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Stroke Treatment: Focus on Neuroprotection and EPO

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ABSTRACT

Erythropoietin (EPO) is a neuroprotective chemical that is produced by the brain in response to stroke-induced hypoxia. Stroke was first described by Hippocrates (460-377 BC) as apoplexy, the Greek term for "struck down by violence". In modern day, the term "brain attack" and the expression "time is brain" have been put forward to emphasize the urgency for medical treatment if a stroke is suspected. Stroke assessment and stroke treatment focus on neuroprotective mechanisms to minimize irreversible brain tissue loss. The following discussion will review scientific evidence for the efficacy of EPO in patients with subacute ischemic or hemorrhagic stroke.

KEYWORDS: Stroke; Neuroprotection; Brain attack; Haemorrhage

INTRODUCTION

Hippocrates (460-377 BC) first described stroke over 2400 years ago. At that time stroke was called apoplexy, the Greek term for "struck down by violence" to describe the sudden paralysis or change in a person's physical and mental well-being [1]. Stroke, the lay term for apoplexy, did not appear in the English language until 1599, a synonym for at the "stroke of Gods hande", conveying the seemingly random, sudden nature of acute events and the subjective experience of people who have been 'struck' [1]. From the time of Hippocrates until the first half of the 20th century, apoplexy was the medical term while stroke was predominantly the lay term used to denote the acute condition in which the patient often suddenly fell to the ground ' without sense' or voluntary movement [1]. The Greek physician Galen (born AD 131) believed that apoplexy was caused by the interruption of the flow of 'vital spirit' to the brain [1]. Wepfer established the relationship between cerebral haemorrhage and apoplexy, in 1658. Apoplectic habitus was one of the predominant medical theories of apoplexy describing the personal risk factors (i.e. large thick heads) or 'the sort of person' (i.e. an indulgent lifestyle) that succumbed to apoplexy [1]. Cerebrovascular disease emerged in the early 20th century following evidence from post-mortem examinations suggesting that the disease is far less dramatic than the term apoplexy suggested and moved the onus of the disease away from the patient [1]. Despite the change in nomenclature, the hope that complete recovery would occur was dim although the benefit of physical rehabilitation was appreciated in 1935 [1]. "Habilitas" is Latin for rehabilitate meaning "to make able again". It is now appreciated that the earlier rehabilitation techniques are employed the better the chance of recovery [2]. Although there was an increased incidence of cerebrovascular accidents occurring post Second World War and an ageing population over the 20th century, stroke did not dictate its own medical specialty until 1962. Although 'stroke illness' was considered a lay term; the term 'stroke' was widely adopted by the medical community due to the convenience of its expression [1]. Recently, however, the term 'brain attack' analogous to heart attack has been introduced along with published warning signs and risk factors to emphasize the urgency for medical treatment if a stroke is suspected [3-8].

