Molecular Medicine

Erythropoietin for the Treatment of Subarachnoid Hemorrhage: A Feasible Ingredient for a Successful Medical Recipe

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Subarachnoid hemorrhage (SAH) following aneurysm bleeding accounts for 6% to 8% of all cerebrovascular accidents. Although an aneurysm can be effectively managed by surgery or endovascular therapy, delayed cerebral ischemia is diagnosed in a high percentage of patients resulting in significant morbidity and mortality. Cerebral vasospasm occurs in more than half of all patients after aneurysm rupture and is recognized as the leading cause of delayed cerebral ischemia after SAH. Hemodynamic strategies and endovascular procedures may be considered for the treatment of cerebral vasospasm. In recent years, the mechanisms contributing to the development of vasospasm, abnormal reactivity of cerebral arteries and cerebral ischemia following SAH, have been investigated intensively. A number of pathological processes have been identified in the pathogenesis of vasospasm, including endothelial injury, smooth muscle cell contraction from spasmogenic substances produced by the subarachnoid blood clots, changes in vascular responsiveness and inflammatory response of the vascular endothelium. To date, the current therapeutic interventions remain ineffective as they are limited to the manipulation of systemic blood pressure, variation of blood volume and viscosity and control of arterial carbon dioxide tension. In this scenario, the hormone erythropoietin (EPO) has been found to exert neuroprotective action during experimental SAH when its recombinant form (rHuEPO) is administered systemically. However, recent translation of experimental data into clinical trials has suggested an unclear role of recombinant human EPO in the setting of SAH. In this context, the aim of the current review is to present current evidence on the potential role of EPO in cerebrovascular dysfunction following aneurysmal subarachnoid hemorrhage.

INTRODUCTION

Cerebral vasoconstriction following aneurysmal subarachnoid hemorrhage (SAH) can produce cerebral ischemia, neurological disability and premature death. To date, there are no truly effective treatments for this condition, and numerous experimental and clinical studies have been performed in the potential development of effective therapeutic strategies. It is well known that cerebral arteries respond to SAH with a biphasic contraction: an acute vasoconstriction that begins minutes following the bleeding and a delayed vasospasm that occurs more than 48 h later (1,2). Although many studies have extensively identified the delayed vasospasm as the major complication in patients affected by aneurysmal SAH, the physiopathological and clinical importance of acute vasoconstriction, a phenomenon well documented in experimental settings (1), remains to be elucidated in humans.

In this regard, considerable evidence has accumulated suggesting that immediate vasoconstriction produces the acute cerebral ischemia that typically follows SAH.

Many theories have been debated to explain the occurrence of SAH-induced acute cerebral ischemia. It is widely accepted that most of the complications following SAH can be attributed to luminal narrowing of the major intracranial vessels. This mechanism, however, cannot account for the diffuse brain ischemia, brain edema, blood–brain barrier (BBB) dysfunction and altered cerebrovascular reactivity that are observed frequently following SAH (3). Therefore, other mechanisms, such as cerebral microcirculatory dysfunction, may be implicated as contributory causes in the pathogenic cascade after SAH. Acute cerebral ischemia has been partially related to a decrease in cerebral perfusion pressure (CPP) (4). However, experimental and clinical studies have shown that CPP does not usually reach the point of perfusion arrest (5,6), suggesting that the decrease in CPP cannot fully explain the SAH-induced acute cerebral ischemia. Furthermore, it has

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been suggested that acute ischemia following SAH can be associated with a sudden and long-lasting decrease in cerebral blood flow (7). It has been pointed out that alteration in the nitric oxide (NO) vasodilatory system may play a role in acute cerebral vasoconstriction and ischemia following aneurysmal SAH (8).

Hence, neuroprotection may be an effective strategy to counteract the damage affecting neurons and glial cells after aneurysmal SAH. Neuroprotective drugs potentially could block the cellular, biochemical and metabolic processes that ultimately lead to brain injury. Accordingly, an ideal addition to the current SAH treatment armamentarium would be a well-tolerated neuroprotective agent with the ability to reduce arterial vasospasm and the delayed ischemic neurologic deficit. Thus far, a few candidate molecules have been investigated and, among these, the hormone erythropoietin (EPO) has shown promising results.

