Suppression of inflammation in ischemic and hemorrhagic stroke: therapeutic options

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Purpose of review

Inflammation is now considered to be a critically important determinant of outcome following acute injury to the CNS, potentially contributing to the development of secondary injury. The current review summarizes the most recent advances in the understanding of inflammatory mechanisms following both ischemic and hemorrhagic stroke, and highlights areas of therapeutic promise.

Recent findings

A prominent inflammatory response occurs following both ischemic and hemorrhagic stroke, thereby exacerbating secondary injury. Recent efforts have been directed toward understanding the mechanisms by which immediate triggers of poststroke inflammation mediate their effects. Inflammatory stimuli administered acutely prestroke are deleterious, but subacute stimuli can be either deleterious or protective; toll-like receptor signaling has been implicated as a regulatory factor. There is growing evidence that systemic inflammation, whether prestroke or stroke-induced, influences stroke outcome and that therapies may need to also attenuate systemic inflammation to be effective. The beneficial effects of stem cell therapy may be mediated, at least in part, by its systemic anti-inflammatory effects.

Summary

Inhibiting inflammation following both ischemic and hemorrhagic stroke remains a promising approach. More sophisticated therapies, with pleiotropic beneficial effects, and more sophisticated targeting of potential recipients, will increase the likelihood of successful clinical translation.

Keywords

cytokines, edema, inflammasome, innate immunity, neuroprotection, therapy, thrombin

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Introduction

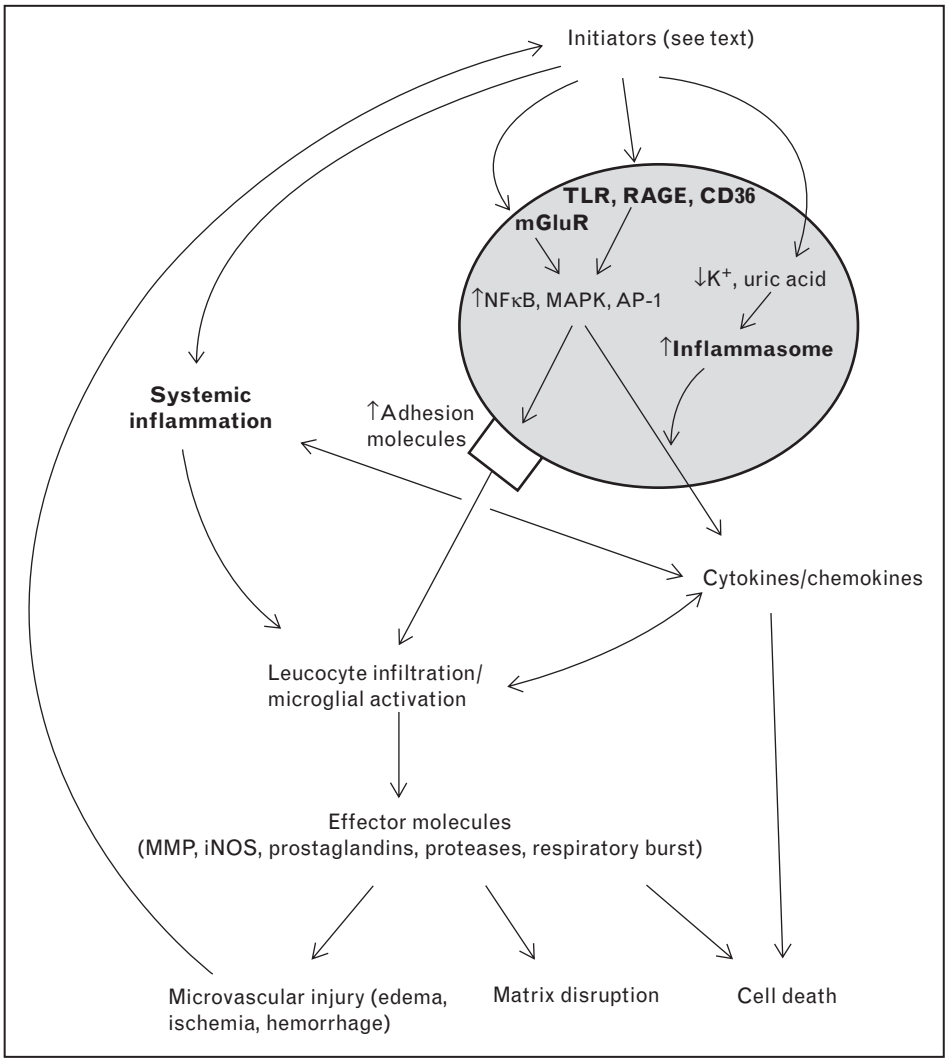
Both ischemic stroke and intracerebral hemorrhage (ICH) elicit brisk inflammatory responses, which appear, on the whole, deleterious [1,2]. However, poststroke inflammation also has beneficial aspects, such as clearing cell debris and iron, and encouraging plasticity, neurogenesis and neovascularization. The inflammatory response is similar in both ischemic and hemorrhagic stroke, with several important differences [1–3]. No anti-inflammatory therapy in either condition has been translated successfully into clinical practice.

