for patients with this disease. Secondary injury mechanisms are known to cause substantial rates of morbidity and mortality following ICH, and the inflammatory cascade is a major contributor to this post-ICH secondary injury. The alpha-7 nicotinic acetylcholine receptor ($\alpha 7$ -nAChR) agonists have a well-established antiinflammatory effect and have been shown to attenuate perihematomal edema volume and to improve functional outcome in experimental ICH. The authors evaluate the current evidence for the use of an $\alpha 7$ -nAChR agonist as a novel therapeutic agent in patients with ICH.
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KEY WORDS • intracerebral hemorrhage • nicotinic acetylcholine receptor • alpha-7 agonist

INTRACEREBRAL hemorrhage accounts for approximately 15% of all strokes in the US and has higher morbidity and mortality rates than any other stroke subtype.^{4,10,11,38,44,57} The prognosis for patients with ICH remains poor, with up to 30% mortality and nearly 90% of patients left with some form of disability.^{4,10,44,58} Although much has been learned about the mechanisms of injury in ICH, successful disease-modifying strategies remain elusive.²⁰ In an effort to identify novel therapeutic interventions, much work has been done to elucidate the underlying pathophysiology of ICH. Although the initial hemorrhage can be devastating, secondary injury mechanisms are responsible for a significant amount of damage to the CNS.^{1,9} Attention has therefore been focused on targeting these secondary injury pathways.

One of these secondary injury pathways is the inflammatory cascade.^{19,53,56} In response to the deposition of immunogenic and biochemically reactive blood products in the perihematomal brain parenchyma, there is a significant amount of cellular infiltration and inflammation.¹ Furthermore, NF- κ B is activated within hours after the onset of hemorrhage,^{1,14,50,51} and in turn results in

the production of a wide variety of inflammatory mediators, including cytokines, adhesion molecules, apoptotic regulators, stress response genes, and inflammatory enzymes.^{3,18,31,36} The inflammatory infiltrate further disrupts the surrounding brain parenchyma, leading to neuronal damage and subsequent cellular apoptosis. This post-ICH inflammatory response has therefore been a target of interest in the quest to develop novel therapeutics for the treatment of ICH.

The $\alpha 7$ -nAChR agonists have been identified as particularly potent antiinflammatory agents, and $\alpha 7$ -nAChRs are expressed in a variety of cell types in the mammalian brain.^{7,26,45} Importantly, the downstream antiinflammatory effects of $\alpha 7$ stimulation involve a downregulation of the ubiquitous NF- κ B,^{21,40,54} suggesting that this class of drug may have a therapeutic benefit in patients with ICH. In this study we evaluate the current evidence for the use of an $\alpha 7$ -nAChR agonist as a novel therapeutic agent in patients with ICH.

Antiinflammatory Effects of $\alpha 7$ Stimulation

There is a highly conserved, endogenous mechanism by which organisms are able to control the magnitude of immune responses and prevent excessive inflammation. This physiological mechanism—the so-called cholinergic antiinflammatory pathway—is mediated by acetylcholine release from the vagus nerve.⁴⁷ It follows, therefore, that

Abbreviations used in this paper: $\alpha 7$ -nAChR = alpha-7 nicotinic acetylcholine receptor; BWC = brain water content; ICH = intracerebral hemorrhage; LPS = lipopolysaccharide; NF- κ B = nuclear factor κ B; PHE = perihematomal edema; PI3K = phosphatidylinositol 3-kinase; TNF- α = tumor necrosis factor- α .

acetylcholine agonists have a well-established antiinflammatory effect. For instance, nicotine—the prototypical nonselective acetylcholine agonist—has antiinflammatory effects in multiple cell types and has been shown to quell inflammation in a variety of inflammation-related processes.^{30,37,55}

The $\alpha 7$ subunit of the nAChR has been identified as the principal mediator of this cholinergic antiinflammatory pathway. Macrophages exposed to antisense oligonucleotides specific to the $\alpha 7$ subunit demonstrate a significantly blunted antiinflammatory response in the presence of cholinergic agonists, compared with control macrophages exposed to antisense oligonucleotides specific to $\alpha 1$ and $\alpha 10$ subunits, which have not been implicated in the cholinergic antiinflammatory pathway.⁵² Furthermore, knock-out mice deficient in the $\alpha 7$ -nAChR subunit have higher serum TNF- α levels following endotoxin exposure compared with wild-type mice, and macrophages isolated from these $\alpha 7$ -deficient mice are refractory to the antiinflammatory effects of cholinergic agonists. The administration of GTS-21, a known agonist at the $\alpha 7$ subunit, reduces serum TNF- α levels and thereby improves outcomes in a murine model of endotoxemia and severe sepsis,³⁵ and GTS-21 also decreases the severity of experimentally induced pancreatitis.⁴⁹ Similarly, tropisetron, another drug with agonist activity at the $\alpha 7$ subunit, inhibits the LPS-induced production of TNF- α and IL-1 β from human monocytes.⁴¹ However, these agents bind to other targets in addition to the $\alpha 7$ -nAChR subunit, therefore making it difficult to ascertain the specific contribution of the $\alpha 7$ subunit to this antiinflammatory effect.