ERYTHROPOIETIN

Erythropoietin is present in all the vertebrates and is a 165-amino acid (~30 kDa) glycoprotein and a member of the type I cytokine superfamily. It is the primary hormone that regulates the differentiation and proliferation of immature erythroid cells (9). This cytokine was cloned in 1985 (10) and rapidly adapted into clinical practice. Subsequently, its recombinant form (rHuEPO) has significantly improved the management of anemia in chronic renal failure and has greatly improved the quality of life for dialysis patients. In the fetus, EPO is produced in the liver until late gestation and, thereafter, a switch is initiated gradually from the liver to the kidney, which is completed after birth (11). In the adult, the kidney is the predominant source of EPO, with about 10–15% being generated by the liver and possibly other organs (11).

During erythropoiesis, EPO acts by binding to its receptor (EPOR) to stimulate the proliferation, differentiation and maturation of erythroid progenitor cells (12). EPOR, a member of the cytokine-receptor type I superfamily and is comprised of an extracellular and intracellular domain. A single EPO molecule binds to two assembled receptor units on the cell surface, and tyrosines located in the intracellular domain are consequently phosphorylated. This process triggers an intracellular signaling cascade that regulates gene expression in the nucleus, which in turn controls cell survival, proliferation and differentiation (13).

The binding of EPO to EPOR is not the only signaling pathway of relevance in EPO function, since the common receptor (βCR), a shared receptor subunit of interleukin-3 (IL-3), IL-5 and granulocyte macrophage colony stimulation factor (GM-CSF) receptors, also mediates several nonhematopoietic effects of EPO in various types of cells by forming a heteroreceptor with EPOR subunits (33,53–55).

At the beginning, EPO was well known for the function in maintaining tissue oxygenation by regulating the number of erythrocytes in a negative-feedback control system that operates between the kidney and the bone marrow. Tissue hypoxia causes a 50-fold or more increase of the EPO level in the serum. EPO is required for erythroid development, allowing maturation of erythroid precursors by inhibiting programmed cell death. The antiapoptotic activity is transduced by a signaling pathway involving the phosphorylation of Janus tyrosine kinase 2 (JAK2). JAK2 in turns activates secondary signaling molecules such as signal transducer and activator of transcription (STAT), Ras–mitogen-activated protein kinase (MAPK) and phosphatidylinositol 3-kinase (PI3K). In erythroid progenitor cells, this mechanism results in the upregulation of antiapoptotic proteins of the B-cell leukemia/lymphoma 2 (BCL2) family, such as BCL-XL (14).

The discovery that EPO has biological functions apart from regulating erythropoiesis was unexpected and is supported by numerous studies. It has been pointed out that EPO and EPOR are localized in brain areas and spinal cord (15,16). Immunohistochemical studies have demonstrated that EPO and its receptors have a particular distribution in the developing human brain since the first stages of human life (17). Recent studies have shown that astrocytes and neurons express both EPO and EPOR (16,18,19). Microglia, although not a source of EPO, produce EPOR mRNA (20) and synthesize EPO protein (21).

Further, hypoxia induces EPO expression in cultured astrocytes (16,22) and rodent and primate brain (15,16,23). The knowledge that cells of the nervous system express EPO and its receptor support a function of EPO as an autocrine–paracrine factor outside the bone marrow.

A crucial mechanism by which EPO exerts neuroprotective actions is via the inhibition of apoptosis in the tissue adjacent to a lesion (24). Further studies have demonstrated that EPO additionally can modulate NO synthesis in the vascular endothelium (25), promote neurotransmitter release (26,27) and counteract the BBB dysfunction that is induced by vascular endothelial growth factor (VEGF) (28) or inflammation (29).

The emerging understanding is that EPO protects and repairs tissue damage that result from the activation of an innate inflammatory response. Primary triggers of the various molecular pathways of the innate inflammatory response are proinflammatory cytokines and the hypoxia-inducible factor (HIF). The latter is a protein that initiates the synthesis of genes encoding for EPO, VEGF, glucose transporters and glycolytic enzymes (30).