The review considers those recent studies in which authors have directly targeted (or elucidated) the poststroke immune response. It focuses on several novel findings and is prefaced by simplified summaries of preexisting knowledge to provide context in which to place these findings. Several comprehensive reviews of inflammation following hemorrhagic and ischemic stroke have been published recently [1–4] and the reader is referred to these articles for more complete background information.

Ischemic stroke overview

The inflammatory response to ischemic stroke is summarized in Fig. 1. Oxygen–glucose deprivation, reactive oxygen species, activated proteases, complement and factors released from necrotic cells rapidly lead to upregulation of proinflammatory pathways in neurons, astrocytes, microglia, oligodendrocytes, pericytes, endogenous mast cells and cerebrovascular endothelial cells. Chemokines and cytokines are secreted, microglia activated and endothelial adhesion molecules expressed. Leucocytes (predominantly neutrophils but also monocytes) marginate and infiltrate in increasing numbers within several hours. Marginating leucocytes may plug cerebrovascular microvessels, worsening microvascular perfusion and/or preventing effective reperfusion. Lymphocytes infiltrate

Figure 1 The inflammatory cascade in ischemic stroke



Initiation of the inflammatory cascade by a variety of factors leads to systemic inflammation or activation of cellular (shaded) proinflammatory pathways in a typical cell (neuron, astrocyte, endothelial cell, etc.). Chemokines and cytokines are consequently secreted, microglia activated and endothelial adhesion molecules expressed activating various effector molecules that initiate cell death, matrix disruption and microvascular injury. The latter provides a positive feedback loop. Recently identified contributors to the pathway are indicated in bold type. AP-1, activator protein-1; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; NFκB, nuclear factor kappa-B; RAGE, receptor for advanced glycosylation end-products; TLR, toll-like receptor.

later and in fewer numbers. Macrophages (both microglia-derived and blood-derived) are visible within 24 h, with decreasing numbers of neutrophils seen within 48 h.

Activated microglia and infiltrating inflammatory cells secrete proinflammatory mediators that amplify the inflammatory response, as well as various effector molecules (proteases, prostaglandins and reactive oxygen species such as nitric oxide via inducible nitric oxide synthase), which can directly damage cells, vasculature or extracellular matrix. Cytokines may also directly lead to cell death. Damage to the endothelium and other components of the blood–brain barrier can lead to uncontrolled vasogenic edema, microvascular ischemia or, if damage is severe enough, hemorrhagic transformation of the infarct. Reperfusion, which may occur spontaneously or therapeutically, can increase reactive oxygen species formation and thus inflammation.

Intracerebral hemorrhage overview

The inflammatory response to ICH overlaps greatly with that following ischemic stroke [1]; however, there are important differences [3]. Blood components, including inflammatory cells, proteases, chemokines and cytokines,

are introduced directly into the brain at hemorrhage onset. Thrombin, in particular, appears pivotal in the initiation of inflammation following ICH; a mass lesion caused by infusion of heparinized blood or red cells without thrombin causes minimal edema, whereas infusion of thrombin rapidly causes intracerebral leucocyte infiltration and edema [5]. Additionally, iron-containing proteins released by hemolysis are potent inflammatory stimuli. These are released from approximately 48 h onwards [6]. Ischemia associated with intracerebral hemorrhage is local and irreversible, with no salvageable perihematomal 'penumbra' [7]. Reperfusion injury, with its acute surge in free radical production, does not occur.

Animal studies: postischemic inflammatory triggers of ischemic stroke

Key initiators of poststroke inflammation have recently been elucidated. Toll-like receptors (TLRs), so-named for their homology to the Drosophila toll receptor, are able to recognise invariant pathogen-associated molecular patterns (PAMPs) and thus initiate the innate immune response to infection. However, many endogenous TLR ligands have been demonstrated. It is clear that TLRs also function as endogenous 'danger sensors', recognizing so-called damage-associated molecular patterns (DAMPs) released in the early phases of tissue injury [8]. Several of these are released early in cerebral ischemia (e.g. heat-shock proteins, heparan sulfate) [9].