ASSESSING STROKE

The expression "time is brain" [9] was coined to underscore that with stroke progression brain tissue is lost and there is a need for immediate medical evaluation, including magnetic resonance imaging (MRI) and possibly pharmaceutical treatment [10-11]. In animals studies cerebral infarction leads to irreversible tissue loss within 1-2 hours post-occlusion [8]. Brain injury following ischemic stroke with resulting interruption of nutrient-rich blood (i.e. glucose and oxygen) flow to select brain regions involves a rapidly occurring complex series of cellular metabolic events including oxidative, inflammatory and excitotoxic cascades [8]. Ischemia following stroke results in adjacent brain tissue atrophy as well as diaschisis in tissue remote from the infarct lesion [12]. With focal impairment of cerebral blood flow, oxygen and glucose levels are reduced resulting in loss of cellular energy and membrane potential. Neurons and glia depolarize, excitatory neurotransmitters and Ca^{2+} accumulate, Na^{+} and Cl^{-} enter the cell with water passively following. Brain edema is one of the earliest markers for stroke pathophysiology and its resolution is key to whether a patient survives the first 72 hours post-stroke [13-14]. Through the failure of ionic homeostasis activation of glutamate receptors and elevations in extracellular Ca^{2+} levels initiate ischemic cell death. Brain excitoxicity occurs within minutes of occlusion following by peri-infarct depolarization 6-8 hours later, inflammation and apoptosis occurring hours and days following stroke [13] and in the days and weeks that follow the ischemic event the final characterization of the injured parenchyma [15]. In an animal model of ischemia administration of antagonists to the NMDA glutamate receptor, an ionotropic receptor permeable to Ca^{2+}, Na^{+}, and K^{+} ions, administered prior to or at the time of middle cerebral artery (MCA) occlusion is neuroprotective blocking the damaging effects of glutamate excitotoxicity and peri-infarct K^{+} depolarization reducing ischemic infarct volume. Compared to the prophylactic and neuroprotective effects of NMDA receptor antagonists the therapeutic window is narrow closing 1-2 hours post-arterial occlusion [13] and would be relatively ineffective in the acute (up to 4 days), subacute (up to 2-3 weeks), consolidation (up to several months), and chronic (years) following injury. There is some evidence that this time-line would be extended somewhat in the human condition [15] as excitotoxicity remains a primary target for stroke interventions [13]. Following ischemic events pathophysiological processes may be categorized into 5 distinct temporal stages including 1. A hyperacute phase (onset to 6 hours thereafter) where the direct effects of ischemia are most prominent 2. An acute phase (up to 4 days) where secondary effects are maximum, 3. A subacute phase (48 hours to 3 weeks) involving subsiding of secondary effects and emergence of neurofunctional plasticity, 4. A consolidation period (up to several months) involving the waning of neurofunctional alterations and 5. The chronic phase where deficits become static [16]. The extent of brain dysfunction and thus the severity of stroke are predicated on the location, severity and duration of focal cerebral ischemia.

Stroke Treatment: Focus on Neuroprotection and EPO

Ischaemic penumbra, first described in the late 1970s, is hypoperfused tissue peripheral to the infarct. The penumbral zone is charged as dynamic responsive to prompt reperfusion techniques to re-establish blood flow with possible restoration of function [8]. At present, in the hyperacute and acute phases of ischemic stroke the primary therapeutic approaches are directed towards the restoration of regional cerebral blood flow to preserve the ischaemic penumbra, protect neurons against further ischaemic damage and to augment brain plasticity to maximizing recovery [3]. The optimal treatment for artery occlusion that underlies ischemic infarction is reperfusion either by opening the blocked artery or circumventing blood flow [18]. Tissue-type plasminogen activator (rt-PA) is an effective thrombolytic agent inducing reperfusion of occluded vessels and better patient neurological outcomes if administered within 3 hours, although studies have extended this to 5 hours with positive results, following ischemic events[3;7;18-19]. However it has been estimated that only 1-3% of stroke patients receive rt-PA due to other factors besides the strict time constraint including restrictive patient selective criteria, increased risk for intracerebral hemorrhage, and lack of availability and/or slow transport to the hospital [8]. The relative success of rt-PA spurred development of new avenues for both acute and chronic stroke management with an increased focus on neuroprotective therapies and therapies directed towards the restoration of cognitive function following an ischemic attack. Cognitive function impairments, including deficits in attention, visuospatial perception, language, memory and executive function are often the most prominent and persistent sequelae of stroke [20]. Erythropoietin (EPO) restores cognitive function post-stroke, may be used in combination with rt-PA or as an alternative strategy for up to 90-97% of patients that are excluded from rt-PA use [8,21].

Erythropoietin (EPO)