The tissue-protective molecular pathways that are triggered by EPO have similarities to, as well as differences from, those activated during erythropoiesis.

It has been proposed that a different receptor than EPOR specifically mediates tissue protection (31). In this regard, the hematopoietic and tissue-protective activities could be separated occurring the hormonal and neuroprotective actions of EPO via a different signaling systems (32). Based on this information, engineered molecules have been developed that mediate tissue protection but do not bind to erythroid progenitors, thereby dissociating the biology of EPO-mediated cytoprotection from that of erythropoiesis (32,33).

ERYTHROPOIETIN SIGNALING PATHWAYS IN THE NERVOUS SYSTEM

The mechanism by which rHuEPO acts in the central nervous system across the BBB remains a matter of controversy. EPO exerts its neuroprotective effects by acting through several signaling pathways. In the bone marrow, the EPO binding to its receptor results in phosphorylation of JAK2. Upon JAK2 phosphorylation and activation, the signaling propagates through the MAPK or the protein kinase B (PKB/Akt)–nuclear factor-κB (NF-κB) pathways (34). In erythroid cells, MAPK activation promotes the accumulation of the antiapoptotic protein BCL-XL by the inhibition of caspases. Also, EPO appears to prevent apoptotic injury through an Akt-dependent mechanism (35).

Substantial evidence also indicates that EPO mediates protective effects by maintaining normal vascular autoregulation. This has clinical relevance in pathologies such as stroke, traumatic brain injury (TBI), spinal cord injury (SCI) and aneurismal SAH. The principal mechanism of this protective mechanism appears to depend upon NO signaling via increased endothelial NO synthase (25), which mediates vascular relaxation and blood flow preservation. This observation could explain the remarkable efficacy of EPO in reversing the vascular spasm that accompanies subarachnoid hemorrhage (36–38) and spinal cord compression (39,40), which, in turn, reduces tissue damage. Moreover, recent studies reported that EPO promotes neovascularization through an Akt-dependent activation of eNOS in endothelial progenitor cells (EPCs) (41,42). Thus, the ability of EPO to counteract cerebral vasospasm may be related to a direct effect on the vascular endothelium rather than a direct action on cerebral parenchyma. The recognized ability of EPO to activate protein kinase B (Akt) and subsequent phosphorylation of eNOS in endothelial cells suggests that increased formation of NO could be an important mechanism underlying the therapeutic effect of EPO, although the definitive interaction between EPO and NO is not fully understood (43). Figure 1 illustrates the main intracellular EPO signaling pathways associated with neuroprotection.

Endothelium-derived NO is one of the main regulators of the vessel tone. The activity of endothelial NO synthase (eNOS) promotes NO production in endothelial cells (ECs) (44). eNOS is regulated by a complex signaling network including kinase-dependent signaling pathways such as PI3K/Akt, Src and JAK2 in response to different stimuli (45-47).

Under normal conditions, NO provides vasodilation of the microcirculation and maintenance of normal vascular tone, antithrombotic effects, prevention of excess platelet adhesion and aggregation, inhibition of endothelial apoptosis, as well as vascular smooth muscle cell hyperplasia. NO released from ECs diffuses to adjacent smooth muscle cells and activates soluble guanylate cyclase (GC). GC, in turn, produces cyclic guanosine monophosphate (cGMP), which activates intracellular calcium pumps sequestering free Ca^{2+} into sarcoplasmic reticulum and relaxing smooth muscle cells (48). Dysregulation of eNOS has been reported in several vascular diseases, including aneurismal SAH. Following hemorrhage, hemoglobin binds NO, thus reducing its availability (49). Accordingly, scavenging of NO results in a decrease of GC activity, which, in return, causes vasoconstriction. Furthermore, hemoglobin has shown to directly inactivate GC by oxidization, thus leading to reduced production of cGMP (50). Furthermore, after SAH, increased oxidative stress can uncouple eNOS via Zn^{2+} thiolate oxidation, or, theoretically, by tetrahydrobiopterin depletion or oxidation, resulting in a paradoxical release of superoxide anion radical, further exacerbating oxidative stress and microvascular damage (51, Figure 2).

