ASIAN JOURNAL OF PHARMACEUTICAL AND CLINICAL RESEARCH

INNOVARE ACADEMIC SCIENCES
Knowledge to Innovation

Vol 9, Issue 3, 2016

Online - 2455-3891
Print - 0974-2441

Research Article

PHOSPHORYLATION OF TAU PROTEIN IN BRAIN REGIONS OF CHRONIC RENAL FAILURE - INDUCED RATS: AMELIORATIVE EFFECT OF ERYTHROPOIETIN

KARTHICK N[1], POORNIMA KN[1], SARAVANAN A[1], ALWIN D[2], VENKATARAMAN P[3]*

[1]Department of Physiology, SRM Medical College Hospital and Research Centre, SRM University, Kattankulathur - 603 203, Tamil Nadu, India. [2]Department of Animal House, SRM Medical College Hospital and Research Centre, SRM University, Kattankulathur - 603 203, Tamil Nadu, India. [3]Department of Medical Research, SRM Medical College Hospital and Research Centre, SRM University, Kattankulathur - 603 203, Tamil Nadu, India. Email: venky_prabhu@hotmail.com

Received: 11 March 2016, Revised and Accepted: 21 March 2016

ABSTRACT

Objectives: Chronic kidney disease (CKD) is a major clinical health problem as it is a systemic disorder that causes widespread organ damage and it is related to significant morbidity and mortality. Numerous studies have shown that, cognitive dysfunction increase in prevalence, due to increase in reactive oxygen species in CKD severity. Tau proteins are proteins that stabilize microtubules. Hyperphosphorylation of tau reduces its ability to bind to microtubule causes dystabilization and production of neurofibrillary tangles (NFT) and neurodegeneration in the brain. Aberrant hyperphosphorylation of tau is critical to the progression of neurodegeneration. Erythropoietin (EPO), a glycoprotein has been in clinical use for millions of anemic patients, and some studies show it has a neuroprotective role. Till now studies on the level of tau protein phosphorylation in brain regions of CKD-induced experimental animals and impact of EPO therapy are scarce. The aim of this study is to determine the impact of CKD and EPO therapy on tau protein phosphorylation in brain regions of experimental rats.

Methods: This study was performed on 48 adult male Wistar rats. Two phases were conducted to find out the difference between simultaneous and posttreatment of EPO. Phase I: 24 adult male Wistar rats were divided into 4 groups (6 animals each): Group 1: Control, Group 2: 0.75% of adenine mixed diet for 4 weeks, Group 3: 0.75% of adenine mixed diet was given for 4 weeks and simultaneous administration of EPO (100 IU/kg btw, ip) thrice weekly. Group 4: EPO alone (100 IU/Kg btw, ip) thrice per week. All the animals were sacrificed uniformly at the end of 4 weeks. In Phase II, 24 animals were maintained separately for 40 days experimental period and divided into 4 groups. Groups 1, 2, and 4 animals were treated as same mentioned in Phase I. Group 3: For EPO posttreatment, adenine mixed diet was given for 4 weeks for chronic renal failure (CRF) induction. After the 4th week, EPO (100 IU/Kg btw.) was administered daily once for 12 days. At the end of the 40 days, all the animals were sacrificed uniformly. In both the phases after the treatment period, the brain tissue was removed and samples were homogenized. Total tau protein and phosphorylated tau protein expressions were analyzed by western blotting method.

Results: In results, both the total tau and phosphorylated tau protein levels were significantly increased all the brain regions of CRF-induced groups when compared to control. In both simultaneous and posttreatment of EPO, the levels were retrieved.

Conclusion: This study proves that EPO supplementation has a promising role in neuroprotection by preventing abnormal phosphorylated tau protein accumulation. This study also proves the clinical usefulness of EPO as a supplemental therapeutic agent in neurotoxicity.

Keywords: Chronic renal failure, Cognitive dysfunction, Hyperphosphorylation of tau protein, Erythropoietin.

INTRODUCTION

Chronic kidney disease (CKD) is a major clinical health problem as it is a systemic disorder that causes widespread organ damage and it is related to significant morbidity and mortality [1]. The uremic state of CKD is characterized by the retention of solutes that are toxic in high concentration such as urea, creatinine, parathyroid hormone, myoinositol, and β2 microglobulin [2]. Several studies have postulated that middle molecules (MW 300 - 12,000 KDa) [3] are the toxins that underlie the development of neurological dysfunction in CKD, yet little evidence exist that such substances are actually neurotoxic [4].

