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HYPOTHESIS

Erythropoietin as a Neuroprotectant: Does Phenytoin Exert its Neuroprotective Functions Through Stimulation of Erythropoietin Production?

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Abstract. Neuropathic pain is caused by various central and peripheral nervous system disorders. Sodium channel up-regulation, γ-amino butyric acid down-regulation, nerve demyelination and sympathetic-somatosensory crosstalk are considered as important pathophysiological mechanisms for neuropathic pain. Pharmacotherapy is currently the mainstay of neuropathic pain treatment. Phenytoin, a classical anticonvulsant, has been shown to provide neuroprotection through sodium and calcium channel blockade and inhibition of glutamate release and glutamate receptors. On the other hand, it has been shown that erythropoietin, the principal regulator of erythropoiesis, has important non-hematopoietic actions such as neurodevelopment and neuroprotection. In this paper, we hypothesize that phenytoin may increase erythropoietin level directly through inhibition of calcium influx and indirectly through an increase in renin secretion and production of angiotensin II which causes hypoxia. Considering the neuroprotective function of erythropoietin, phenytoin may provide neuroprotection partially through stimulation of erythropoietin production.

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Abbreviations used: Epo, erythropoietin; GABA, γ-amino butyric acid; NMDA, N-methyl-D-aspartate.

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1. Introduction

The International Association for the Study of Pain (IASP) defines neuropathic pain as "Pains resulting from disease or damage of peripheral or central nervous system" [1]. Persistent neuropathic pain usually alters a patient's quality of life by interfering with sleep, work, recreation, and emotional well-being [2]. According to a new Pfizer funded global survey carried out in seven countries, neuropathic pain is estimated to affect from 2.8% to 4.7% of the global population, with an increasing incidence with age [3]. Despite the heterogeneity in etiology, anatomy and manifestation, neuropathic pains share certain characteristics [4].

Several cellular and molecular mechanisms are thought to be contributing to neuropathic pain, as summarized below:

1. Ectopic and spontaneous impulse generation from the cell bodies of myelinated fibers following injury which is thought to be secondary to accumulation and novel expression of sodium channels in neuromas, dorsal root ganglion (DRG) and areas of demyelination [5].
2. Anatomical reorganization of dorsal horn in which myelinated afferent fibers located in deeper layers of the dorsal horn cells (laminae III and IV) sprout to outer laminae of the dorsal horn (laminae I and II) where most nociceptive-specific neurons are located [6].
3. Increased sympathetic nervous system activity due to expression of new α-adrenergic receptors on damaged primary afferent axons, and release of noradrenalin from sympathetic terminals result in sympathetic communication with somatosensory afferents in neuromas and DRGs and induction of ectopic discharges from DRGs [7].
4. Neuroplastic changes including a decrease in spinal inhibitory pain signals via decreasing the expression of inhibitory neurotransmitters and their receptors [*e.g.* γ-amino butyric acid (GABA) and glycine] [8] and increasing glutamatergic transmission through augmentation of glutamate release from primary afferents and up-regulation of its receptors [*e.g.* N-methyl-D-aspartate (NMDA) and amino-3-hydroxy-5-methyl-isoxazol propionic acid (AMPA)] [9].

The management of neuropathic pain includes treating any underlying condition, providing symptomatic relief from pain and disability and preventing recurrence [10]. Effective treatment usually combines nonpharmacologic approaches with medication. Nonpharmacologic approaches are usually used as an adjunct to medication [11].

Pharmacotherapy is the mainstay for treating neuropathic pain [12]. Medications from several different drug classes are used to treat neuropathic pain [11]. In recent years, erythropoietin (Epo), a key endogenous hematopoietic growth factor, has been recognized as a novel neuroprotectant in the central and peripheral nervous system [13]. However, US Food and Drug Administration (FDA) approved only six medications for neuropathic pain including the 5% lidocaine patch, gabapentin and pregabalin for postherpetic neuralgia, pregabalin and duloxetine for painful diabetic neuropathy, carbamazepine for trigeminal neuralgia and ziconotide for severe chronic pain in patients for whom intrathecal therapy is warranted and who are intolerant of or refractory to other treatments [12,14].