Erythropoietin (EPO) is a neuroprotective chemical that is produced by the brain in response to stroke-induced hypoxia. Endogenously produced EPO levels are elevated 3 to 20 fold in the brain and up to 200-fold in the kidney in response to cellular hypoxia through upregulation of its transcription factor hypoxia-inducible factor 1 (HIF-1) and 2 (HIF-2), oxygen sensing molecules in tissue and brain transcribed in response to lowered oxygen levels [22-26]. In patients with mild cognitive impairment and patients with early Alzheimer disease the EPO receptor is upregulated in astrocytes in the temporal cortex and hippocampus presumably reflective of an EPO CSF deficiency in these states [27-28]). With age endogenous CSF EPO levels decline [29] perhaps indicative of increased white matter lesion and stroke risk. When given as a treatment, EPO can improve the functional outcome of stroke recovery. EPO an endogenous pleiotropic cytokine hormone secreted from the kidney, liver and spleen was initially documented for its hematopoietic function and its use as an erythropoietic stimulating agent in the treatment of anemia [30]. It was first recognized as early as 1987 that recombinant human EPO (rHu-EPO) as well as structural derivatives of EPO including darbepoietin alfa have been reported to possess cognitive enhancing properties in anemic patients following kidney failure and chemotherapy; medical conditions punctuated by low endogenous EPO and lymphocyte counts [31-35]. Paradoxically, hematological side-effects associated with exogenous EPO treatment include increased hematocrit levels and increased blood viscosity with resultant increased risk for cardiovascular accidents [36,37] including cerebral stroke, due to accompanying decreased brain oxygen levels which can be averted with the use of low dose EPO. New more selective EPO agents are possible following the identification of two distinct EPO receptor systems, one for erythropoietic and the other for the tissue-protective properties of EPO [38-40] such as non-erythropoiesis-stimulating EPO derivatives such as asialo-rHu-Epo [32,35] or carbamylated EPO (CEPO) [41]. Nonerythropoietic EPO analogues possess tissue neuroprotective effects without stimulating hematopoiesis (i.e. increased hematocrit) in a focal cerebral ischemic model in rats, and like EPO are correlated with a reduction in overall microglial activation, neuroinflammation and secondary tissue damage and increasing functional recovery as measured in the sensorimotor and foot-fault tests [32,38,40-42]. Notwithstanding cognitive performance in patients with chronic renal failure was noticeably improved at EPO doses without any measurable effect on hematocrit [31].

EPO RECEPTOR

The EPO receptor is found on brain capillaries and glial capillary end-feet [44] and recombinant human EPO (molecular weight over 30 000 Daltons) is capable of crossing an intact blood-brain barrier (BBB). Both EPO and its receptor (EPOr) are expressed by neurons and astrocytes in the developing and albeit at lower levels in the adult human cerebral cortex, cerebellum, hippocampus, amygdala, pituitary and spinal cord [45-48]. The EPO receptor is abundantly expressed on dopaminergic neurons in the adult rat and promotes proliferation and differentiation of dopamine neurons in cell culture [49]. The distribution of EPO and its receptor on CNS dopaminergic neurons in the adult brain reflects the biology of the therapeutic efficacy of EPO and is consistent with its neurotrophic effect in vivo in models of stroke [49-50].

PRECLINICAL STUDIES

Among healthy human subjects and normal rats, elevated levels of CSF EPO can be detected 3.5 hours following a bolus injection [31]. Pharmacokinetic studies performed on intact mice and rats indicate that murine EPO, human EPO and darbapoetin alpha, a glycosylation analogue of human EPO with an extended half-life cross the blood-brain barrier by a specific receptor mediated transport mechanism in amounts sufficient to activate brain erythropoietin receptors and account for EPO's neuroprotective effects [51-52]. It should be noted, however, that CSF EPO levels following ischemia exceed levels of intact animals [52]. Administration of exogenous EPO exerts beneficial CNS effects by increasing blood oxygen levels ameliorating attention deficits, psychomotor slowing and improves memory [35]. EPO and darbepoietin alfa provides neuroprotection, prevents global brain atrophy, ameliorates behavioral abnormalities including spatial memory deficits up to 9 months post-lesion and increases cognitive function in neurological and neuropsychiatric models including experimental models of brain and spinal cord injury including blunt head trauma [53], kainate-induced seizures, autoimmune encephalomyelitis [52-54], unilateral parietal lesion [55], bilateral ventral hippocampal lesions [56] and stroke [57-62]. EPO may represent a new therapeutic class of agents for the treatment of a vast array of distinct neurological disorders [53].