To date, endothelial mechanisms are considered to be the main contributors to induced vasospasm (52). It has been reported that NO level decreases acutely within 10 min after SAH both in experimental models and in humans, leading cerebrovascular relaxation impairment and ischemic neuronal damage (52). The main responsible agent in causing endothelial impairment appears to be either oxyhemoglobin or bilirubin (49), with oxyhemoglobin proposed as able to scavenge NO, inactivate guanylate cyclase (GC) or increase production of oxygen radicals.

It has been reported that βCR may play an integrative role in the EPO signaling-mediated activation of eNOS in ECs through a Src/JAK2/Akt pathway (56). Taking the classic EPOR and βCR interaction collectively, substantial evidence has shown that EPO has several protective effects on the vascular system through the NO system, although the detailed molecular mechanisms underlying such interaction remain to be determined (42,57,58). In this regard, EPO has shown to promote EC angiogenesis by an eNOS-dependent mechanism (59–61). Furthermore, EPO protects cardiac myocytes against ischemia-induced injury and apoptosis by increasing eNOS activation or protein expression (62,63). In an isolated rat heart ischemia-reperfusion model, pretreatment with EPO provides cardioprotection that is dependent on NO (64).

It has been demonstrated that EPO promotes proliferation, migration and tube formation in ECs (41,42). Blocking βCR and EPO-induced activation of Src/JAK2/Akt signaling pathway inhibited the EPO-induced increase in proliferation, migration and tube formation, which indicates the critical role of βCR in EPO-mediated beneficial effects on ECs (56). In experimental intracerebral hemorrhage, EPO reduced inflammation around the hematoma and activated eNOS, leading to improved perfusion (65).