The use of antiepileptic drugs for the control of neuropathic pain started over 50 years ago [15]. Use of antiepileptic drugs to treat neuropathic pain is based on the fact that there is a notable similarity between the pathophysiology and biochemical mechanisms of epilepsy and neuropathic pain [16]. The pathophysiologic processes that underlie the wind-up phenomenon caused by nerve injury and in the kindling of hippocampal neurons in epilepsy are remarkably similar; both appear to result from activation of NMDA receptors [17,18]. Antiepileptic drugs generally enhance inhibitory neurotransmission, reduce excitatory neurotransmission and regulate cation channel conductance [19].

Phenytoin, an anticonvulsant agent, is known as a neuroprotective medication and has been administered for this purpose for many years. In spite of several probable suggested

mechanisms which will be discussed later, the precise pathway in which phenytoin provides neuroprotection has not been completely understood. We hypothesize that phenytoin may exert its neuroprotective activity partially through the induction of Epo production.

2. Phenytoin and Epo: Two neurotherapeutic agents

2.1. Phenytoin as a Neuroprotectant

In the UK and USA, phenytoin is indicated for the control of tonic-clonic seizures (grand mal epilepsy), partial seizures (focal including temporal lobe) or a combination of these, and the prevention and treatment of seizures occurring during or following neurosurgery and/or severe head injury [20]. Phenytoin was the first drug to be used for trigeminal neuralgia but its efficacy is based only on uncontrolled studies [4]. Pain relief is obtained in approximately 60% of patients initially. As tachyphylaxis may develop within a short time, only about 20-30% of patients will experience sustained pain relief [21]. However, a recent double-blind, placebo-controlled, crossover study reported that IV phenytoin at doses of 15 mg/kg when administered as 2-h infusion reduced acute attacks of neuropathic pain including trigeminal neuralgia [22]. Orally administered phenytoin at an average dose of 300 mg/day was more effective than aspirin 1700 mg/day and placebo in patients with Fabry's disease [23]. Two clinical trials of phenytoin for treatment of diabetic neuropathy showed conflicting results. Phenytoin was found to be superior to placebo in one of these studies with an NNT (number-needed-to-treat) of 2.1 [24] while ineffective in the other study [25]. The availability of phenytoin for intravenous infusion makes it a good choice for treatment of acute attacks of neuropathic pain [15].

According to the current data, the effectiveness of phenytoin in the treatment of neuropathic pain is attributed to the following mechanisms:

1. Phenytoin blocks sodium channels and consequently suppresses sustained, repetitive, high frequency neuronal firing [26,27]. In addition, phenytoin increases Na^+/K^+ ATPase activity [28,29]. Stimulation of Na^+/K^+ ATPase as well as blockade of sodium channels inhibit sodium influx into neurons, lowering intracellular sodium concentration and prevent subsequent deleterious events including chloride and water entry which causes osmotic swelling and intracellular calcium accumulation [30]. Calcium accumulation is due to activation of NMDA receptors and reversing the operation of Na^+/Ca^{2+} exchanger [31]. This will prevent secondary damages that are due to high intracellular calcium concentration [32-34].
2. Phenytoin attenuates glutamate excitotoxicity through inhibition of calcium-dependent presynaptic glutamate release [35] and blocking NMDA receptors [36].
3. Phenytoin has an inhibitory effect on T-type calcium channels [37], which are responsible for sensory transmission and pain processing (nociception) [38]. Therefore, the effectiveness of phenytoin on various forms of neuropathic pain such as trigeminal neuralgia and diabetic neuropathy may be partially due to blocking T-type calcium channels in DRG [37].

2.2. Epo as a Neuroprotective Cytokine

Epo, a 34-kDa glycoprotein hormone, is characterized as the principal regulator of erythropoiesis by inhibiting apoptosis and by stimulating the proliferation and differentiation of erythroid precursor cells. Epo is initially produced in the liver but shortly after birth, its production is shifted to the kidney [39]. The glomerular tuft [40,41], tubular [42] and peritubular (interstitial and endothelial) [43] cells have been the postulated sites of Epo production in the kidney. The FDA approved Epo use in 1989 [39], and it is now widely used for boosting erythropoiesis before autologous blood transfusion [44] and treatment of anemia associated with renal failure, cancer, prematurity, chronic inflammatory disease and human immunodeficiency virus (HIV) infection [39].