THERAPEUTIC EFFECTS OF EPO

Under hypoxic conditions increased production of EPO and its receptor (EpoR) in vascular endothelial cells,

microglia, astrocytes and neurons in the brain including the hippocampus, cortex, capsula interna and midbrain areas protects neuronal integrity and function [52,63-65]. In post-mortem brain infarct tissue of stroke patients a massive increase in EPO and EPO receptor expression was observed in neurons, astrocytes and vascular endothelial cells [66]. Cytoprotection afforded by EPO on post-mitotic neuronal cells in the CNS includes decreased cell death in the penumbra region secondary to activation of penumbral EPO receptors, reduction of inflammatory infiltrate, protection of neural cells and the promotion of neurovascular repair [57,63,67-68] following hypoxic/ischemic events. The therapeutic effects of EPO have been linked to reduction in cellular apoptosis through lessening of cytotoxicity (i.e. reduction of glutamate release and Ca^{2+} influx) glutamate in hippocampal cells [69] as well as activation of survival pathways [70-72], inhibition of caspases [70] and suppression of death-receptor complexes in peri-infarct areas [72-74]. The neuroprotective effects of the EPO/EpoR system on post-mitotic CNS neuronal integrity and function in clinical and animal models of neurological disease have been coupled to anti-apoptotic, anti-inflammatory, anti-oxidant and neurotrophic signaling pathways [46,71,75-76] including brain-derived neurotrophic factor (BDNF) driven synaptic plasticity [77]. EPO protected hippocampal CA1 neurons from ischemic cell death and improved hippocampal spatial learning 6 and 24 hours following stroke and traumatic brain injury (TBI) in rats [77-79]. In rats with bilateral ventral hippocampal lesions sustained as neonates or as young adults 500 or 5000 U/kg intraperitoneal darbepoetin alfa (Aranesp®) administration improved working memory in the novel object recognition task [56] while a three week EPO regimen (5000 IU/kg every other day for three weeks) enhanced long-term potentiation (LTP) in the CA1 region of the hippocampus and memory-related neuronal networks in healthy mice [80]. EPO treatment lessened hippocampal cell loss, increased angiogenesis and neurogenesis in damaged parietal and temporal cortex including the hippocampus following TBI in young rats and mice [55]. In neural progenitor cells EPO increases angiogenesis by activating the pro-survival Akt and ERK1/2 signaling pathways resulting in the increased production and release of vascular endothelial growth factor (VEGF) [24,81]. In aged animals VEGF production is impaired resulting in reduced angiogenesis post-ischemia relative to fetal and juvenile animals [82]. In acute-stage (within 24 h of stroke onset) ischaemic stroke patients serum VEGF levels were increased in patients following large vessel disease relative to small vessel disease (white matter lesions; lacunar infarcts) [83]. Elevated VEGF levels predicted favorable prognosis 3 months post-ischaemic stroke based as assessed by the National Institute of Health Stroke Scale (NIHSS) [83-84]. The National Institute of Health Stroke Scale (NIHSS) 13 days after stroke predicts physical performance and activities of daily living 3-6 months post-stroke in patients with subcortical and to lesser extent cortical lesions in both the right and left hemispheres. NIHSS predicted depression partnered with right sided lesions but could not predict cognitive outcomes among patients with infarcts limited to the right hemisphere [85] suggesting that validated fMRI investigations would be a valuable adjunct for charting the course of cognitive deficits in stroke. EPO (5,000 U/kg intraperitoneal administration 1, 2, and 3 days post injury appreciably enhanced functional outcome in neurological severity and sensorimotor functional outcome as measured by the foot fault test and spatial learning in the Morris water maze independent of hematocrit [86]. Human recombinant EPO (5000 IU/kg) also improved Morris water maze spatial learning in fimbria-fornix-transected rats and improved search strategy in intact animals [87]. EPO administration is associated with increased BDNF expression, increased brain levels of vascular endothelial growth factor (VEGF) [88], enhanced hippocampal acetylcholine (ACh) release, increased striatal DA release in vitro [89] and increased hippocampal nitric oxide (NO) release in vivo [90] which underlies angiogenesis, neural stem cell differentiation and survival in vitro [91-92] and forebrain neural stem cells in vivo [91] and may increase neurogenic synaptic events concordant with memory consolidation and learning [93-94]. EPO facilitates improvement of cognitive function following TBI or ischemic/embolic stroke [88,95-96]. In experimental stroke models, penumbral endothelial expression of the EPOr was increased 3 to 21 days following focal cerebral ischemia in mice [97] and global ischemia in rats [98]. In post-mortem neuropathological control brain tissue weak neuronal EPO and EPO receptor immunoreactivity was present while in ischemic brain EPO and the EPO receptor were upregulated in neurons, astrocytes and the vasculature epithelium surrounding cerebral infarcts [66]. EPO treatment promoted neurogenesis in a model of neonatal stroke [99]. EPO administration was also associated with reduced microglial infiltration and suppressed proinflammatory cytokine release following middle cerebral artery occlusion in rats [100]. Additionally EPO reduced microglial infiltration, decreased caspase-3 expression and sustained neurological (motor and cognitive) improvement within injured spinal cord [101], benefits lasting 3 to 14 days in an experimental model of closed head injury inducing focal TBI [102] in the rat.

