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The role of inflammation and potential use of sex steroids in intracranial aneurysms and subarachnoid hemorrhage

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Abstract

Background: Aneurysmal subarachnoid hemorrhage (aSAH) continues to be a devastating neurological condition with a high risk of associated morbidity and mortality. Inflammation has been shown to increase the risk of complications associated with aSAH such as vasospasm and brain injury in animal models and humans. The goal of this review is to discuss the inflammatory mechanisms of aneurysm formation, rupture and vasospasm and explore the role of sex hormones in the inflammatory response to aSAH.

Methods: A literature review was performed using PubMed using the following search terms: “intracranial aneurysm,” “cerebral aneurysm,” “dihydroepiandrosterone sulfate” “estrogen,” “hormone replacement therapy,” “inflammation,” “oral contraceptive,” “progesterone,” “sex steroids,” “sex hormones” “subarachnoid hemorrhage,” “testosterone.” Only studies published in English language were included in the review.

Results: Studies have shown that administration of sex hormones such as progesterone and estrogen at early stages in the inflammatory cascade can lower the risk and magnitude of subsequent complications. The exact mechanism by which these hormones act on the brain, as well as their role in the inflammatory cascade is not fully understood. Moreover, conflicting results have been published on the effect of hormone replacement therapy in humans. This review will scrutinize the variations in these studies to provide a more detailed understanding of sex hormones as potential therapeutic agents for intracranial aneurysms and aSAH.

Conclusion: Inflammation may play a role in the pathogenesis of intracranial aneurysm formation and subarachnoid hemorrhage, and administration of sex hormones as anti-inflammatory agents has been associated with improved

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functional outcome in experimental models. Further studies are needed to determine the therapeutic role of these hormones in the intracranial aneurysms and aSAH.

Key Words: Estrogen, inflammation, intracranial aneurysms, progesterone, sex hormones, subarachnoid hemorrhage

INTRODUCTION

Patients surviving an aneurysmal subarachnoid hemorrhage (aSAH) often develop cerebral vasospasm and delayed ischemic neurological injury. Approximately two-thirds of patients with aSAH develop angiographic vasospasm 3–14 days after rupture of an aneurysm. Following aSAH, inflammatory cells enter the central nervous system (CNS) leading to a decrease in cerebral blood flow (CBF) and endothelial cell death. Inflammation, increase in endothelin-1 (ET-1), and depletion of nitric oxide (NO) from endothelial dysfunction are associated with the onset of vasospasm. Sex differences in the inflammatory and apoptotic response to brain injury induced by SAH have been shown to exist in experimental models and sex hormones such as estrogen and progesterone have been shown to have beneficial effects on inflammation and edema after SAH. Treatment with estrogen has been shown to decrease ET-1 and increase NO. Mortality has also been shown to be significantly reduced in progesterone treated SAH animals. Even though the incidence of SAH is generally found to be higher in females, there have been conflicting results on the different gender outcomes associated with aSAH. The purpose of this review is to explore the relationship between inflammation and vasospasm in the setting of aSAH, as well as the potential benefits of sex hormones as a therapeutic anti-inflammatory intervention.

Role of inflammation in intracranial aneurysms and subarachnoid hemorrhage

Evidence of inflammation in aneurysm formation and rupture

Factors leading to abnormal vascular remodeling and weakening of the vessel wall are not well understood, but chronic inflammation and infiltration of inflammatory cells has been shown to be an early histologic hallmark for aneurysms. The number of macrophages within the aneurysmal wall increases as intracranial aneurysms (IAs) develop, while macrophage-depleted mice have much lower rates of IA formation compared to controls. Widespread macrophage infiltration with accelerated extracellular matrix degradation was also shown to correlate with increased rates of aneurysmal rupture. T-cells, mast cells, and humoral response were also shown to be involved in the formation of IAs. Chemokines and cell adhesion molecules such as monocyte chemotactic protein-1 (MCP-1) and vascular cell adhesion molecule-1 (VCAM-1) play a role in the recruitment of monocytes/macrophages to early sites of aneurysm formation and arterial wall degeneration. Along with inflammatory cell infiltration, endothelial dysfunction and induction of proinflammatory cascades such as activation of NF-kB, increased expression of IL-1B, and elevated TNF-alpha have been suggested to play a role in IA development. Increased levels of cyclooxygenase within the walls of ruptured and unruptured aneurysms, as well as a reduction in rate of rupture with aspirin administration was also demonstrated. In animal studies, loss of mural cells, increased neutrophil accumulation in intraluminal thrombus, adventitial fibrosis, and inflammation were some of the characteristics of progressing and ruptured IAs in rats. In human IA samples, epithelial denudation of the aneurysm wall, apoptosis of mural cells, luminal thrombosis, T-cell, and macrophage infiltration were associated with rupture.