Toll-like receptors activate the nuclear factor kappa-B (NFκB) and mitogen-activated protein kinase (MAPK) pathways in various brain cell types, leading to the synthesis and secretion of proinflammatory cytokines such as interleukin (IL)-1, IL-6 and tumour necrosis factor-α (TNF-α), chemokines and the expression of leucocyte adhesion molecules. TLRs can also up-regulate anti-inflammatory factors [such as interferon-β via interferon-regulatory factor (IRF) pathway up-regulation] [8]. Both TLR-4 and TLR-2 knockout are beneficial in experimental ischemic stroke [9,10]. The identity of these key TLR ligands is not yet known.

High mobility group box-1 protein (HMGB-1), a ubiquitous and abundant DNA-binding protein, is a ligand for TLR-2 and 4. It is also binds the receptor for advanced glycosylation end-product (RAGE), which, like the TLRs, potently up-regulate NFκB signaling. HMGB-1 is released from necrotic and penumbral cells within the first hour after cerebral ischemia and up-regulates TNF-α and adhesion molecules *in vitro* [11]. HMGB-1 inhibition or gene knockout is strikingly neuroprotective [12]. Inhibition of HMGB-1 in $RAGE^{-/-}$ mice gives no additional benefit, suggesting that HMGB-1 primarily acts through RAGE in the setting of ischemic stroke.

CD36 may also serve as a 'danger sensor', responding to early post-ischemic stroke formation of oxidized low-density lipoprotein (LDL) and diacylglyceride [13]. CD36 is a receptor for advanced glycosylation end-products and oxidized LDL involved in the development of atherosclerosis-related inflammation. CD36 may form signaling complexes with TLRs [14] and has recently been demonstrated to play a deleterious role in ischemic stroke [13]. CD36 null mice have dramatically decreased lesion size and leucocyte infiltration post-ischemic stroke [15]. Neutrophil infiltration induced by IL-1 in these mice is, however, unchanged, suggesting that CD36 acts upstream of IL-1. The interplay between RAGE, TLRs and CD36 in ischemic stroke has yet to be elucidated.

The liver X receptor (LXR), which recognizes products of cholesterol oxidation and antagonizes NFκB signaling, may be an early counterbalance to the proinflammatory receptors listed; two groups have recently reported beneficial effects of LXR agonists [16,17]. Heat-shock protein-70, which has been recognized for some time to be neuroprotective in the setting of focal ischemia [18], may also exert its protective effects by early inhibition of the NFκB pathway [19].

Although both RAGE and TLRs cause up-regulation of cytokine secretion, pro-IL-1β, one of the main products and a major proinflammatory cytokine, is inactive unless cleaved. The process responsible has only recently been unravelled. IL-1β is cleaved by the so-called 'inflammasome', a protein assembly incorporating caspase-1, analogous to the 'apoptosome' which cleaves caspases-8 and 9 [20]. Whereas the TLRs sense predominantly extracellular DAMPs, upstream components of the inflammasome sense intracellular danger signals (such as uric acid and low potassium). Targeting the inflammasome may prove a fruitful avenue of stroke research [21••].

Glutamate, an excitatory neurotransmitter released early following ischemic and hemorrhagic stroke, may also play a role in the early initiation of inflammation. In-vitro studies of a recently developed model of the ischemic penumbra suggest that glutamate from neurons exposed to oxygen/glucose deprivation up-regulates microglial NFκB via their group II metabotropic glutamate receptors [22], causing microglia to release neurotoxic levels of TNF-α.

Prestroke inflammation: potentiation versus protection

In human stroke, there is an association between prestroke infection and worse outcome (reviewed recently by McColl and colleagues [23]). Several studies have recently examined potential mechanisms. Infarct volumes and neurological deficits following focal ischemia were greatly increased by acute prestroke administration of either

lipopolysaccharide (LPS) or IL-1 [24•]. Depletion of neutrophils prior to prestroke LPS administration almost completely attenuated this enhanced injury. Further research performed by the same group demonstrated that prestroke LPS challenge transformed the bi-phasic blood–brain barrier opening usually seen following ischemia into a sustained disruption, and that this was due to enhanced activity of neutrophil-derived matrix metalloproteinase-9 (MMP-9) [25]. High levels of C-reactive protein (CRP), which is an acute-phase protein released by the liver in response to IL-6, are linked to worse outcome following stroke [26], and acute prestroke administration of human CRP is deleterious in experimental models [27], perhaps by enhancing complement-mediated neutrophil chemotaxis and degranulation.