More recently, $\alpha 7$ -subtype-selective nAChR agonists have been used to investigate the antiinflammatory effect of the $\alpha 7$ subunit more directly. The administration of a subtype-selective $\alpha 7$ -nAChR agonist, A-833834, causes a reduction in LPS-induced TNF- α release in mouse peritoneal macrophages, as well as in human whole blood in vitro and in mouse serum in vivo.²⁵ Another selective $\alpha 7$ agonist, AR-R17779, leads to an improvement in clinical arthritis and a reduction in synovial inflammation in experimental (collagen-induced) arthritis in mice, and this is accompanied by a reduction in TNF- α levels in both plasma and synovial tissue.⁴⁸ In the brain in particular, rats treated with a selective $\alpha 7$ agonist following traumatic brain injury demonstrate reduced microglial activation and increased cortical tissue sparing as well as improved performance on functional outcome assessments.¹³ In addition, nicotine leads to an attenuation of microglial activation and a reduction in TNF- α levels in rodents following LPS-induced inflammation of the substantia nigra, and this effect is blocked by the addition of α -bungarotoxin, a selective $\alpha 7$ antagonist.³³

Inflammation as a Driver of Post-ICH Edema

Perihematomal edema is a well-known sequela of ICH, and increased PHE volume has been identified as an independent predictor of worse clinical outcomes.³⁹ It has been demonstrated that edema formation in experimental ICH is dependent on circulating white blood cells and platelets,¹⁷ leading to the hypothesis that inflamma-

tion plays a causative role in PHE formation. More recently, TNF- α has been shown to increase in the serum and plasma of patients with spontaneous ICH, and the degree of rise is correlated with the magnitude of PHE.^{5,8,16} Furthermore, the downregulation of cytokine activity via adenovirus-mediated transfer of the *IL-1 receptor antagonist* gene into the brain prior to hematoma induction in experimental ICH causes a significant reduction in PHE volume.^{28,29} In humans, the antiinflammatory agent celecoxib has been shown to reduce PHE volume in patients with ICH compared with controls.³³

Results of $\alpha 7$ Stimulation in Experimental ICH

Given that the inflammatory reaction is a major driver of the development of post-ICH edema, and that the $\alpha 7$ -nAChR mediates downstream antiinflammatory effects, it follows, therefore, that $\alpha 7$ -nAChR stimulation may lead to a reduction in post-ICH edema. This hypothesis has been tested in experimental ICH.

Krafft et al.²³ administered a subtype-selective $\alpha 7$ agonist—either PHA-543613 or PNU-282987—1 hour after induction of hemorrhage in a murine model of ICH. To identify more precisely the molecular pathways involved in any observed effects of the experimental treatments, select cohorts of mice also received methyllycaconitine, an $\alpha 7$ -nAChR antagonist, and/or wortmannin, an inhibitor of the PI3K-Akt signaling pathway located downstream of the $\alpha 7$ -nAChR. These experiments demonstrated a significant attenuation in behavioral deficits and a reduction in BWC in mice receiving $\alpha 7$ -nAChR agonist, and these effects were abolished by coadministration of methyllycaconitine and/or wortmannin, suggesting that the observed effect is mediated by $\alpha 7$ -induced PI3K-Akt signaling. Importantly, the PI3K-Akt signaling pathway leads to a reduction in neuronal apoptosis in a variety of disease models,^{7,43,45} and so it is likely that the observed effects of $\alpha 7$ -nAChR stimulation are at least partially due to a reduction in post-ICH apoptosis.

In a similar series of experiments, Hijioka et al.¹⁵ demonstrated an increase in the number of surviving neurons and an attenuation of the inflammatory cell infiltrate in the perihematomal region, but no significant effect on BWC (functional outcome was not assessed in this study). This lack of effect on BWC is in contrast to the findings of Krafft et al., as described above. As suggested by Hijioka et al., it is possible that these contradictory findings may be attributable to a difference in the administered dose (10 mg/kg vs 12 mg/kg) or a difference in the particular mouse strains used. However, it is likely that the experimental methods used in this study also contributed to the lack of effect on BWC. In particular, experimental ICH was induced using a collagenase injection model, which is less representative of human spontaneous ICH than the alternative autologous blood injection model, as was used by Krafft et al. Mechanistically, the collagenase injection model leads to hemorrhage by proteolytically destroying blood vessel walls in the region of the injection. This leads to massive edema that is not inflammation driven, and so the lack of effect of an antiinflammatory agent (that is, an $\alpha 7$ agonist) on BWC is not surprising. In addition, the protocol used by Hijioka

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et al. for the determination of BWC involved drying brain samples at a lower temperature (75°C vs 100°C) and for less time (12 hours vs 24 hours) compared with Krafft et al., as well as with the vast majority of other research groups using this technique for calculation of BWC.^{2,12,24,27,46} It follows, therefore, that Hijioka et al. probably underestimated the effect of $\alpha 7$ stimulation on BWC.

Clinical Feasibility of an $\alpha 7$ -nAChR Agonist as a Novel Therapeutic Agent in Patients With ICH

Although the safety of $\alpha 7$ -nAChR agonists has not been specifically evaluated in the population of patients with ICH, a number of different $\alpha 7$ agonists have been administered to humans for other indications and have been well tolerated.^{6,22,32,42} Furthermore, although the optimal timing of drug administration is not currently known, the preclinical data support postictus drug administration. In particular, in the study described above, Hijioka et al. administered PNU-282987 at different initial time points relative to ICH onset (1 hour prior, and 3, 6, and 12 hours after), and demonstrated a time-dependent decline in efficacy that remained statistically significant until 6 hours after ICH onset, with the greatest efficacy being observed with initial dosing at 3 hours after ICH onset.

Conclusions

There is strong preclinical evidence for a therapeutic benefit of an $\alpha 7$ -nAChR agonist in the setting of experimental ICH, and there is clinical evidence of safety and tolerability of such an agent in humans. More thorough investigation is necessary to evaluate the optimal timing of drug administration after ICH onset, and Phase I clinical trials are warranted to evaluate the safety of this drug in the population of patients with ICH.

Disclosure

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