For many years, it has been believed that

Epo acts exclusively on erythroid precursor cells [45]. However, several lines of evidence indicated that Epo receptors are expressed in non-erythroid blood cell lines such as myeloid cells, lymphocytes and megakariocytes [46] and also in a wide variety of non-haematopoietic cells such as endothelial cells [47], enterocytes [48], muscle (skeletal, smooth and cardiac) [49-51], neuronal and nonneuronal cells (astrocytes, oligodendrocytes and microglia) of the nervous system [52,53]. Further *in situ* studies demonstrated that Epo and its receptor localize in different sites of central (temporal and frontal cortex, the hippocampus, cerebellum and amygdala in brain [54] and motor neurons within the ventral horn in spinal cord [55]) and peripheral (axons and Schwann cells) nervous system [56].

Similar to the kidney, in both astrocytes and neurons, an oxygen sensing system may control Epo production [57,58]. However, hypoxia is not the only factor affecting Epo expression in the nervous system [46]. Metabolic disturbances such as hypoglycemia or strong neuronal depolarization [59], insulin and insulin-like growth factors [60] may also increase Epo expression through hypoxia-inducible factor-1 (HIF-1). Neuronal Epo and its receptor expression change during brain development indicating the importance of Epo in neurodevelopment [46]. Epo drives the production of neuronal progenitors from neural stem cells [61] and stimulates progenitor cell differentiation into neurons [62]. Epo treatment has shown protective effects in experimental models of Parkinsonism [63], subarachnoid hemorrhage [64], traumatic brain injury [65], spinal cord ischemia [55] and injury [66], retinal ischemia [67], peripheral nerve injury [68], multiple sclerosis [69] and diabetic neuropathy [70], suggesting that Epo may have neuroprotective function. In line with these experimental *in vivo* studies, Ehrenreich *et al*. conducted the first clinical trial with recombinant human Epo (rHuEpo) in patients with cerebral ischaemic stroke. The group of patients having received 100,000 IU rHuEpo in three intravenous injections, 8 h following the stroke and 24 and 48 h later, showed significant neurologic improvement, better functional recovery as determined by Barthel Index and a less prominent neurologic deficit at 1-month follow up [71]. Morever, according to the preliminary reported data of a pilot study, addition of rHuEpo to a standard chemotherapy protocol in breast cancer was associated with improvement in some cognitive functions [72].

Although the exact mechanism for the neuroprotective effect of Epo has not been elucidated yet, several mechanisms have been suggested:

1. Epo reduces the nitric oxide (NO)-mediated formation of free radicals or antagonizes their toxicity through increasing the activities of antioxidant enzymes such as superoxide dismutase, glutathione peroxidase and catalase in neurons [73,74]. Epo also reduces the level of tissue injury through attenuation of glutamate toxicity by inhibition of glutamate release [75].
2. Epo improves blood flow and tissue perfusion in ischemic areas (including the nervous system) through two mechanisms: a) modulation of angiogenesis via stimulation of endothelial cells to produce proteases, cell proliferation [76] and activation of vascular endothelial growth factor (VEGF) [77] b) reversing acute vasoconstriction by inducing the constitutive form of NO synthase (NOS) [74,78].
3. Epo attenuates the apoptosis of endothelial and neural cells by controlling the balance between pro- apoptotic (*e.g.* Bak) [79] and anti-apoptotic [*e.g.* nuclear factor-κB (NFκB) [80] and Bcl-x_L [79]] molecules.
4. Epo decreases inflammation by inhibiting leukocyte infiltration in the ischemic areas and reducing the levels of pro-inflammatory cytokines such as TNF-α, IL-6 and monocyte chemoattaractant protein-1 (MCP-1) [81].
5. Epo is involved in synaptic plasticity via modification of various neurotransmitters such as GABA, dopamine and acetylcholine in an NO-dependent [74] or independent [82] manner.
6. Epo induces the synthesis of neuroglobin [83], a new identified globin expressed mainly in the brain, which exerts its neuroprotective effects through controlling oxy-

gen affinity and kinetics [84].

7. Epo also regulates neurogenesis by controlling the proliferation and differentiation of neuronal stem cells localized in subventricular zone [61].