Evidence of inflammatory markers in the systemic circulation and cerebrospinal fluid after subarachnoid hemorrhage

Inflammatory markers increase in the systemic circulation as well as in cerebrospinal fluid (CSF) following SAH and are predictive of poor outcomes. Inflammatory markers include acute phase proteins, such as C-reactive protein (CRP), which is a more precise measure of inflammatory activity. CRP was found to be significantly associated with poor outcomes determined by Glasgow Outcome Scale at 3 months. Zhong et al. showed that higher levels of IL-6 and IL-10 24 hours after admission is associated with severe EBI, and increased the susceptibility to infections such as pneumonia. The rate of change of IL-6 and erythrocyte sedimentation rate (ESR) levels were also associated with DCI. Red blood cell distribution width (RDW), an emerging inflammatory marker, was also found to be significantly higher in SAH patients and associated with poor outcome.
shown be increased starting from day 2 and was highest approximately 9–10 days after SAH. Higher levels of macrophage migration inhibitory factor (MIF), soluble CD40 ligand (sCD40L), and platelet-derived growth factor (PDGF)-BB were also correlated with poor outcome. aSAH patients may also experience cardiopulmonary complications as part of the systemic reaction. Pulmonary edema that occurs after SAH was associated with cardiac failure in the early phase and inflammatory response in the delayed phase. It is worth noting that thromboelastography maximum amplitude (MA), a marker of platelet activation, was shown to be higher in patients with severe EBI and DCI, and the association between MA and clinical outcome was reported to be stronger than that between traditional biomarkers.

Several studies have investigated various inflammatory mediators in cerebrospinal fluid (CSF) following aSAH, with some conflicting reports. Higher levels of TNF-α, though other studies have found increased levels of interleukin (IL)-6 and IL-8 but not TNF-α. One recent study found detectable levels of TNF-α in 50% of patients after SAH, suggesting that the amount and type of inflammation may vary considerably in different patients. In animal models of SAH, blockage of TNF-α has been shown to reduce apoptosis in the hippocampus after SAH. Another inflammatory marker found throughout many studies is endothelin-1 (ET-1), and monocytes isolated from CSF of these patients are capable of producing ET-1. As with several other pro-inflammatory molecules, the