Studies have shown that the accumulation of toxic metabolites in renal failure may lead to excessive production of free radicals or depletion of antioxidant capacity [5]. Cognitive impairment has been increasingly recognized in CKD - affecting up to 80% of patients [6]. Cognitive dysfunction increases in prevalence, due to increase in reactive oxygen species (ROS) in CKD severity [7]. In addition to chronic cognitive dysfunction and dementia, acute disturbances of cognition are also prevalent in CKD.

Dementia is a primary neurodegenerative disorder and it leads to a complete psychological and physical dependency and finally to death within one to two decades. It involves aberrant protein processing characterized by the presence of both intraneuronal protein clusters composed of extracellular Aβ protein aggregates (senile plaques) and bundles of intracellular paired helical filaments of abnormal pTau (neurofibrillary tangles [NFT]) [8].

Tau proteins are proteins that stabilize microtubules. Tau is central to the dynamics of microtubule assembly and hence maintains neuronal physiology [9]. Hyperphosphorylation of tau reduces its ability to bind to the microtubule, causes dystabilization, production of NFT and neurodegeneration in the brain [10]. Hyperphosphorylation of tau is a physiologically reversible response of the brain to some stressful conditions such as heat shock, starvation, or ischemia [11]. Tau hyperphosphorylation affects the morphology and biological functions of the neurons. The disorganization of the neuronal skeletal contributes to neuronal malfunction, neuronal cell death and eventually dementia [12]. Although the precise significance of these pathological findings remains elusive, the number of NFT's strongly correlates with the degree of dementia [13]. Aberrant

hyperphosphorylation of tau is, therefore, critical to the progression of neurodegeneration [14].

Erythropoietin (EPO), a glycoprotein was the first characterized as a hematopoietic growth factor and has been in clinical use for millions of patients over the decade for the treatment of anemia [15]. Studies also show EPO indirectly reduces cellular oxidative stress by increasing number of circulating young red blood cells and clinical reports also confirmed that EPO therapy increases the level of antioxidant enzymes in erythrocytes [16]. It acts as a tissue protective cytokine, especially within the nervous tissue, kidney and cardiac muscle, and its receptor is widely distributed in variety of tissues [17]. EPO protects neurons from apoptosis in cell culture studies and in animal models of CNS injury [18]. In mice, EPO improves hippocampus-dependent memory by modulating plasticity, synaptic connectivity and activity of memory-related neuronal networks [19]. Till now studies on EPO treatment in CKD-induced neurotoxicity and the mechanism of tau phosphorylation is scarce. Hence, we intended to do the study on the impact of EPO therapy on CKD-induced changes in tau protein phosphorylation.

METHODS

Chemicals

Adenine and other chemicals were purchased from Sisco Research Laboratory, India. EPO was purchased from Serum Institute of India, Chennai. Primary and secondary antibodies to detect total tau and phosphorylated tau protein by western blotting method were purchased from Cell Signaling Technology Inc., USA. To detect total tau protein, mouse monoclonal (mAb) tau (Tau46) and to detect phosphorylated tau protein, rabbit polyclonal phospho-tau (ser202) were used. Anti-rabbit and anti-mouse HRP conjugated secondary antibodies were used to detect both of these antibodies. For control, rabbit monoclonal (mAb) GAPDH was also purchased from Cell signaling technology Inc., USA.

Experimental design

This study was performed on 48 adult male Wistar rats with 120-150 g in weight. After 10 days of acclimatization, the animals were randomly assigned to either the experimental groups or control group. Animals were housed in the Central Animal House of SRM Medical College Hospital. Institutional ethical committee approval was obtained for this study. Each 3 animal has given individual labeled cages, and the animals were maintained under standard laboratory conditions of 12 hrs dark/light cycle, 20-22°C temp. Adenine is mixed with the feed at a conc. of 0.75%, w/w, for 4 weeks to induce chronic renal failure (CRF). Two phases were conducted to find out the difference between simultaneous and posttreatment of EPO in CRF-induced changes in protein expression in brain regions of experimental animals. The dose of adenine (0.75%) mixed diet for CRF induction was selected according to Ali *et al*. (2013) [20]. Dose and treatment procedure for EPO was selected according to Bagnis *et al*. (2001) [21] and Lee *et al*. (2009) [22].

Phase I: A total of 24 male Wistar rats were used in this phase, and the animals were divided into 4 groups (6 animals each): Group I: Control animals without treatment, Group II: Animals which was given adenine 0.75% in feed for 4 weeks (28 days), Group III: Animals were treated by adenine 0.75% mixed diet for 4 weeks and simultaneous administration of EPO (100 IU per kg body weight) thrice weekly, in that period. Group IV: Epo alone has given (100 IU/kg btw) thrice per week for 4 weeks. All the animals in this phase were sacrificed after 4 weeks.