3. Possible Mechanisms of Phenytoin in Triggering Epo Production

3.1. Role of Phenytoin in Renin Secretion

In addition to its anticonvulsant effect, phenytoin has been shown to affect membrane transport in a wide variety of tissues other than the nervous system [85-87]. One example is the kidney [88]. Phenytoin accumulates in high concentration in the kidney [89]. Churchill *et al*. demonstrated that phenytoin stimulates renin secretion from rat renal cortical slices. The stimulatory effect of phenytoin was antagonized by blocking Na^+/K^+ ATPase via incubating slices in potassium-free medium or adding ouabain to the medium. Furthermore, decreasing the extracellular sodium concentration of the incubation medium resulted in inhibition of renin release [90]. Given that the secretory activity of juxtaglomelular apparatus, the site of renin synthesis, storage and release is directly related to the transmembrane sodium gradient or reciprocally related to intracellular sodium [91-93], the authors suggested that the stimulatory effect of phenytoin on renin secretion may be attributed to a decrease in intracellular sodium or an increase in sodium gradient [90]. An *in vivo* experiment by Migdal *et al*. which investigated the effect of phenytoin on renin release indicated that infusion of phenytoin into the renal artery of anesthetized dogs significantly altered renal hemodynamics. Results of this study showed a decrease in renal vascular resistance, increase in renal blood flow, renal plasma flow, diuresis and natriuresis. A stimulatory effect on renin release was also recognized. The authors could not clarify the precise mechanism by which phenytoin produced renal vasodilation, natriuresis and diuresis. Consistent with the conclusion obtained from the study performed by Churchill *et al*., the authors assumed that renin rise in response to phenytoin is due to a decrease in intracellular sodium concentration [94]. In contrast, in another study, infusion of phenytoin into the renal artery of either denervated or innervated kidneys of anesthetized dogs did not change systemic or renal hemodynamics including renal blood flow and renal vascular resistance. Renin secretion did not change in dogs with denervated kidneys following phenytoin infusion, whereas in dogs with innervated kidneys, renin release was increased. These results suggested that phenytoin-stimulated renin release is mediated by or requires the presence of renal nerves rather than renal hemodynamic changes [95]. In line with this view, the results of the study which examined the effects of adrenergic blockers in phenytoin-mediated renin release in five groups of anesthetized dogs showed that beta-1 selective inhibitors such as metoprolol and atenolol antagonize the stimulatory effect of phenytoin on renin release [96]. These data strongly confirmed the role of renal nerves in phenytoin-mediated renin secretion.

On the other hand, two lines of evidence suggest a causal relationship between phenytoin and calcium on renin release.

First, a series of studies found that in addition to sodium influx, phenytoin can inhibit calcium influx in neurons, brain slices and isolated presynaptic nerve endings (syanaptosomes) of rat [97-99]. Furthermore, phenytoin decreases intracellular calcium both at rest and during repetitive stimulation in lobster axons [100]. In agreement with the observations previously published by Yaari *et al.* [101], Ferrendelli and Daniels-McQueen stimulated calcium intake into syanaptosomes by veratridine (a sodium channel activator) and found that phenytoin attenuated this effect [102]. In murine neuroblastoma, the calcium-dependent portion of the action potential was shown to be inhibited in the presence of phenytoin [103] and a similar observation was made in spinal cord neurons [104]. Intracellular and extracellular application of phenytoin in cells of the clonal mouse neuroblastoma X rat glioma hybrid line 108CC5 depressed the inward calcium current in a concentration-dependent manner [105]. These studies could not elucidate the mechanisms responsible for the interfering action of phenytoin on voltage-dependent cal-

cium channels. Twombly *et al*. conducted an experiment on neuroblastoma cells (N1E-115) of C1300 mouse tumor in order to determine whether phenytoin directly blocks type I, "T-type" (low-threshold) or type II, "L-type" (high-threshold) calcium channels and evaluate the mechanisms that contribute to the blocking action of phenytoin. This study showed that phenytoin at concentrations from 3 to 100 μM suppressed type I calcium channel currents without altering the time course of current inactivation. In contrast, type II calcium channel currents are insensitive to phenytoin. Voltage-dependent and frequency-dependent suppressing actions of phenytoin suggest that phenytoin shifts the calcium channel population toward the inactivated state allowing fewer channels to open with depolarization [106].

Second, extensive studies defined the role of calcium in mediating renin release. High extracellular calcium inhibits [107] whereas low extracellular calcium stimulates [108] renin release. When calcium is excluded from a medium with identical high potassium ion, renin release is increased [109]. Calcium chelating agents such as ethylenediamine tetraacetic acid (EDTA) and/or ethylene glycol tetraacetic acid (EGTA) stimulate renin release in several *in vitro* preparations including rat glomeruli [110], rat [111] and pig [109] renal cortical slices and isolated perfused rat kidneys [112]. On the other hand, calcium ionophore which promotes entry of calcium into cells inhibits renin release [110,113]. Taken together, intracellular calcium acts as an inhibitory second messenger in the renin secretory process.