<table>
<thead>
<tr>
<th>Marker</th>
<th>Location</th>
<th>Timing</th>
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<tbody>
<tr>
<td>White blood cell (WBC) count</td>
<td>Serum and cerebrospinal fluid (CSF)</td>
<td>-Days 1, 4, 7, 10, and 14 after SAH in serum</td>
<td>-Correlated with delayed cerebral ischemia (DCI) occurrence. WBC count remain elevated for 14 days in SAH patients. No difference was found in the CSF cell counts in patients with DCI vs those without.</td>
</tr>
<tr>
<td>C-reactive protein (CRP)</td>
<td>Serum and CSF</td>
<td>-Days 1, 3, 5, 7, 9, 11, and 13 in serum</td>
<td>-Significant differences were found between patients that developed vasospasm vs those that did not on days 1, 3 and 5.</td>
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<td>-Days 0, 1, 2, 3, 5, 7 in CSF</td>
<td>-Peak value was observed on day 3 in both serum and CSF levels. Higher CRP levels correlated with vasospasm and poor outcome.</td>
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<td>High-sensitivity c-reactive protein (hs-CRP)</td>
<td>Serum</td>
<td>Not available</td>
<td>Significant association was found between high hs-CRP levels and poor Glasgow Outcome Scale score.</td>
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<tr>
<td>Erythrocyte sedimentation rate (ESR)</td>
<td>Serum</td>
<td>Up to 15 days</td>
<td>Time-independent association between ESR and DCI was found.</td>
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<tr>
<td>Interleukin-1beta receptor antagonist</td>
<td>Serum and CSF</td>
<td>-First two weeks in both serum and CSF</td>
<td>Development of systemic inflammatory response syndrome post-SAHI and organ failure were correlated with significant increase in serum only.</td>
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<td>IL-6</td>
<td>Serum and CSF</td>
<td>-Up to 15 days in serum</td>
<td>-Rate of change in IL-6 was associated with DCI. IL-6 was elevated in patients with global cerebral edema, SAH early brain edema score ≥3 and Hunt and Hess ≥4.</td>
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<td>-In less than 48 hours in serum</td>
<td>-IL-6 in CSF was increased in patients with vasospasm. Levels between 530 and 3100 pg/mL were associated with increased likelihood of vasospasm.</td>
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<td>IL-8</td>
<td>Serum and CSF</td>
<td>-Serially over 14 days in both serum and CSF</td>
<td>Patients experiencing vasospasm had significantly higher CSF levels of IL-8 on days 5 and 7.</td>
</tr>
<tr>
<td>Tumor necrosis factor-α (TNF-α)</td>
<td>Serum and CSF</td>
<td>-Up to 2 weeks in serum</td>
<td>Elevated TNF-α on days 2 and 3 and global elevation were correlated with poor outcome but not vasospasm.</td>
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<td>-Up to 12 days in serum</td>
<td>-No association was found between TNF-α and DCI.</td>
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<td>-Day 2 in serum and meta-analysis</td>
<td>-Serum levels of TNF-α were increased in relation with vasospasm and correlated with Hunt and Hess grade.</td>
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expression of ET-1 is highly variable. In one study, mRNA expression of ET-1 levels were found to be present in 46% of the patients with SAH 5 days after the start of symptoms versus none detectable in the CSF of control participants. A study from a different group, however, failed to detect ET-1 after SAH at various time points using radioimmunoassay for big endothelin. The variation found in these inflammatory markers reflects the heterogeneity of complications associated with aSAH. The conflicting findings in these studies may stem from the time CSF levels of these markers were measured and the diagnostic method used following SAH or the difference in inflammatory response experienced by each participant.

Evidence for inflammation as a cause of vasospasm after subarachnoid hemorrhage

Several clinical studies have attempted to correlate fever and inflammation in the absence of infection with vasospasm. Pro-inflammatory agents, such as lipopolysaccharide (LPS), have been administered using the intracisternal route to show that vasospasm can occur in the absence of blood. This has demonstrated that the presence of red blood cells (RBCs) or hemoglobin (Hgb) are not necessary for the induction of vasospasm. Among the cellular adhesion molecules, E-selectin has also been shown to correlate well with the patients’ response to SAH. E-selectin was found to be in higher concentrations in the CSF of SAH patients who develop moderate or severe vasospasm. In addition, inhibition of E-selectin with an inhibitory antibody was shown to decrease vasospasm in rodent models. Other adhesion molecules have been implicated as well. In one study Mac-1 monoclonal antibodies and anti-LFA-1 antibodies were administered systemically, and were shown to reduce vasospasm in rat, rabbit, and primate SAH models. Similar results have been shown with anti-ICAM1 monoclonal antibodies in a rodent model. Among other pro-inflammatory cytokines, TNF-α levels in patients with lower grade SAH were shown to correlate with severity of vasospasm. This has been further studied as TNF-α inhibitors were shown to attenuate vasospasm in animal models. The levels of other inflammatory cytokines such as IL-1B, IL-6, IL-8, and MCP-1 were also shown to be increased in the artery wall, serum, and CSF correlating with vasospasm and severity of SAH. Signaling pathways have been examined as well in the induction of vasospasm, namely, mitogen-activated protein-kinase (MAPK) and nuclear factor kappa-B (NF-κB). Other studies have suggested that oxidative stress and complement pathway activation could play an important role in the induction of vasospasm as well.