Phase II: A total of 24 male Wistar rats were used in this phase, and the animals were divided into 4 groups (6 animals each): Group I: Control animals without treatment, Group II: Animals which was given adenine 0.75% in feed for 4 weeks, Group III: In EPO posttreatment group, animals were treated by adenine 0.75% mixed diet for 4 weeks for CRF induction. After the 4th week, EPO (100 IU/kg btw.) was administered for the next 12 days, daily once. Group IV: EPO alone has given (100 IU/kg btw) thrice per week for 4 weeks. All the animals in this phase were sacrificed after 40 days.

Tissue collection and preparation

About 24 hrs after last treatment, the animals were sacrificed and brain was immediately removed and washed in ice-cold physiological saline repeatedly, and brain was dissected over ice-cold glass slides to the following regions: Cerebral cortex, cerebellum, and hippocampus [23]. Regions from each of the brain tissue were blotted, weighed accurately. The samples were homogenized using a Potter-Elvehjem homogenizer to produce 10% homogenates.

Western blotting

Total tau protein and phosphorylated tau protein expressions were analyzed by western blot. Tissues were homogenized with three volumes of lysis buffer containing 10 mmol/L HEPES, 1 mmol/L ethylenediaminetetraacetic acid, 100 mmol/L KCl, 1% triton X-100, pH 7.5 and protease inhibitors cocktail (1: 100), and the homogenates were centrifuged at 600 g for 10 minutes. The supernatants were further centrifuged at 45 000 g for 30 minutes at 4°C and stored at −80°C until use. The protein concentration of the tissue homogenates was determined by the standard method of Lowry *et al.* 1951. About 50 µg of total protein was mixed with 6X sample buffer and boiled for 5 minutes. The sample mixture was run on 10% sodium dodecyl sulfate - polyacrylamide gel electrophoresis gel in 1X running gel buffer at 100 V for 1 hr. and then transferred to polyvinylidene difluoride membrane. Then, the membrane was blocked in blocking buffer containing 5% skimmed milk powder for overnight. After overnight, the blocked membranes were incubated with the specific primary antibodies for detection of total tau (mouse monoclonal, dilution 1:1000) and phosphorylated tau (rabbit polyclonal phospho-tau ser202, dilution 1:1000). Phospho-tau (ser 202) antibody recognizes the endogenous level of tau protein only when phosphorylated at Ser 202. Rabbit monoclonal GAPDH (dilution 1:1000) was used as an internal control. Suitable secondary antibodies were added to detect each protein expression and incubated for 1 hr. The following two intermittent washes with 1X TTBS and TBS, membranes were developed using ECL and Chemi Doc Imaging System to detect signal. Then, the quantification of the band was done using image J software.

Statistical analysis

The statistical analysis of the results was conducted using SPSS version 21, one-way analysis of variance and the independent t-test followed by Turkey's multiple comparison tests. The $p \leq 0.05$ is considered as statistically significant. Results were expressed as a mean ± standard error of the mean.

RESULTS

Figs. 1 and 2 show the effect of EPO supplementation on CRF-induced changes in pTau and total tau protein expressions in the cerebral cortex. Both pTau and total tau protein levels were significantly increased in CRF-induced groups when compared to control. The level was significantly restored only after simultaneous treatment of EPO but restoration is not significant in posttreatment of EPO.

Figs. 3 and 4 show the effect of EPO supplementation on CRF-induced changes in pTau and total tau protein expressions in the cerebellum. Here, both the tau protein levels were increased in CRF-induced groups significantly when compared to control and it was significantly restored in both the simultaneous and post EPO supplementation. Figs. 5 and 6 show the impact of EPO supplementation on CRF-induced changes in pTau and total tau levels in the hippocampus. The same trend was observed significantly in this region as in cerebellum.