NO also has an important role as a neuroprotectant in traumatic brain injury (TBI). In an experimental model of TBI, administration of L-arginine increased NO levels in cerebral microdialyzate, restored CBF to near preinjury levels and reduced the contusion volume (66). Pretreatment with EPO administration resulted in a significant CBF recovery in the penumbra within 2 h after the injury by a NO-dependent mechanism (67).
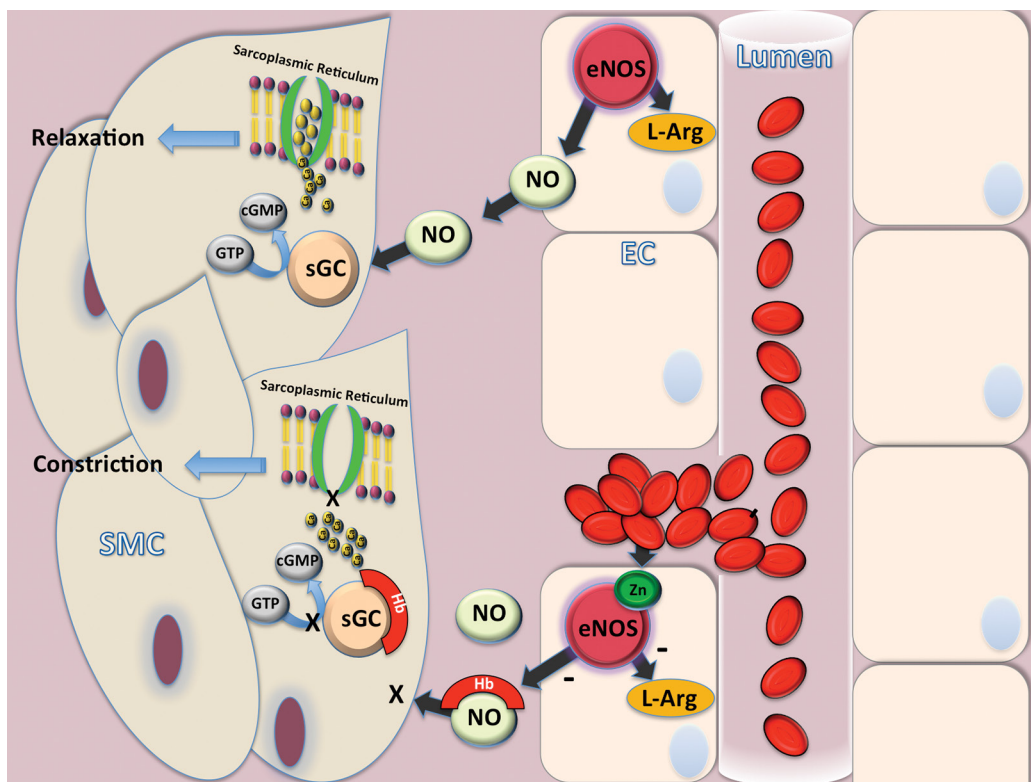
Figure 1. Main EPO signaling pathways. Following the EPO binding with the complex EPO receptor (EPOR) and β common receptor (βCR), Janus tyrosine kinase 2 (JAK2) is activated. At this time, a secondary signaling pathway involves mitogen-activated protein kinase (MAPK), phosphatidylinositol 3-kinase (PI3K) and nuclear factor-κB (NF-κB). Signal transduction and activator of transcription 5 (STAT5) can also be triggered. These processes allow an increase in antiapoptotic proteins of the B-cell leukemia/lymphoma (BCL) family. Some of the pathways act directly, while others indirectly by inhibiting the activity of a group of enzymes called caspases. These are key mediators of apoptosis and are responsible for the degradation of antiapoptotic proteins. EPO also inhibits glycogen synthase kinase 3β (GSK3β), which, in turn, prevents the mitochondrial permeability transition pore, a key factor of cell death, through caspase activation. Finally, EPO modulates the activity of calcium channels through phospholipase C (PLC), thus reducing the release of excitatory neurotransmitters and increasing the production of nitric oxide (NO). Moreover, recent studies reported that EPO promotes neovascularization through an Akt-dependent activation of eNOS in endothelial progenitor cells. The recognized ability of EPO to activate protein kinase B (Akt) and subsequent phosphorylation of eNOS in endothelial cells suggests that increased formation of NO could be an important mechanism underlying the therapeutic effect of EPO.