Recent studies have been done to explore a possible genetic predisposition to vasospasm. One promising avenue has been the study of haptoglobin proteins, which are responsible for removal of free hemoglobin from CSF that may be the cause of inflammation. Haptoglobin (Hp) has three known distinct phenotypes in humans – Hp1-1, Hp2-1, and Hp2-2. In humans, the haptoglobin proteins with α-2 subunits are associated with higher rates of vasospasm compared to other haptoglobin types (α1-α1). This is consistent with animal models that demonstrate more severe vasospasm and worst outcome after SAH in genetically altered Hp2-2 rodents.

Changes in NO have also been extensively studied in the induction of vasospasm. Increase in the levels of endothelial nitric oxide synthase (eNOS) and inducible nitric oxide synthase were detected in mice after SAH, and this physiological response to SAH is decreased in pro-inflammatory Hp2-2 transgenic mice compared with Hp1-1 mice. This further supports the evidence that Hp2-2 genotypes are associated with a worse outcome in SAH, as these participants would have less NO, which is involved in signaling pathways that lead to vasodilation and cytoprotection. Studies have also suggested that an alteration dubbed “eNOS uncoupling” may lead to production of superoxides instead of NO following SAH.

ET-1, a potent vasoconstrictor, is thought to play a role in the inflammatory response after SAH. Increase in ET-1 levels in patients with SAH and symptomatic vasospasm has been documented in several studies, and the amount of blood found within the cisterns correlated well with the level of ET-1 in CSF. However, other studies found no significant elevation of ET-1 after SAH, and similarly, no correlation between ET-1 levels and vasospasm. Similar administration of anti-ET-1 monoclonal antibodies was effective in decreasing vasospasm in some studies. A rodent study suggested that transgenic mice overexpressing ET-1 experienced more severe vasospasm and edema. Some studies have attempted the use of clazosentan, a synthetic endothelin receptor antagonist (ETRA), to reduce vasospasm in rodent SAH models, however, the overall morbidity from vasospasm was unchanged.

Several studies have shown a relationship between glutamate, as well as a synthetic analog N-methyl-D-aspartate (NMDA), and vasodilation under physiological conditions. These effects appear to result from neuronal NMDA receptor activation subsequent neuronal depolarization, and production of neuronal NO, which diffuse to cerebral arterioles and arteries leading to vasodilation. Glutamate receptors were not shown on human and rat microvascular endothelial cells, further suggesting that the effect of glutamate on vasodilation is indirect through diffusion of substances from cells surrounding the cerebral vessels. On the other hand, synaptic glutamate receptor 1 (GluR1) has been shown to be reduced in mice 24 hours after SAH.
which also corresponds to peak vasoconstriction in mice. Bell et al. have demonstrated reduction in surface glutamate receptor 2 (GluR2) in rats 7 days after SAH in areas proximal to microthrombosis and thrombin activated platelets. Such reduction in glutamate receptors possibly contributes to vasoconstriction after SAH. A study utilizing S-4-carboxyphenylglycine (S-4-CPG), a glutamate receptor antagonist, inhibited vasospasm in haptoglobin 2-2 mice after SAH induction. Similarly, combination therapy with a GluN1/GluN2B NMDA receptor and metabotropic glutamate receptor 1 negative allosteric modulator was neuroprotective by attenuating apoptosis and improving functional outcome after SAH. Neutrophil depletion, which has been associated with reversal of vasospasm, caused a shift in the NMDA receptor subunit composition toward a memory sparing phenotype, and enhanced memory after experimental SAH. These findings illustrate the complex relationship between glutamate and NMDA receptors and SAH-induced vasospasm. The mechanisms mediating these interactions need to be further explored and scrutinized to clarify the contribution of these receptors to the pathophysiology of SAH-induced inflammation and brain injury.

The impact of sex hormones on intracranial aneurysms and subarachnoid hemorrhage

Estrogen
Estrogen is the primary female sex hormone responsible for the development and regulation of the female reproductive system, although it has been found to play a role in male physiology as well. Estrogen receptor-alpha (ER-α) and estrogen receptor-beta (ER-β) genes encode estrogen receptors (ER) inside the nuclear membrane. Estrogen alters glumatamergic and GABAergic neuronal activity in many steroid-sensitive brain regions and may stimulate mitogen-activated protein kinase (MAPK) signal transductive pathways to protect cells in a manner similar to as growth factors.