Not all prestroke inflammation is deleterious, however. It has long been recognized that administration of LPS (a TLR-4 ligand) more than 24 h prestroke is neuroprotective via a TNF-α-dependent process [28]. Direct stimulation of TLR-2 [29] and 9 [30] more than 24 h prestroke is likewise beneficial. Whereas acute prestroke systemic inflammatory challenges potentiate cerebral inflammation, inflammatory stimuli administered at least 24 h prior to stroke precondition the brain's response to ischemia. This response is analogous to (and overlaps with) ischemic or hyperthermic preconditioning, in which sublethal prestroke stimuli up-regulate a coordinated network of protective genes [31]. Chronic prestroke inflammation can also be protective: chronic preischemic stroke infection of mice with *Toxoplasma gondii* reduced the subsequent proinflammatory cytokine response, improving outcome and lessening infarct size [32]. Similar results were also shown more recently following induced periodontitis in rats [33].

Other chronic inflammatory states can be deleterious, however. Atherosclerosis, a chronic inflammatory state, is linked to worse outcome in experimental stroke, mediated in part by stimulation of the CD36 receptor [34] (see above). Subacute psychological stress worsens stroke outcome in rodents, at least partially through a TLR-4-dependent inflammatory mechanism [35••]. Similarly, rats with chronic hypertension, obesity and diabetes, all of which are associated with systemic inflammation, have worse outcomes after experimental ischemic stroke [36–38].

Thus, inflammatory stimuli administered acutely prestroke are deleterious, but subacute stimuli can be deleterious or protective. The reasons for this are unclear. Modulation of TLR signaling has been proposed as a unifying hypothesis for ischemic, inflammatory and hyperthermic preconditioning [39]. As TLRs induce both proinflammatory and anti-inflammatory pathways [8], varied prestroke inflammatory stimuli may tip the balance of subsequent TLR signaling one way or the other. It is hoped that further elucidation of these mechanisms will identity further pivotal proinflammatory and anti-inflammatory pathways and help rank their importance.

The interaction between systemic and cerebral inflammation

Evidence is accumulating that peripheral immune activation influences stroke outcome. It has previously been demonstrated that peripheral splenocytes are activated postischemia [40]. Ajmo and colleagues [41•] investigated the effects in rats of prestroke splenectomy, demonstrating a dramatic improvement in outcome, with a marked reduction both in infarct size and inflammatory infiltrate. Rats were splenectomized 2 weeks prestroke; it is thus unlikely that the beneficial effects of splenectomy were mediated by inflammatory preconditioning. Pre-ICH splenectomy has a similarly beneficial anti-inflammatory effect [42••] (see below).

In a recent study demonstrating deleterious effects of RANTES (CCL5; regulated on activation, normal T-cell expressed and secreted), a chemotactic factor, bone-marrow chimeras negative for RANTES demonstrated equivalent neuroprotection to RANTES$^{-/-}$ mice, providing further evidence for the importance of systemic immune activation in intracerebral poststroke inflammation [43].

Animal studies: intracerebral hemorrhage

Although ICH accounts for around 15–20% of stroke and causes disproportionate death and disability, it receives, relative to ischemic stroke, less research attention than its contribution to disease burden warrants [44]. The last 12 months has been no exception.

Plasminogen is the precursor of plasmin, an endogenous fibrinolytic protease produced following activation of the coagulation cascade. Plasminogen can be produced intracerebrally, as well as enter the brain at the time of hemorrhage. As thrombin inhibition has been previously demonstrated as the crucial step in initiating post-ICH edema, a significant role of plasma-derived plasmin has been largely discounted. A recent study, however, suggests that plasmin may augment thrombin-mediated injury [45•]. As plasmin inhibition would be expected, if anything, to decrease bleeding, this approach may prove more beneficial than thrombin inhibition, which may do the opposite.