DISCUSSION

CKD has a prevalence of 15% in developed nations [24]. Chronic renal failure in humans has been shown to cause several alterations in

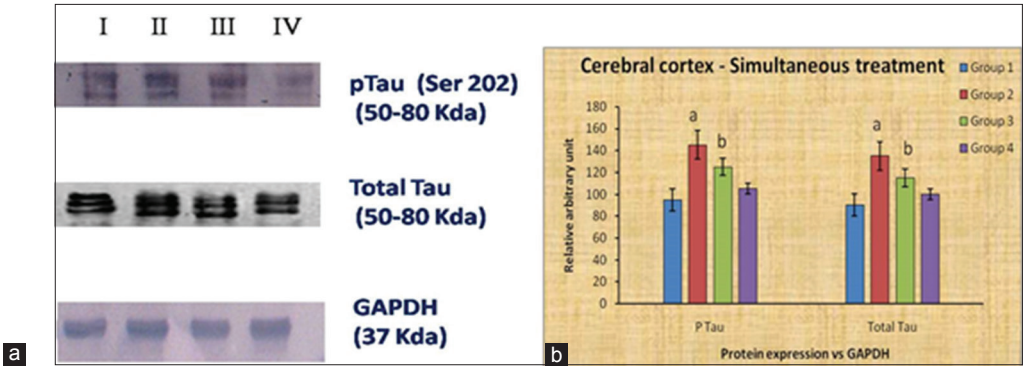

Fig. 1: Simultaneous treatment of erythropoietin (EPO) in cerebral cortex. (a) Protein levels of pTau and total tau in rat cerebral cortex during chronic renal failure (CRF) and simultaneous treatment of EPO. The protein levels were measured by western blot assay. (b) Graphical representation of quantification of pTau and total tau in rat cerebral cortex of CRF and treatment groups. Results are expressed as mean ± standard error of the mean of 3 observation in each group; (a) p<0.001, (b) p<0.01, (c) p<0.05. Statistical significance compared between Groups 2 and 4 versus Group 1, Group 3 versus Group 2, respectively

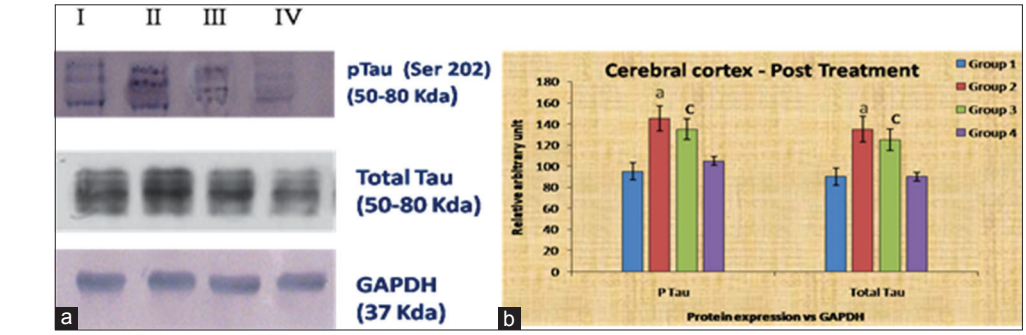

Fig. 2: Posttreatment of erythropoietin (EPO) in cerebral cortex. (a) Protein levels of pTau and total tau in rat cerebral cortex during chronic renal failure (CRF) and posttreatment of EPO. The protein levels were measured by western blot assay. (b) Graphical representation of quantification of pTau and total tau in rat cerebral cortex of CRF and treatment groups. Results are expressed as mean ± standard error of the mean of 3 observation in each group; (a) p<0.001, (b) p<0.01, (c) p<0.05. Statistical significance compared between Groups 2 and 4 versus Group 1, Group 3 versus Group 2, respectively

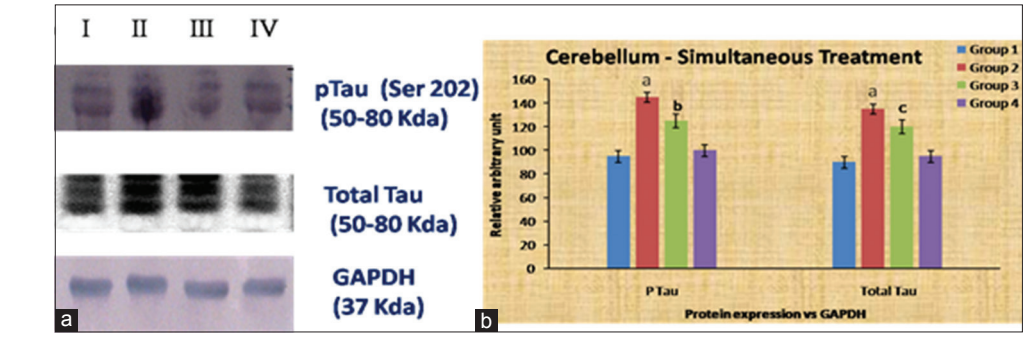

Fig. 3: Simultaneous treatment of erythropoietin (EPO) in cerebellum. (a) Protein levels of pTau and total tau in rat cerebellum during chronic renal failure (CRF) and simultaneous treatment of EPO. The protein levels were measured by western blot assay. (b) Graphical representation of quantification of pTau and total tau in rat cerebellum of CRF and treatment groups. Results are expressed as mean ± standard error of the mean of 3 observation in each group; (a) p<0.001, (b) p<0.01, (c) p<0.05. Statistical significance compared between Groups 2 and 4 versus Group 1, Group 3 versus Group 2, respectively

behavior [25]. Accumulation of toxic metabolites in renal failure leads to excessive production of free radicals [26], and the brain is highly sensitive to oxidative stress.