Estrogen is thought to play a role in aneurysm formation. Females have been shown to develop intracranial aneurysms at higher rates than males and, experimental animal studies support the hypothesis that induced estrogen deficiency via bilateral oophorectomy in rats causes an increase in the frequency of aneurysm formation and augment the aneurysm size. Moreover, reversal of this induced deficiency with continuous-release pellets of 17-β estradiol reduces the frequency of aneurysm formation. These effects are attributed to estrogen’s protective role on endothelial cell growth and function. ERβ agonist was also shown to reduce the frequency of aneurysm formation in wild type ovariectomized mice but not in ovariectomized ERβ knockout mice suggesting that ERβ receptors found on aneurysm walls are involved in the protective effects of estrogen. Whether estrogen may be used as a pharmacological agent to reduce the chronic inflammation and loss of mural cells in the aneurysm wall must be elucidated in future studies.

Evidence obtained from animal studies suggests that continuous estrogen treatment in SAH-induced rats may decrease the rate and severity of vasospasm by inhibiting endothelin-1 production, increasing iNOS expression, and preserving eNOS expression. Mechanistically, estrogen’s attenuation of cerebral vasospasm may be related to its potent vasodilatory action. Estrogen has also been shown to be a potent neuroprotective agent in ischemic stroke, particularly in premenopausal women. In the CNS, estrogen is known to reduce lipid peroxidation and protect against oxidative stress. Decrease the production of reactive oxygen species and interrupt the accumulation of intracellular peroxide in an ER-dependent manner. Moreover, a growing number of studies demonstrate that exogenous estradiol reduces tissue damage resulting from experimental ischemic stroke in both sexes. Female reproductive steroids also may ameliorate ischemic injury through promotion of γ-aminobutyric acid type A (GABA (A)) receptor-mediated mechanisms, as well as through suppression of excitatory amino acid toxicity. Estrogen inhibits inflammatory signaling through the inhibition of NF-κB, an important pro-inflammatory pathway activated after SAH. In addition, tamoxifen, a selective estrogen receptor modulator, was found to modulate TLR4/NF-κB signaling pathways and improve the cognitive and behavioral outcome of SAH rats.

Progesterone
Progestosterone (PROG) is another sex steroid naturally synthesized by neurons and oligodendrocytes in the CNS. In addition to its hypothalamic receptors involved in the regulation of female reproductive physiology, PROG receptors are constitutively expressed in other parts of the brain including the cerebral cortex, hippocampus, basal ganglia, and cerebellum. The classical progestosterone receptor-mediated genomic actions of progestosterone occur via activation of nuclear progestosterone receptor-A (PR-A) and PR-B. The nonclassical signaling of progestosterone is mediated by membrane progestosterone receptors such as mPRα, mPRβ, mPRγ, mPRδ, and mPRe. On the other hand, allopregnanolone, the metabolite of progestosterone, mediates its effects as a potent positive allosteric modulator of GABA (A) receptors in the CNS. PROG and its metabolite allopregnanolone were shown to have strong anti-inflammatory, anti-apoptotic and neuroprotective properties in various neurological injury models including traumatic brain injury (TBI) ischemic stroke, neonatal hypoxic brain injury, diabetic neuropathy, and demyelinating disorders. A water soluble progestosterone analogue, which would facilitate its delivery in emergency conditions, has been recently developed.
Progesterone may play a critical role in altering the pathogenesis of SAH, and has already been proven to be beneficial in few studies of experimental SAH.\(^{[12]}\) Chang et al.\(^{[21]}\) have shown that rats treated with progesterone one hour after SAH induction show reduced vasospasm and greater levels of eNOS compared to controls. Progesterone-mediated increase in eNOS is thought to be related to the Akt signaling pathway, which has also been implicated in estrogen-mediated vasodilation. Progesterone stabilizes the blood–brain barrier (BBB)\(^{[149]}\) and significantly reduces the mortality associated with SAH in experimental animals.\(^{[141]}\) Progesterone treatment was shown to increase appetite scores of SAH rats, decrease proinflammatory cytokines such as IL-1β, TNF-a, and IL-6 in the intestines, and improve the gut structure.\(^{[135,136]}\) Our group has shown that progesterone prevented basilar artery vasospasm, reduced iba-1 expression in the cortex and cerebellum, and restored functional synapses in the hippocampus in mice after SAH.\(^{[135]}\) Progesterone has also improved motor performance on rotarod and grip strength testing 6 and 9 days after SAH, respectively.\(^{[135]}\) Future studies are necessary to clarify the role of progesterone as a pharmacological agent to reduce chronic inflammation in intracranial aneurysms and to reduce vasospasm and inflammation-induced brain injury seen after SAH.