Previous investigations have demonstrated an acute decrease in cerebral NO levels after SAH (8) and a significant improvement of NO system activity after administration of EPO (27,68,69), suggesting that EPO could act against vasospasm by enhancing the endothelial release of NO during the early stage of SAH. In experimental SAH, gene transfer of EPO following intracisternal blood injection increased the phosphorylation of Akt and eNOS, resulting in increased NO production in the basilar arteries, suggesting the beneficial effect of EPO during SAH (70).

Further studies are necessary to better understand the potential roles exerted by EPO and its derivatives in the plasticity and tissue protection of the nervous system. Although the exact mechanisms underlying the interaction between EPO and NO system are unclear, overall these findings have implications for treatment of brain injury following SAH.

FROM BENCH TO BEDSIDE

During the past several years, interest has focused on the efficacy of recombinant human EPO (rHuEPO) as a neuroprotective agent against neurological

injury produced in several experimental models of brain insult. In this regard, many studies have shown a neuroprotective effect of EPO in models of cerebral ischemia (21,71–75), concussive brain injury (76,77), experimental autoimmune encephalomyelitis (78) and kainate-induced seizures (79). In these experimental studies, EPO has been administered both intrathecally or systemically, provoking controversy regarding the ability to cross the BBB. It has been reported that systemic administration of rHuEPO, following experimental SAH, reduces the mortality rate, improves functional
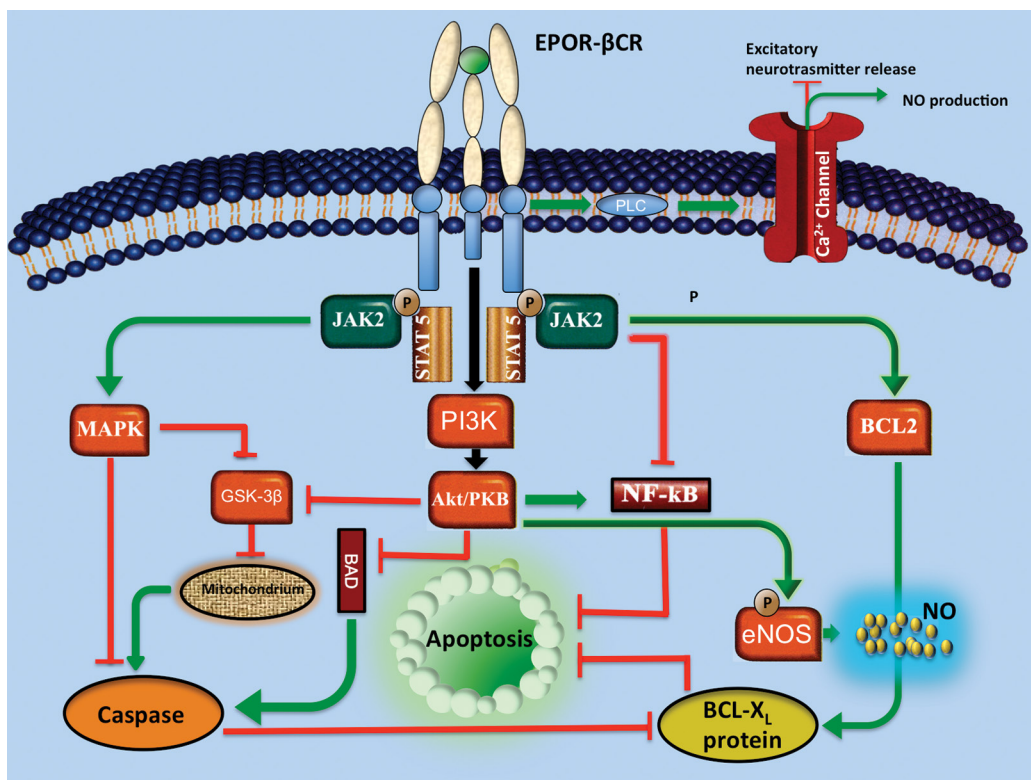
Figure 2. Main metabolic pathways involving NO in SAH. In endothelial cell (EC), NO is produced by NO synthase (eNOS) action. The soluble NO released from endothelial cells diffuses to adjacent smooth muscle cell (SMC) and activates soluble guanylate cyclase (GC). GC, in turn, produces cyclic guanosine monophosphate (cGMP), which activates intracellular calcium pumps thus sequestering free Ca^{2+} into intracellular sarcoplasmic reticulum and relaxing SMC. Following aneurysm bleeding hemorrhage, hemoglobin (Hb) binds NO thus reducing its availability. Hence, scavenging of NO results in a decrease of GC activity, which in return causes vasoconstriction. Furthermore, Hb directly inactivates GC by oxidization, thus leading to reduced production of cGMP. Finally, increased oxidative stress uncouples eNOS via Zn^{2+} thiolate oxidation resulting in a release of superoxide anion radical, further exacerbating oxidative stress and microvascular damage.

outcome, and prevents brain ischemic damage (37,38,80–88). Given these findings, and according to reports that have demonstrated that EPO enhances the NO system activity (27,68,69), and neuroprotective effects on cerebral cortical neurons from *N*-methyl-D-aspartate receptor-mediated glutamate toxicity (71), we further investigated the potential protective effects of rHuEPO in a rabbit model of SAH. Briefly, after experimental SAH has been induced by intracisternal blood injection, the results showed an improvement in functional recovery and mortality rate following systemic rHuEPO administration (1,000 IU/kg) for 72 h post-SAH (81). Interestingly, all EPO-treated animals survived, while 42.9% of placebo-treated animals died within 72 h. An open-field

test performed at 24, 48 and 72 h after SAH showed an increase in locomotor activity at 72 h in the placebo-treated group, while no increase in locomotor activity was observed in rabbits treated with rHuEPO.