Tau protein belongs to a group of proteins referred to as microtubule-associated proteins that in common are heat resistant and limitedly affected by acid treatment without loss of their function [27]. Tau protein promotes tubulin assembly into microtubules, one of the major components of the neuronal cytoskeleton that defines the normal morphology and provides structural support to the neurons [28]. Alterations in the amount or the structure of tau protein can affect stabilization of microtubules and other processes related to this protein [29,30]. A normal level of phosphorylation is required for the optimal function of tau, whereas the hyperphosphorylated state makes tau to lose its biological activity [31]. In pathological conditions, not only does abnormal phosphorylation of tau protein decrease its tubulin binding capacity leading to microtubule disorganization but also this protein self-polymerize and aggregates in the form of NFTs [32,33]. In this study, both the total tau protein and phosphorylated tau (at ser 202 site) were increased in chronic

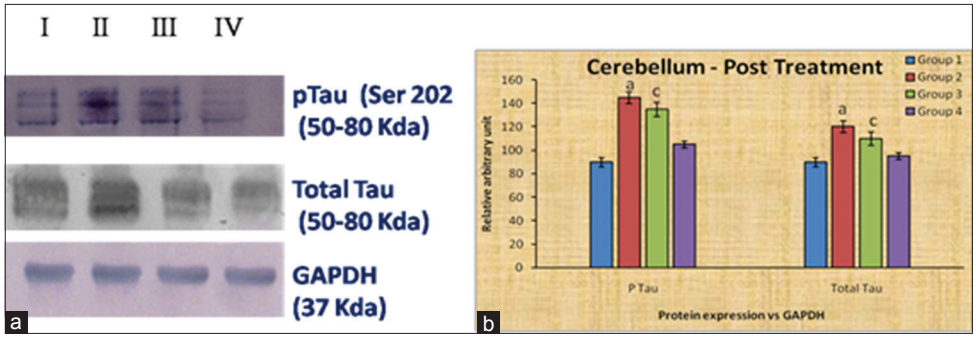

Fig. 4: Posttreatment of erythropoietin (EPO) in cerebellum. (a) Protein levels of pTau and total tau in rat cerebellum during chronic renal failure (CRF) and posttreatment of EPO. The protein levels were measured by western blot assay. (b) Graphical representation of quantification of pTau and total tau in rat cerebellum of CRF and treatment groups. Results are expressed as mean ± standard error of the mean of 3 observation in each group; (a) p<0.001, (b) p<0.01, (c) p<0.05. Statistical significance compared between Groups 2 and 4 versus Group 1, Group 3 versus Group 2, respectively

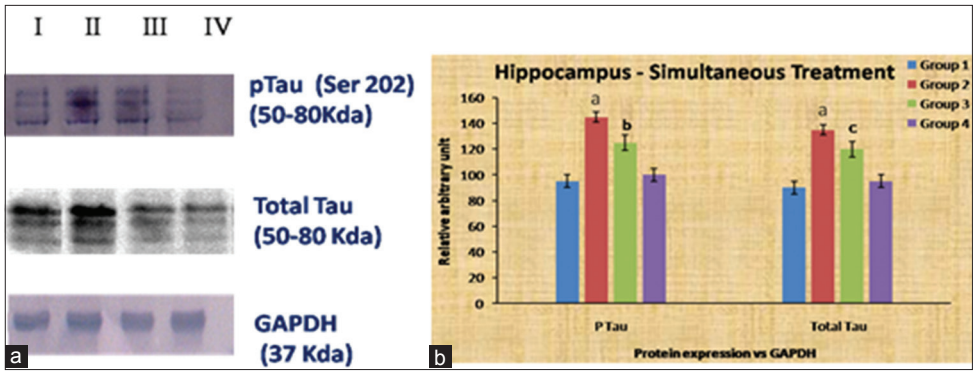

Fig. 5: Simultaneous treatment of erythropoietin (EPO) in hippocampus. (a) Protein levels of pTau and total tau in rat hippocampus during chronic renal failure (CRF) and simultaneous treatment of EPO. The protein levels were measured by western blot assay. (b) Graphical representation of quantification of pTau and total tau in rat cerebellum of CRF and treatment groups. Results are expressed as mean ± standard error of the mean of 3 observation in each group; (a) p<0.001, (b) p<0.01, (c) p<0.05. Statistical significance compared between Groups 2 and 4 versus Group 1, Group 3 versus Group 2, respectively