**Testosterone and dihydroepiandosterone sulfate**

Testosterone, another gonadal sex steroid, also plays important roles in the CNS, but its direct role in SAH is still unclear.\(^{[11]}\) Testosterone is physiologically secreted by the testes and adrenal glands and transported by the sex hormones binding globulins (SHBG) and albumins.\(^{[11,141]}\) It acts through activation of androgen receptors (AR)\(^{[11]}\) found in multiple neurons throughout the brain.\(^{[10]}\) Testosterone may also play a neuroprotective and anti-inflammatory role in the CNS.\(^{[85,106]}\) In the setting of SAH, testosterone was shown to inhibit vasospasm in SAH rabbits; however, the exact mechanism is unclear.\(^{[48]}\) Future studies are needed to investigate the role of testosterone in aneurysm formation and SAH. Dihydroepiandosterone sulfate (DHEAS) is another sex steroid recently associated with favorable outcomes in human SAH.\(^{[53]}\) Higher serum levels of DHEAS were correlated with favorable neurological outcomes after SAH. In the same cohort, favorable outcomes were also associated with lower levels of IL-6,\(^{[53]}\) although DHEAS levels were only studied in peripheral circulation.\(^{[94]}\)

**Oral contraceptives and hormone replacement therapy**

Several population-based studies have failed to show a strong association between risk of SAH and the use of oral contraceptives.\(^{[12,28,88]}\) The results on the influence of hormonal replacement therapy (HRT) on incidence of SAH, on the other hand, are conflicting. Several studies have shown that HRT reduces the risk of SAH in postmenopausal women with odds ratios ranging from 0.6 (0.4–0.8) to 0.47 (0.26–0.86) with the greatest risk reduction in women with a history of smoking.\(^{[18,77,88]}\) On the contrary, other studies showed no influence of HRT on the incidence of SAH in women.\(^{[15,101]}\)

**Translation from bench to bedside and remaining challenges with clinical trials**

Though there is promising data alluding to sex hormones as potential therapeutic agents for vasospasm and neuroprotection in aSAH patients, the gap between animal studies and human trials is still large. Concern surrounding the failure of clinical trials evaluating progesterone in TBI in humans despite extensive supporting data in animal models calls for more precise outcome measures and alternative clinical trial methodologies.\(^{[50]}\) Potential challenges for failure of randomized clinical trials in SAH are thought to include functional ineffectiveness of the tested therapies, timing and dose of the treatment, inadequate sample size, insensitive or inappropriate outcome measures, the confounding effect of rescue therapies in placebo groups, treatment-associated side effects, and variations in practice across different centers.\(^{[80]}\) The lessons learned from the failed phase III randomized clinical trial of progesterone for the treatment of TBI is that careful evaluation of dosage needed to treat the patients is critical and that outcome measures need to be further improved to detect the efficacy of future therapeutic agents.\(^{[127]}\) Extensive neurobehavioral testing is also needed to ensure the functional effectiveness of a therapeutic agent before proceeding to a randomized clinical trial.\(^{[114]}\)

**CONCLUSION**

Inflammation in the CNS is a major contributing force behind vasospasm and early brain injury in aSAH patients. Though this link has been made in many animal experiments, human trials with anti-inflammatory agents have not been successful in reducing morbidity and mortality and improving functional outcome. Evaluation of sex hormones as potential therapeutic agents to stabilize intracranial aneurysms and improve functional outcome in aSAH patients is promising as many preliminary animal studies indicate the safety and effectiveness of the sex steroids to cross the BBB. Future studies are warranted to determine the role of sex hormones in treatment of these conditions.

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