Intracerebral hemorrhage accounts for approximately 15% of all strokes in the US and has higher morbidity and mortality rates than any other stroke subtype. The prognosis for patients with ICH remains poor, with up to 30% mortality and nearly 90% of patients left with some form of disability. Although much has been learned about the mechanisms of injury in ICH, successful disease-modifying strategies remain elusive.

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. Attention has therefore been focused on targeting these secondary injury pathways.

One of these secondary injury pathways is the inflammatory cascade. 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. Furthermore, NF-κB is activated within hours after the onset of hemorrhage, 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. 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 α7-nAChR agonists have been identified as particularly potent antiinflammatory agents, and α7-nAChRs are expressed in a variety of cell types in the mammalian brain. Importantly, the downstream antiinflammatory effects of α7 stimulation involve a downregulation of the ubiquitous NF-κB, 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 α7-nAChR agonist as a novel therapeutic agent in patients with ICH. The authors evaluate the current evidence for the use of an α7-nAChR agonist as a novel therapeutic agent in patients with ICH.

**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 α7-nicotinic acetylcholine receptor (α7-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 α7-nAChR agonist as a novel therapeutic agent in patients with ICH.

**KEY WORDS** • intracerebral hemorrhage • nicotinic acetylcholine receptor • alpha-7 agonist
acetycholine agonists have a well-established antiinflammatory effect. For instance, nicotine—the prototypical nonselective acetycholine 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 α7 subunit of the nAChR has been identified as the principal mediator of this cholinergic antiinflammatory pathway. Macrophages exposed to antisense oligonucleotides specific to the α7 subunit demonstrate a significant blunted antiinflammatory response in the presence of cholinergic agonists, compared with control macrophages exposed to antisense oligonucleotides specific to α1 and α10 subunits, which have not been implicated in the cholinergic antiinflammatory pathway.23 Furthermore, knockout mice deficient in the α7-nAChR subunit have higher serum TNF-α levels following endotoxin exposure compared with wild-type mice, and macrophages isolated from these α7-deficient mice are refractory to the antiinflammatory effects of cholinergic agonists. The administration of GT-257-1, a known agonist at the α7 subunit, reduces serum TNF-α levels and thereby improves outcomes in a murine model of endotoxemia and severe sepsis,39 and GT-257-1 also decreases the severity of experimentally induced pancreatitis.39 Similarly, tropisetron, another drug with agonist activity at the α7 subunit, inhibits the LPS-induced production of TNF-α and IL-1β from human monocytes.41 However, these agents bind to other targets in addition to the α7-nAChR subunit, therefore making it difficult to ascertain the specific contribution of the α7 subunit to this antiinflammatory effect.

More recently, α7-subtype-selective nAChR agonists have been used to investigate the antiinflammatory effect of the α7 subunit more directly. The administration of a subtype-selective α7-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 α7 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.45 In the brain in particular, rats treated with a selective α7 agonist following traumatic brain injury demonstrate reduced microglial activation and increased cortical tissue sparing as well as improved performance on functional outcome assessments.11 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 α7 antagonist.39

**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.30 It has been demonstrated that edema formation in experimental ICH is dependent on circulating white blood cells and platelets,25 leading to the hypothesis that inflammation 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 adeno-virus-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.24,29 In humans, the antiinflammatory agent celecoxib has been shown to reduce PHE volume in patients with ICH compared with controls.33

**Results of α7 Stimulation in Experimental ICH**

Given that the inflammatory reaction is a major driver of the development of post-ICH edema, and that the α7-nAChR mediates downstream antiinflammatory effects, it follows, therefore, that α7-nAChR stimulation may lead to a reduction in post-ICH edema. This hypothesis has been tested in experimental ICH. Krafft et al. administered a subtype-selective α7 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 α7-nAChR antagonist, and/or wortmannin, an inhibitor of the PI3K-Akt signaling pathway located downstream of the α7-nAChR. These experiments demonstrated a significant attenuation in behavioral deficits and a reduction in BWC in mice receiving α7-nAChR agonist, and these effects were abolished by coadministration of methyllycaconitine and/or wortmannin, suggesting that the observed effect is mediated by α7-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 α7-nAChR stimulation are at least partially due to a reduction in post-ICH apoptosis.

In a similar series of experiments, Hijjoka et al. 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., described above. As suggested by Hijjoka 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 α7 agonist) on BWC is not surprising. In addition, the protocol used by Hijjoka...
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e 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 Krafif et al., as well as with the vast majority of other research groups using this technique for calculation of BWC.2,12,24,27,42 It follows, therefore, that Hijioka et al. probably underestimated the effect of α7 stimulation on BWC.

Clinical Feasibility of an α7-nAChR Agonist as a Novel Therapeutic Agent in Patients With ICH

Although the safety of α7-nAChR agonists has not been specifically evaluated in the population of patients with ICH, a number of different α7 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 α7-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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References


47. Shaffer CL, Gunduz M, Scialis RJ, Fang AF: Metabolism and disposition of a selective alpha(7) nicotinic acetylcholine receptor agonist in humans. *Drug Metab Dispos* **35**:1188–1195, 2007