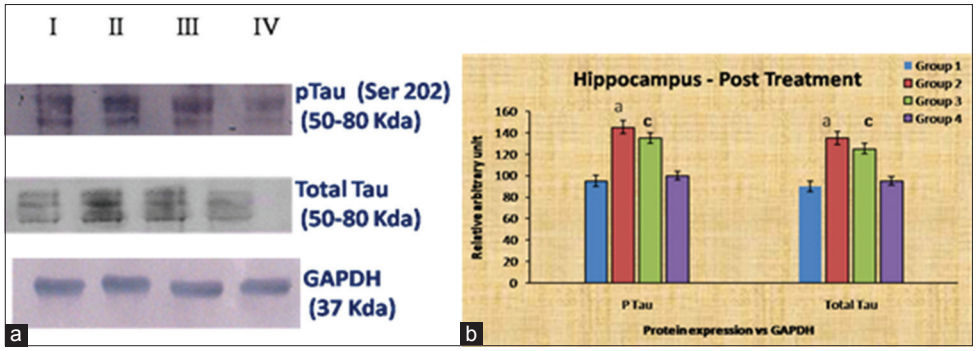

Fig. 6: Posttreatment of erythropoietin (EPO) in hippocampus. (a) Protein levels of pTau and total tau in rat hippocampus during chronic renal failure (CRF) and posttreatment of EPO. The protein levels were measured by western blot assay. (b) Graphical representation of quantification of pTau and total tau in rat cerebellum of CRF and treatment groups. Results are expressed as mean ± standard error of the mean of 3 observation in each group; (a) p<0.001, (b) p<0.01, (c) p<0.05. Statistical significance compared between Groups 2 and 4 versus Group 1, Group 3 versus Group 2, respectively

renal failure induced experimental animals when compared to control.

Maintenance of energy homeostasis in the brain requires a distinct molecular circuitry which provides tight coupling between energy consumption and production during the performance of sensory, motor and cognitive tasks [34]. Creatine kinase (CK), a crucial enzyme is highly sensitive to free radicals [35]. In our previous study, increased serum CK level and its decreased activity in brain regions such as cerebral cortex, cerebellum, and hippocampus of chronic renal failure induced animals were observed [36]. It is also known that a decrease in CK activity is associated with neurodegenerative pathways that result in neuronal death in brain ischemia, neurodegenerative diseases bipolar disorder, and other pathological states [37]. In this study, the increased levels of both total and phosphorylated tau in brain regions of CRF-induced rats (Figs. 1-6) may be due to decreased CK activity in the same.

The clinical relevance of the use of EPO as a neuroprotective agent was enhanced when it was found to cross the blood - brain barrier after peripheral administration [15]. EPO was first characterized as a hematopoietic growth factor and has been in clinical use for millions of patients over the decade for the treatment of anemia [38]. Epo exerts a remarkable neuroprotection in both cell cultures and in animal models [39,40]. Epo may indirectly reduce cellular oxidative stress by increasing number of circulating young red blood cells, clinical reports have confirmed that Epo therapy could increase the level of erythrocyte antioxidative enzymes [19]. Several studies have indicated that it may protect neurons from glutamate toxicity by activating calcium channels and limiting the production of tissue-injuring molecules such as ROS, leading to the increased activity of antioxidant enzymes in neurons [41].

In our study, we found that there is a significant change in the hyperphosphorylated, and total tau proteins were observed in selected brain regions of CRF-induced experimental animals after the supplementation of both simultaneous and posttreatment of EPO. In our previous study, the decreased CK activity in brain regions of CRF-induced animals was significantly retrieved after the supplementation of simultaneous and posttreatment of EPO. This study proves that EPO promotes neuroprotection by preventing the abnormal phosphorylated tau protein accumulation through the activation of CK system in CRF-induced experimental rats.

CONCLUSION

This study proves the clinical usefulness of EPO as a supplemental therapeutic agent in neurotoxicity. It also proves that EPO supplementation has a promising role in preventing neurotoxicity through the abnormal protein accumulation in CKD-induced animals.