Stem cell therapy is a promising treatment for both ischemic stroke and ICH. It has been previously assumed that replacement of dead or damaged cells is the major

benefit of treatment. However, it has become increasingly clear that stem cells have multiple mechanisms of action, including the inhibition of inflammation [46]. Lee and colleagues [42••] recently studied the effects of intravenous and intracerebral neuronal stem cell (NSC) injections postcollagenase ICH. Intriguingly, only intravenous injections were beneficial, with marked reductions in inflammatory infiltration, edema and neurological deficits. These beneficial effects were evident within the first few days post-ICH, at which time-point very few intracerebral NSCs were demonstrated. Most were residing in the spleen. Pre-ICH splenectomy without NCS injection also led to decreased inflammation and edema. NSC injection provided no additional benefit in this latter group. The authors concluded that the neuroprotective effect of NSC injection in their experiment was mediated by amelioration of a deleterious spleen-induced inflammatory response. Similar findings were recently reported following hematopoietic stem cell transplantation in ischemic stroke [47]; beneficial effects were associated with a blunting of the splenic, but not CNS, poststroke up-regulation of proinflammatory gene transcription.

Many, but not all, anti-inflammatory therapies proven beneficial in experimental ischemic stroke are beneficial in ICH. Mirroring work in ischemic stroke, complement inhibition [48], antioxidant treatment [49] and inhibition of neutrophil infiltration [50] have all recently proven beneficial.

Recent and ongoing human studies of poststroke inflammation

Table 1 summarizes clinical and experimental approaches to the treatment of poststroke inflammation, including, when available, 'levels of evidence' from internationally published guidelines [51,52] (a recent meta-analysis of hemicraniectomy for post-ischemic stroke edema [53] postdates these guidelines).

There are no proven directly anti-inflammatory treatments for human stroke, although several recent studies have helped confirm concepts derived from experimental stroke.

Recent microarray analysis of human perihematomal brain tissue confirms that both proinflammatory and anti-inflammatory gene networks are highly up-regulated in human ICH [54], strongly overlapping with previous work in experimental ICH [55]. NFκB pathway-associated genes are particularly prominent.

The body of evidence in both human and animal models for neutrophil-mediated secondary damage continues to accumulate, despite the failure to prove the beneficial effects of neutrophil inhibition in human stroke [56]. Matrix metalloproteinase (MMP) inhibition is an attractive therapeutic target poststroke, although both the identity and origin of deleterious MMP has been a matter of debate. Recent evidence obtained by the analysis of

Table 1 Investigational and proven therapies to limit inflammation in human and experimental stroke

Inflammatory pathway	Recommended therapy	Investigational therapy
Inflammatory triggers	Thrombolysis (1A[b])	CD36 antagonist; **thrombin antagonist**; RAGE antagonist; TLR antagonist
Intracellular pathways		NFκB pathway antagonist; MAPK pathway antagonist; AP-1 pathway antagonist; inflammasome inhibition; LXR agonist; HSP-70; **interferon β1a**; **hypothermia**[a]; **erythropoietin**[a]; **ACE antagonist**[a]; **angiotensin II antagonists**[a]
Inflammatory mediators		Cytokine antagonist (e.g. IL-1β); anti-inflammatory cytokine (e.g. IL-10, TGF-β); chemokine inhibition (e.g. IL-8); omega-3 PUFA (**albumin therapy**[a]); mast cell inhibition; substance P inhibition; bradykinin inhibition; lipoxygenase inhibition; microglial modulation (**minocycline**[a])
Leucocyte trafficking		Adhesion molecule inhibition (e.g. **ICAM-1**, CD11b, **?statins**[a])
Systemic inflammatory response	Prevent and treat infectious complications (1B[b]); avoid indwelling catheters (3C[b])	Splenectomy; **stem cell therapy**[a]; CRP antagonist; complement antagonist
Effector molecules		MMP antagonist; **COX-2 antagonist**; iNOS antagonist; **free radical scavengers**; NOX antagonist
Limitation of consequences	Hemicraniectomy for massive edema [53]; antipyretics for fever (IC[b])	

?, perhaps (it is disputed by some in the literature); ACE, angiotensin-coverting enzyme; AP-1, activator protein-1; COX-2, cyclo-oxygenase-2; CRP, C-reactive protein; HSP-70, heat-shock protein-70; IL, interleukin; iNOS, inducible nitric oxide synthase; LXR, liver X receptor; MAPK, mitogen-activated protein kinase; MMP, matrix metalloproteinase; NFκB, nuclear factor kappa B; NOX, NADPH oxidase; PUFA, polyunsaturated fatty acids; RAGE, receptor for advanced glycosylation end-products; TGF-β, transforming growth factor-β; TLR, toll-like receptor. Therapies in bold type have been, or are currently being trialled in humans.