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ANIMAL STUDIES

Recombinant human EPO (rhEPO) treatment decreased cerebral, cerebellar, hippocampal and brain stem infarct volumes, decreased hemispheric volume loss [57,103], increased local cerebral blood flow [77,97,103], restoration of blood-brain barrier (BBB) integrity [104] and increased post-stroke neurogenesis [99,105]. Deletion of EPO and/or its receptor is embryonically lethal early in development due to severe anemia, systemic hypoxia and deficits in embryonic neurogenesis in null mice [105-106]. Among EPO receptor conditional knock-out mice, brain specific EPO receptor deletion resulted in impaired post-stroke neurogenesis secondary to reduced subventricular cellular proliferation and impaired sensorimotor peri-infarct neuroblast migration 7 days post-injury [105]. In contrast, among transgenic mice that overexpressed EPO specifically in the brain (*tg*21) permanent MCA occlusion translated to smaller parietal cortex infarct volumes relative to wild-type littermates [107]. In the same study transgenic mice that overexpressed EPO in both brain and plasma (*tg*6) had increased infarct volumes rooted to reduce penumbra reperfusion secondary to high hematocrit levels and increased blood viscosity [107]. Exogenous EPO improved sensory neglect, facilitates recovery of sensorimotor functions [57,108] and asymmetry of fore-limb use which was accompanied by preserved hemispheric volume and decreased unilateral expansion of the subventricular zone following neonatal [57] and adult [88] stroke. EPO also preserved mesencephalic tyrosine hydroxylase-positive indicative of dopamine neuroprotection in the substantia nigra pars compacta and ventral tegmental area accompanied by abolishment of apomorphine-induced rotational asymmetry and decreased sensory neglect 4 weeks post-stroke in a neonatal model of hypoxic-ischemic brain injury [109]. In rats as assessed in the Morris water maze (MWM) alterations in brain plasticity following EPO administration was concordant with an improvement in neurological outcome and promotion of functional recovery following traumatic brain injury [78] and stroke with employment of MRI to monitor changes associated with the reduction of neurological deficits in the injured brain [110-113]. In stroke-prone spontaneously hypertensive rats with permanent occlusion of the left middle cerebral artery, cerebroventricular administration of EPO lessened ischemic-induced place navigation deficits as measured by the Morris water maze as well as cortical, hippocampal and thalamic infarct volumes [114]. EPO has a favorable therapeutic time-window, positive pharmacokinetics, multifactorial mechanism of action, enhances progression of white matter reorganization as revealed by T2- and diffusion-weighted MR imaging and reduces infarct volumes in both ischemic and hemorrhagic stroke [115-118]. Nonetheless the beneficial effects of EPO on neurological parameters and functional recovery in patients with subacute ischemic or hemorrhagic stroke are not known for certain, although results to date showing the neuroprotective efficacy of EPO in human cerebral ischemia are promising.