Churchill explained the stimulatory effect of phenytoin on renin release on the basis of the hypothetical model of juxtaglomerular cells [114]. Since juxtaglomerular cells are derived from smooth muscles [115] and considering the presence of Na^+/Ca^{2+} exchanger and also active calcium transporter in smooth muscle cells [116,117], he postulated that these mechanisms of calcium efflux may also exist in juxtaglomerular cells [114]. Additionally, as mentioned previously, it has been shown that phenytoin stimulates Na^+/K^+ ATPase and maintains sodium and potassium concentration gradients [28,29]. According to this explanation, phenytoin increases the activity of Na^+/K^+ ATPase located on the cell membrane of juxtaglomerular cells and therefore, intracellular sodium concentration decreases. Subsequently, Na^+/Ca^{2+} exchangers activate and extrude calcium and as a result, intracellular calcium level depletes. As mentioned previously, intracellular calcium is an inhibitory second messenger in renin release and therefore, phenytoin may stimulate renin secretion via this pathway [114].

In summary, in the presence of the renal nerves, phenytoin may stimulate renin release through decrease in intracellular calcium level of juxtaglomerular cells directly via blocking voltage sensitive calcium channels or indirectly through activating Na^+/Ca^{2+} exchanger via stimulation of Na^+/K^+ ATPase.

3.2. Relationship between Renin-Angiotensin System and Epo

Several experimental and clinical observations suggest that there is a relationship between renin-angiotensin system (RAS) and Epo production. The infusion of renin [118] or angiotensin II (Ang II) [119] into rats significantly increases Epo production in response to hypoxemia. There is a direct correlation between plasma renin activity and serum Epo in patients with normal renal excretory function who have glomerulonephritis or pyelonephritis [120]. Type 1 diabetic patients with hyporeninemic hypoaldosteronism and mild renal insufficiency have anemia secondary to Epo deficiency [121]. Clinical observations with the use of angiotensin-converting enzyme inhibitors (ACEIs) offer further support for the existence of a relationship between RAS and Epo production. Renal transplant patients who develop polycythaemia due to excess Epo production can be successfully treated using ACEIs [122]. In hypertensive patients treated with ACEI, an approximately 7-10% fall in hemoglobin occurs [123]. Subjects with Type 1 diabetes taking ACEI have significantly reduced hemoglobin compared with controls [121]. In patients with chronic renal failure, treatment with enalapril is associated with worsening anemia and decreased plasma Epo levels [124]. Furthermore, several controlled experi-

ments conducted on healthy human volunteers demonstrated that Ang II can be a direct stimulant of Epo production. Blocking the stimulatory effect of Ang II on Epo production by Ang II subtype 1 receptor (AT_1R) antagonists (losartan or valsartan) suggests that AT_1R receptors play a role for the control of Epo production in humans [125-127].

The mechanism by which the RAS may influence Epo production is not completely elucidated. Based on Ang II physiology, it causes preferential vasoconstriction of the efferent arteriole at the glomerulus, resulting in an increase in the filtration fraction [128]. At the proximal tubule, Ang II increases sodium reabsorbtion both passively by effects on the Starling forces and actively, by stimulation of the Na^+/H^+ exchanger [129]. The tubular reabsorbtion of sodium accounts for 80% of the oxygen consumption of the human kidney [130]. In other words, Ang II decreases oxygen delivery to the tubular interstitial tissue due to diminished blood flow in peritubular capillaries while at the same time increasing oxygen consumption in the proximal tubule [129]. Regarding the fact that production of Epo is hypoxia-sensitive, once a critical level of hypoxia is perceived by oxygen sensors, Epo gene transcription is initiated in Epo producing cells [131]. Ang II may also have a direct effect on Epo gene transcription [131,132]; however it is not known whether Epo producing cells in renal interstitial tissue have receptors for Ang II. These cells are not available for *in vitro* experiments yet [133], therefore, intracellular effects of Ang II on the transcription of Epo gene remain hypothetical [131].