[a] These agents have pleiotropic actions, influencing more than one mechanism of inflammation, other cell death mechanisms, or both.

[b] Levels of evidence drawn from recent internationally published guidelines [51,52] [references for therapies listed in text, or can be obtained from referenced reviews (space limitations preclude full referencing)].

autopsy material strongly links neutrophil-derived MMP-9 with fatal hemorrhagic transformation [57]. Buck and colleagues [58] have recently demonstrated a strong relationship between lesion volume and peripheral neutrophil count. It remains to be demonstrated whether this is a cause and/or effect of severe stroke in humans. However, it suggests that targeting antineutrophil treatments to stroke patients with a peripheral neutrophilia may increase the chance of success.

Hyperthermia poststroke is both a result of the inflammatory response to stroke, and can itself enhance secondary injury through inflammatory and other pathways [59]. A recent meta-analysis confirms that elevated temperature is associated with worse outcome in all forms of acute brain injury, including ischemic stroke and ICH [60].

As inflammation is only one component of the injury cascade, and injury responses are more heterogenous in unselected human stroke patients than in the tightly controlled laboratory environment, agents which target multiple components of the secondary injury cascade are more likely to prove effective in human trials. This concept receives support from several recent human studies, which have examined for a possible protective effect of prestroke therapies.

Prestroke therapy with angiotensin-converting enzyme (ACE) inhibitors and statins appear to lessen stroke severity [61–64] and cessation of prestroke statin therapy at the time of stroke appears deleterious [65]. It is perhaps no coincidence that both of these agents have pleiotropic effects: in the case of ACE inhibitors, anti-inflammatory, thrombolytic and vasculoprotective effects [66] and in the case of statins, an anti-inflammatory effect [67] as well as up-regulation of endothelial nitric oxide. A pilot study of acute simvasatin therapy poststroke suggests a possible benefit in recovery, although there was a concerning trend towards increased poststroke infections in the treatment group [68]. In addition, therapeutic anticoagulation with warfarin is not only linked to decreased incidence of stroke, but also decreased stroke severity [69]. Whereas the most likely explanations for this effect are decreased clot burden and/or enhanced thrombolysis, it is possible that anti-inflammatory effects of lowered thrombin concentrations may contribute. This contention is supported by a recent comparison of warfarin-associated and spontaneous ICH; the latter group had significantly more early perihematomal edema [70•], confirming in humans the functional significance of the coagulation cascade in edema formation [5].

Lower body temperature, which has anti-inflammatory, antiexcitotoxic and antiapoptotic activity, is linked to better outcome poststroke. Induced hypothermia seemed to reduce poststroke edema in a recent trial [71]. Minocycline has pleiotropic beneficial actions (anti-inflammatory, antiapoptotic and protease inhibitory) [72]. Recently, a trial of poststroke minocycline therapy was reported [73], and suggested a substantial treatment benefit. Although this was an open-label study, results (as well as extensive animal data) were sufficiently encouraging to warrant testing in a large randomized, blinded study.

In this vein, there are ongoing phase one or two trials of hypothermia (COAST-II, CHILI, ICTuS-L) and minocycline (MINO) [74]. Phase three trials of albumin therapy (ALIAS), erythropoieitin and angiotensin receptor antagonism (SCAST) are ongoing [74]: all three are pleiotropic agents with anti-inflammatory properties [75–77]. Combining multiple pleiotropic therapies (such as statins combined with hypothermia [78]) may be a worthwhile future approach.

Few anti-inflammatory therapies are currently in clinical trials for patients with ICH. As mentioned previously, statins have anti-inflammatory activities; recent retrospective analyses link pre-ICH statin therapy to decreased perihematomal edema [79]. However, it is unclear whether functional outcome is improved [80,81]. A phase two trial looking at the effect of simvastatin on perihematomal edema is currently underway [74].

Conclusion

There is a growing body of evidence that inflammation after both ischemic stroke and ICH is predominantly deleterious. However, as for potentially neuroprotective therapies, inhibition of inflammation following stroke in humans has not, as yet, proven successful. Nonetheless, a greater understanding of the mechanisms underlying inflammation will improve chances of identifying promising therapeutic approaches, with experimental stroke models continuing to further our understanding of these underlying inflammatory mechanisms. Adherence to broadly disseminated guidelines for stroke animal research [82] will help in the selection of the most promising agents. Successful translation of therapies into the clinical context will be made more likely by targeting treatments to patients likely to have the most severe inflammatory response, as well as using therapies with pleiotropic beneficial actions.

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