Subsequently, the efficacy of rHuEPO has been evaluated on acute cerebral ischemia following experimental SAH in a rabbit model (36,80). Histological analysis performed 24 h following injury documented a reduction in brain ischemic damage in animals given rHuEPO (1,000 IU/kg). In particular, analysis of cortical neurons showed that the EPO-treated rabbits presented with a significant decrease in the amount of necrotic neurons compared with the untreated and placebo-treated animals (80). Another

important finding provided by this study was the observation that the concentration of EPO in the cerebrospinal fluid (CSF), assessed before euthanization of the animals, was significantly higher in the rHuEPO-treated animals than in the other groups, suggesting for the first time that systemically-administered EPO by crossing the BBB can result in increased EPO concentrations within the CSF. In a subsequent study, rabbits were given intraperitoneal injections of rHuEPO (1,000 IU/kg) starting 5 min after the induction of SAH and repeated every 8 h for 72 h (37). The authors aimed to investigate the ability of exogenous administered EPO to exert a vascular effect and, in particular, to counteract the spastic response of the cerebral arteries during SAH. By

morphometric analysis of the basilar artery, the authors observed that the administration of rHuEPO significantly reduced the vasoconstriction in SAH-plus-rHuEPO-treated animals compared with other animals that underwent SAH. Pathological findings also showed that EPO attenuated SAH-induced brain injury. Further evidence for the beneficial effect in the setting of SAH has also been provided by an experimental SAH model where a single dose of EPO (400 IU/kg) given subcutaneously prevented SAH-induced impairment of CBF autoregulation (89). Further evidence confirmed this observation (90). An additional experiment has confirmed the neuroprotective properties exerted by EPO by measuring the S-100 protein concentration in CSF of SAH-injured rabbits (38). The findings of this study indicated that high levels of S-100 protein correlated with mortality rate, neurological outcome, and ischemic brain damage. Animals treated with rHuEPO were found to have significantly lower levels of S-100 protein in their CSF, no deaths, favorable neurological outcome and significant protection against brain ischemic damage.

In a recent experimental study, recombinant adenoviral vectors (10^9 plaque-forming units per animal) encoding genes for human EPO (AdEPO), and β-galactosidase were injected immediately after injection of autologous arterial blood into the cisterna magna of rabbits, resulting in significant reversal of arterial vasospasm (70). Subsequent experimental studies have tried to provide new insight into the mechanisms underlying EPO-mediated neuroprotection. In particular, Cheng and collaborators (91) investigated whether rhEPO administration influenced the endothelial cell apoptosis in the basilar artery after SAH in the rabbit. They also investigated the modulation of rhEPO on the activity of JAK2 and STAT3 as part of the signaling in apoptotic mechanisms. As a result, they found that administration of rhEPO could activate JAK2 and STAT3 in the basilar artery and decrease the apoptosis index of endothelial cells following SAH. Moreover, the antiapoptotic genes,

such as bcl-2 and bcl-xL, were upregulated after injections of rhEPO. Recently, the effect of EPO and darbepoetin-α (DA), a novel EPO-derived agent with an extended circulatory half-life and an increased *in vivo* biological activity greater than EPO, were assessed in a rabbit model of SAH (92). Both erythropoietin and darbepoetin α treatments were found to attenuate cerebral vasospasm and provide neuroprotection after SAH.

Based on the experimental evidence suggesting an efficacious EPO-based therapy in SAH (86), clinical trials blossomed, however, with uncertain results (93,94). In this regard, the first clinical trial was terminated preliminarily, with inconclusive results, because of a lower than expected inclusion rate (93). Seventy-three patients were randomized to treatment with EPO (500 IU/kg/day for three days) or placebo. The primary endpoint was Glasgow Outcome Score at six months. Surrogate measures of secondary ischemia, that is, transcranial Doppler (TCD) flow velocity, symptomatic vasospasm, cerebral metabolism and jugular venous oximetry, biochemical markers of brain damage and blood–brain barrier integrity were assessed. The study failed in assessing the primary endpoint due to the limited sample size. Furthermore, except for an increased EPO concentration in the CSF of the EPO-treated group, there were no statistically significant group differences in the primary or secondary outcome measures.

A recent Phase II, proof-of-concept trial tested the hypothesis that acute systemic EPO therapy in patients following aneurismal SAH can reduce cerebral vasospasm and shorten impaired autoregulation as primary endpoints, which subsequently decrease delayed ischemic deficits (DIDs) and improve clinical outcome as secondary endpoints (94). Within 72 h following aneurysm bleeding, 80 patients were randomized to receive intravenous EPO (30,000 U) or placebo every 48 h for a total of 90,000 U. Primary endpoints were the incidence, duration and severity of vasospasm and impaired autoregulation on transcranial

Doppler ultrasonography. Secondary endpoints were incidence of delayed ischemic deficits and outcome at discharge and at 6 months. As result, although no differences were demonstrated in the incidence of vasospasm and adverse events between the two groups, patients receiving EPO had a decreased incidence of severe vasospasm, reduced DIDs, a shortened duration of impaired autoregulation and more favorable outcome at discharge.