REFERENCES

1. Ikeda R, Imai Y, Maruyama W, Mizoguchi K. Systemic disorders of calcium dynamics in rats with adenine induced renal failure: Implication for chronic ksidney disease related complications. Nephrology (Carlton) 2010;15(1):54-62.
2. Meyer TW, Hostetter TH. Uremia. N Engl J Med 2007;357(13):1316-25.
3. Babb AL, Ahmad S, Bergström J, Scribner BH. The middle molecule hypothesis in perspective. Am J Kidney Dis 1981;1(1):46-50.
4. Vanholder R, De Smet R, Hsu C, Vogeleere P, Ringoir S. Uremic toxicity: The middle molecule hypothesis revisited. Semin Nephrol 1994;14(3):205-18.
5. Sener G, Sakarcan A, Sehirli O, Ekshioglu-Demiralp E, Sener E, ErcanF, *et al*. Chronic renal failure – induced multiple – organ injury in rats is alleviated by the selective CysLT1 receptor antagonist montelukast. Prostaglandins Other Lipid Mediat 2007;83(4):257-67.
6. Murray AM. Cognitive impairment in the aging dialysis and chronic kidney disease populations: An occult burden. Adv Chronic Kidney Dis 2008;15(2):123-32.
7. Hailpern SM, Melamed ML, Cohen HW, Hostetter TH. Moderate chronic kidney disease and cognitive function in adults 20 to 59 years of age: Third National Health and Nutrition Examination Survey (NHANES III). J Am Soc Nephrol 2007;18(7):2205-13.
8. Butterfield DA, Reed T, Newman SF, Sultana R. Roles of amyloid β-peptide-associated oxidative stress and brain protein modifications in the pathogenesis of Alzheimer's disease and mild cognitive impairment. Free Radic Biol Med 2007;43(5):658-77.
9. Alonso AC, Grundke-Iqbal I, Barra HS, Iqbal K. Abnormal hyper phosphorylation of tau and the mechanism of Alzheimer neurofibrillary degeneration: Sequestration of microtubule-associated proteins 1 and 2 and the disassembly of microtubules by the abnormal tau. Proc Natl Acad Sci USA 1997;94(1):298-303.
10. Noble W, Pooler AM, Hanger DP. Advances in tau-based drug discovery. Expert Opin Drug Discov 2011;6(8):797-810.
11. Castro-Alvarez JF, Gutierrez-Vargas J, Darnaudéry M, Cardona-Gómez GP. ROCK inhibition prevents tau hyperphosphorylation and p25/CDK5 increase after global cerebral ischemia. Behav Neurosci 2011;125(3):465-72.
12. Taylor JP, Hardy J, Fischbeck KH. Toxic proteins in neurodegenerative disease. Science 2002;296(5575):1991-15.
13. Gomez-Isla T, Hollister R, West H, Mui S, Growdon JH, Petersen RC, *et al*. Neuronal loss correlates with but exceeds neurofibrillary tangles in Alzeimer's disease. Eur J Biochem 1997;244:414-25.
14. Yang Y, Zhang J, Ma D, Zhang M, Hu S, Shao S, *et al.* Subcutaneous administration of liraglutide ameliorates Alzheimer-associated tau hyperphosphorylation in rats with type 2 diabetes. J Alzheimers Dis 2013;37(3):637-48.
15. Sirén AL, Ehrenreich H. Erythropoietin – A novel concept for neuroprotection. Eur Arch Psychiatry Clin Neurosci 2001;251(4):179-84.
16. Mimic-Oka J, Simic T, Djukanovic L. Epoetin treatment improves red blood cell and plasma antioxidant capacity in hemodialysis patients. Ren Fail 2002;24(1):77-87.
17. Brines M, Cerami A. Discovering erythropoietin's extra-hematopoietic functions: Biology and clinical promise. Kidney Int 2006;70(2):246-50.
18. Digicaylioglu M, Lipton SA. Erythropoietin-mediated neuroprotection involves cross-talk between Jak2 and NF-κB signalling cascades. Nature 2001;412(6847):641-7.
19. Adamcio B, Sargin D, Stradomska A, Medrihan L, Gertler C, Theis F, *et al.* Erythropoietin enhances hippocampal long-term potentiation and memory. BMC Biol 2008;6:37.
20. Ali BH, Al-Salam S, Al Za'abi M, Waly MI, Ramkumar A, Beegam S, *et al.* New model for adenine-induced chronic renal failure in mice, and the effect of gum acacia treatment thereon: Comparison with rats. J Pharmacol Toxicol Methods 2013;68(3):384-93.