STROKE TRIALS

The Gottigen EPO proof of principle stroke trial, restricted to a small number of patients, assessed the effects of EPO on patients (≤ 80 years of age) within eight hours (average five hours) of symptoms post-MCA territory stroke (i.e. acute phase) as indicated by a clear lesion demarcated by diffusion weighted MRI imaging while T-2 weighted fluid attenuated inversion-recovery (FLAIR) imaging was normal at the start of the trial [31]. Diffusion weighted MRI (DWI) is extremely sensitive in the early detection (i.e. within minutes) of acute cerebral ischemia, small embolic infarctions and assessing the risk for infarct progression [119]. DWI is able to image swollen cells (cytotoxic edema) as a measure of Brownian motion of molecules and differences in diffusion of water molecules within the cytoarchitecture of the brain [119]. In comparison, perfusion weighted imaging (PWI) assesses the amount and latency of cerebral blood flow to various brain areas identifying structurally intact but functionally aberrant brain tissue perfusion. In the acute stages of stroke a PWI/DWI mismatch defined as areas with adequate diffusion but poor perfusion outlines the ischemic penumbra [120]. Human recombinant EPO reduces inflammatory cytokine release in response to injury and resulting cerebral inflammation and cytotoxic edema within 1 hour of therapeutic administration as measured by diffuse weighted MRI and T1 mapping in the neocortex and striatum; benefits that persist for protracted intervals (3-14 days) following diffuse traumatic brain injury in rats [121-122]. Cytotoxic edema is thought to occur within 12 hours of the cerebrovascular incident and is key to the pathogenesis of secondary brain injury following TBI and stroke [121,123,124]. As "time is brain" DWI is an invaluable clinical tool in the management of patients with suspected infarcts for the identification of ischemic penumbra within the hyperacute stage (within 12 hours) of human stroke and immediate need for treatment [119,125]. DWI is finely tuned to movement of water between extra- and intracellular space. While extracellular fluid is able to diffuse with relatively unrestricted motion, the cell restricts

intracellular fluid. The first metabolic failure in stroke is Na/K pump, following reduced ATP levels, leading to intracellular swelling (relative increase in intracellular versus extracellular fluid) [126]. So what you are seeing with DWI is the beginning of metabolic failure following intracellular edema. The DWI signal characteristics of acutely damaged tissue decrease with temporal evaluation while on conventional imaging (Flair, T2) the lesion will slowly appear. In other words, DWI may be used to identify areas of brain tissue with restricted diffusion prior to becoming ischemic as well as ischaemic penumbra [119,125]. Conventional MRI (Flair and T2) does not reliably detect cerebral infarction within the hyperacute stage of stroke (i.e. within the first few hours following the insult) but detects permanent changes or infarcts or extracellular edema [119,127]. Concomitant use of DWI and Flair permits the visualization of new infarctions amongst chronic lesions [119]. Diffusion tensor imaging is sensitive to injury-induced microstructural changes in white matter as well as identifying and quantifying the degree of changes occurring in neural tissue during recovery processes [128-130]. Diffusion-Tensor-Imaging (DTI) in patients attending a memory clinic for verbal memory decline with mild cognitive impairment revealed microstructural alterations in hippocampal areas and left hippocampal atrophy relative to age- and gender-matched healthy controls. Microstructural abnormalities as indicated by DTI and ROI analyses may be employed during the initial stages of cognitive decline indicative of early dysfunction in hippocampus temporal lobe gray matter, and corpus callosum and as a promising tool for initial detection of neurodegenerative cognitive function [131-132].