3.3. Calcium and Epo Secretion/Biosynthesis

Calcium ions are important in controlling the production of, including gene transcription, mRNA stabilization, glycosilation secreted proteins at different levels and secretion [134-136]. Similar to renin, calcium level in both kidney and liver cells may be very important in the regulation of Epo biosynthesis and/or secretion [137]. Low extracellular calcium level (0.3 mmol/l) in the culture medium significantly enhances Epo secretion by human renal carcinoma cells. Treatment of the culture with trifluoperazine (a calmodulin inhibitor) also results in 53% increase per day in Epo secretion [138]. McGonigle *et al*. demonstrated that verapamil (5, 10 and 20 mg/kg intraperitonally) produced significant increase in mean serum Epo levels in rats exposed to hypoxia [139]. Cobalt which is known to decrease calcium entry into cells also stimulates Epo production [140,141]. On the other hand, increase in medium level of calcium ionophore, A23187, decreases Epo secretion in Epo-producing renal carcinoma cells *in vitro* [137]. Furthermore, several prospective studies in hemodialysis patients with secondary hyperparathyroidism indicated that parathyroidectomy significantly improves renal anemia. Parallel with the improvement in anemia after parathyroidectomy, there was either a gradual increase in serum Epo level or a decrease in the required weekly dose of rHuEpo [142-145]. In addition to the direct inhibitory effect of parathyroid hormone on erythropoiesis, Ureña *et al*. suggested that the chronically elevated serum calcium concentration during secondary hyperparathyroidism as well as hyperphosphatemia could contribute to the decreased Epo production and conversely reduction of plasma calcium concentration after parathyroidectomy could lead to Epo enhancement [142].

The precise molecular role of calcium on Epo secretion has not been elucidated. One suggested mechanism is based on the fact that inositol triphosphate (IP_3) increases intracellular calcium mobilization from the endoplasmic reticulum and activates calcium-calmodulin (Ca CAM) kinase. Activation of Ca CAM kinase may increase the level of an inhibitory phosphoprotein resulting in a decrease in Epo secretion [137]. Compatible with this view, hypoxia which is the major stimulant for Epo secretion, decreases IP_3 [146]. Interestingly, a recent study provided evidence that lowering intracellular calcium concentration can activate HIF-1. Similar to hypoxia, decreasing the intracellular calcium level by a calcium chelator, 1,2-bis (2-aminophenoxy) ethane-N,N,N′,N′-tetraacetic acid (BAPTA), induced HIF-1α nuclear accumulation and greatly enhanced expression of HIF-1 target gene in the neuroblastoma cell line, SH-SY5Y, under nor-

moxic conditions. In contrast, elevation of cytosolic calcium level by thapsigargin, an endoplasmic reticulum Ca^{2+}-ATPase inhibitor, which depletes intracellular calcium stores and elicits elevation of cytosolic free calcium concentration, reduced the BAPTA-mediated effects [147].

4. The Probable New Pathway for the Neuroprotective Function of Phenytoin

Based on the above data, it can be hypothesized that due to the calcium channel blocking activity of phenytoin and its ability to accumulate in the kidney, it may decrease intracellular concentration of calcium in kidney cells, resulting in renin secretion. This, increases Ang II formation and may result in an increase in Epo secretion. Phenytoin also can increase Epo release directly through inhibition of calcium influx. Based on the neuroprotective functions of Epo, we suggest that in addition to its previous mechanisms, phenytoin may exert its neuroprotective effect through stimulation of Epo production.

In order to establish our hypothesis, determining endogenous Epo levels before and after phenytoin administration in patients with neuropathic pain is recommended. If our hypothesis was confirmed with experimental and clinical studies, Epo may be used in place of phenytoin for treatment of neuropathic pain. Considering the complications of phenytoin administration including its side effects (*e.g.* sedation, motor and hematologic disturbances) and its unpredictable and complex pharmacokinetic profile [4], together with the fact that rHuEpo is a relatively safe and well tolerated drug and has a favorable pharmacokinetic profile [148], this replacement may be advantageous. However, it is worthy of note that all of the currently available information due to the safety of rHuEpo in humans comes from its use in other clinical disorders and generalization of such safety information from the treatment of anemia to neuropathic pain may be dangerous and unreasonable. Morever, other issues such as optimum dose and duration of treatment should be considered.

Given the evidence from cell culture and animal experiments mentioned before, Epo has a wide therapeutic potential range which varies from acute and chronic neurodegenerative diseases (stroke, Parkinson's disease, Alzheimer disease, amyotrophic lateral sclerosis, multiple sclerosis, neurotrauma and perinatal asphyxia) to psychiatric disorders such as schizophrenia. Theoretically, it is reasonable that any endogenous or exogenous agent that has Epo-biosynthesis/secretory ability may have a therapeutic spectrum similar to Epo. The probable effect of phenytoin on Epo production represents a novel paradigm for neuroprotection.

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