21. Bagnis C, Beaufils H, Jacquiaud C, Adabra Y, Jouanneau C, Le Nahour G, *et al.* Erythropoietin enhances recovery after cisplatin-induced acute renal failure in the rat. Nephrol Dial Transplant 2001;16(5):932-8.
22. Lee DW, Kwak IS, Lee SB, Song SH, Seong EY, Yang BY, *et al.* Post-treatment effects of erythropoietin and nordihydroguaiaretic acid on recovery from cisplatin-induced acute renal failure in the rat. J Korean Med Sci 2009;24 Suppl: S170-5.
23. Glowinski J, Iversen LL, Axelrod J. Storage and synthesis of norepinephrine in the reserpine-treated rat brain. J Pharmacol Exp Ther 1966;151(3):385-99.
24. Krishnan AV, Kiernan MC. Neurological complications of chronic kidney disease. Nat Rev Neurol 2009;5(10):542-51.
25. Chilcot J, Wellsted D, Vilar E, Farrington K. An association between residual renal function and depression symptoms in haemodialysis patients. Nephron Clin Pract 2009;113(2):c117-24.
26. Kliem V, Johnson RJ, Alpers CE, Yoshimura A, Couser WG, Koch KM, *et al.* Mechanisms involved in the pathogenesis of tubulointerstitial fibrosis in 5/6-nephrectomized rats. Kidney Int 1996;49(3):666-78.
27. Cleveland DW, Hwo SY, Kirschner MW. Physical and chemical properties of purified tau factor and the role of tau in microtubule assembly. J Mol Biol 1977;116(2):227-47.
28. Kosik KS. The molecular and cellular biology of tau. Brain Pathol 1993;3(1):39-43.
29. Mandelkow EM, Stamer K, Vogel R, Thies E, Mandelkow E. Clogging of axons by tau, inhibition of axonal traffic and starvation of synapses. Neurobiol Aging 2003;24(8):1079-85.
30. LaPointe NE, Morfini G, Pigino G, Gaisina IN, Kozikowski AP, Binder LI, *et al*. The amino terminus of tau inhibits kinesin-dependent axonal transport: Implications for filament toxicity. J Neurosci Res 2009;87(2):440-51.
31. Sergeant N, Delacourte A, Buée L. Tau protein as a differential biomarker of tauopathies. Biochim Biophys Acta 2005;1739(2-3):179-97.
32. Avila J. Tau kinases and phosphatases: Commentary. J Cell Mol Med 2008;12(1):258-9.
33. Iqbal K, Grundke-Iqbal I. Alzheimer neurofibrillary degeneration: Significance, etiopathogenesis, therapeutics and prevention: Alzheimer review series. J Cell Mol Med 2009;12(1):38-55.
34. Barinaga M. What makes brain neurons run? Science 1997;276(5310):196-8.
35. Sangkabutra T, Crankshaw DP, Schneider C, Fraser SF, Sostaric S, Mason K, *et al.* Impaired K+ regulation contributes to exercise limitation in end-stage renal failure. Kidney Int 2003;63(1):283-90.
36. Karthick N, Alwin D, Poornima KN, Chitra V, Saravanan A, Balakrishnan D, *et al*. Neurobehavioral alterations and brain creatine kinase system changes in chronic renal failure induced male wistar rats: Impact of erythropoietin supplementation. J Bioequiv Availab 2015;7(2):074-81.
37. Aksenov M, Aksenova M, Butterfield DA, Markesbery WR. Oxidative modification of creatine kinase BB in Alzheimer's disease brain. J Neurochem 2000;74(6):2520-7.

38. Brines ML, Ghezzi P, Keenan S, Agnello D, de Lanerolle NC, Cerami C, *et al.* Erythropoietin crosses the blood-brain barrier to protect against experimental brain injury. Proc Natl Acad Sci USA 2000;97(19):10526-31.
39. Zhang Y, Xiong Y, Mahmood A, Meng Y, Qu C, Schallert T, *et al.* Therapeutic effects of erythropoietin on histological and functional outcomes following traumatic brain injury in rats are independent of hematocrit. Brain Res 2009;1294:153-64.
40. Mogensen J, Miskowiak K, Sørensen TA, Lind CT, Olsen NV, Springborg JB, *et al.* Erythropoietin improves place learning in fimbria-fornix-transected rats and modifies the search pattern of normal rats. Pharmacol Biochem Behav 2004;77(2):381-90.
41. Ozturk E, Demirbilek S, Kadir But A, Saricicek V, Gulec M, Akyol O, *et al.* Antioxidant properties of propofol and erythropoietin after closed head injury in rats. Prog Neuropsychopharmacol Biol Psychiatry 2005;29(6):922-7.