FUNCTIONAL MRI

Treatment of patients with subacute ischemic or hemorrhagic stroke with an acute intravenous high dose of EPO is extremely well tolerated, results in increases in CSF EPO concentrations 60-100 times that of untreated patients, reduced neurological impairment [30,133-136], decreased infarct size as measured by diffusion weighted MRI [111,137], reduced cerebral edema as observed with fluid-attenuated inversion recovery sequences (FLAIR) [137] and increases functional outcome scores one month post stroke [133,135,137]. EPO treatment decreased neurological deficits, increased functional outcome scores as assessed by the Barthel Index, modified Rankin Scale and National Institute of Health (NIH) stroke scale as well as decreased glial damage as measured by S100B serum levels. Importantly EPO did not increase hematocrit and red blood cell counts exceeding normal values [31,137-139]. Acute EPO (40, 000 IU) administration in healthy volunteers was associated with increased verbal fluency and enhanced working memory as assessed with a verbal fluency task and n-back working memory (WM) paradigm three and seven days post-administration. Functional MRI during the working memory task revealed time dependent alterations in frontal-parietal neuronal networks with increased activation in left frontal and cingulate cortices and decreased right parietal cortex BOLD signals [140]. Parenthetically, evidence suggests that a cortico-cerebellar circuit consisting of the ventrolateral and dorsolateral prefrontal cortex, parietal cortex, pre-supplementary motor area and anterior cingulate is involved in working-memory as assessed in a 2-back task in a blocked-design [141]. In contrast one week post-EPO administration the neural response was increased in right frontal-parietal neuronal networks and decreased in left homologous areas suggestive of a delay in the employment of task relevant spatial strategies [140]. During picture retrieval EPO administration enhanced hippocampal BOLD response, relative to placebo. One week following EPO (40,000 IU) administration increased activity in the hippocampus independent of hematocrit changes in response to picture encoding and retrieval relative to placebo was demonstrated in healthy volunteers. The protracted increase in hippocampal activity in response to EPO administration is consistent with EPO's neurotrophic actions and upregulation of hippocampal BDNF [140]. In healthy volunteers one week following EPO(40,000 IU) administration recognition of fearful faces only as well as the BOLD response to fearful relative to neutral faces was reduced in the occipito-parietal cortex. In addition there was a reported increase in mood three days post-administration. Both the BOLD changes in response to fearful faces as well as mood improvement are reminiscent of conventional SSRI antidepressant action [142]. EPO modulates fear processing and memory-relevant hippocampal response and improves mood for three days as assessed by the Positive Affect Negative Affect Schedule (PANAS) following administration. During the processing of happy faces EPO increased activation in the right precuneus and left amygdala in healthy volunteers three days post-administration and was associated with increased positive emotional processing, facial recognition and mood [143]. As an aside, EPO is being considered as an adjunctive therapy for the treatment of cognitive dysfunction in schizophrenia as weekly treatment with EPO over 3 months improved cognitive function [31,144]. The cognitive enhancing effects of EPO occurred without changes in hematological measures. The direct enduring neurobiological actions of EPO inducing neurochemical changes and inciting neurotrophic signaling in

neural networks affected by neurological and neuropsychiatric illness supports the clinical utility of EPO for the treatment of neuropsychiatric brain disorders characterized by deficits in cognitive function [145].

Use of serum markers in conjunction with imaging analyses would be a smart strategy for the evaluation and monitoring of new pharmacological inducers, such as the therapeutic efficacy of EPO, post-stroke. Moreover, EPO may lower the impact of environmental risk factors on stroke. For example, EPO has a beneficial effect in cardiovascular disease increasing cardiac function in the ischemic heart [146] and may have a prophylactic role in the prevention of cardiac precipitated silent stroke, white matter lesions and cognitive dysfunction [8;147].While the first clinical stroke study by Ehrenreich and colleagues [112,148] has demonstrated the safety and improvement of functional recovery in acute stroke the efficacy of EPO in standardized measures of stroke rehabilitation and brain plasticity will need to be demonstrated. Subsequently EPO efficacy in acute stroke has been evaluated in a multi-center study involving eight university hospitals in Germany [115].

Since the Gottingen trial, review of the literature suggests the field is steadily progressing highlighting both the benefits and controversies surrounding the use of EPO for the treatment of stroke. The double-blind, placebo-controlled, randomized German Multicenter EPO Stroke Trial (Phase II/III; ClinicalTrials.gov Identifier: NCT00604630) revealed major safety concerns with the use of EPO in humans suggesting the translation of data from bench to bedside needs to be reevaluated [149]. A recent review of Souvenir et al. in 2015 [150] echoes the promising effects of EPO as outlined in our discussion above while also highlighting the controversies surrounding the use of EPO including the need for additional clinical phase II safety studies.

CONCLUSION

Due to the heterogeneity of inter-individual stroke deficits it may be clinically and scientifically naïve to believe that a single therapeutic agent may be employed for as an intervention directed at functional deficits in the treatment of stroke. However, pharmaceutical agents may be employed to render the brain receptive to supporting functional recovery, to exploit the brain's capacity for neuroregeneration and adaptive neuroplasticity, to minimize the impact or influence of maladaptive behaviors and to maximize the efficiency of cognitive rehabilitation techniques. One such agent may be erythropoietin which has been shown to have a multifaceted mechanism of action and effects on a multitude of neurotransmitter systems involved in brain repair. While the data supporting the efficacy of EPO in preclinical and human studies is well-documented further controlled clinical trials are needed to clarify the inclusion/exclusion criteria of patients to mitigate serious unexpected adverse drug reactions.

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