In spite of some limitations, including a small number of cases, a single EPO dose, a single center and a lack of scheduled computed tomographic scan examinations, the study demonstrates in humans what was already observed in experimental studies (88). EPO administration can be effective in limiting cerebral vasospasm and ischemia after aneurysmal SAH.

The interesting features observed in experimental and clinical studies suggest new therapeutic strategies. Some issues, however, should be considered. The first concern is the safety of recombinant human EPO administration in the setting of SAH. It should be considered that the current information about the safety of this drug in humans comes from its use in anemic patients. Translating such information from anemic therapy to SAH-affected patients can be critical, since many pieces of information regarding the interaction and influence between EPO and physiologic variables, as well as with common therapy administered to patients with SAH, are unknown.

Second, several lines of evidence suggest that chronic EPO administration can produce hypertension, hypertensive encephalopathy, accelerated atherosclerosis, seizures and thrombotic/vascular events (82). In a model of embolic stroke in rats, EPO (5,000 U/kg) in combination with tissue plasminogen activator (tPA) exacerbated tPA-induced brain hemorrhage without reduction of ischemic brain damage when administered 6 h after stroke by upregulating matrix metalloproteinase-9, NF-κB, and interleukin-1 receptor-associated

kinase-1 (95). In the recent prospective, randomized, double-blind, placebo-controlled trial, the safety and efficacy of a single intravenous bolus of epoetin alfa (60,000 U) in patients with acute ST-segment elevation myocardial infarction (STEMI) was evaluated (96). In the efficacy cohort, EPO administration within 4 h of percutaneous coronary intervention did not reduce infarct size and was associated with higher rates of adverse cardiovascular events among older patients.

Although in the SAH clinical studies so far reported, no adverse effects during the EPO treatment have been observed, the short-term treatment and low EPO dosage used in these pioneering studies should be considered. In preclinical studies, recombinant human EPO treatment at a dose of 1,000 IU/kg administered every 8 h was effective in reducing cerebral vasospasm and cerebral ischemia, and in significantly improving neurological performance. The dosage used in the clinical setting is the lowest dose considered effective following SAH. It can be argued that the uncertain results from the first clinical trial (93) and the weak findings of the second clinical study (94) can find answer in the low dosage used and frequency of treatment. Accordingly, it is widely accepted that vasospasm and cerebral ischemia after aneurysmal SAH follow a different time course between humans and animals. In experimental settings, EPO has been found to be effective at a dosage starting from 400 IU to 1,000 IU/kg with a duration of 24 to 72 h (37).

Although in experimental studies and some clinical reports, an early, short-lived phase of vasospasm occurring immediately after SAH has been observed, the subsequent phase that is prolonged or chronic, noted on an angiogram in 40–70% of patients in the second week after hemorrhage, appears to be most important clinically. Accordingly, to achieve stronger effects, there is a rationale for starting and continuing neuroprotection for at least 14 d following the onset of SAH.

CONCLUSION

To date, all phase III trials using neuroprotective drugs have failed in demonstrating efficacy, thus suggesting that great optimism can lead to premature clinical trials driven by wishful thinking instead of detailed scientific evidence.

Further studies must be tailored and performed to assess the safety of EPO in the setting of this delicate clinical application. Optimal tolerated dosages, therapeutic time window and duration of therapy must be clearly identified. Furthermore, since increased blood viscosity and thrombotic events appear to be the major complications for chronic EPO administration, new EPO-derived drugs without erythropoietic effects (32,97) developed and experimentally tested with efficacy at present, should be further investigated to tailor successful future clinical trials.

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DISCLOSURE

The authors declare that they have no competing interests as defined by *Molecular Medicine*, or other interests that might be perceived to influence the results and discussion reported in this paper.

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