Title: Erythropoietin pretreatment effect on blood glucose and its relationship with interleukin -1α & 6 after brain ischemic-reperfusion injury in male wistar rats

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To appear in: Basic and Clinical Neuroscience

Received date: 2017/08/31

Revised date: 2018/04/5

Accepted date: 2018/04/30

Please cite this article as:

Gholamzadeh, R. Mostafavi, H. Eskandari, M. Bigdeli, M. (In Press). Erythropoietin pretreatment effect on blood glucose and its relationship with interleukin -1α & 6 after brain ischemic-reperfusion injury in male wistar rats. *Basic and Clinical Neuroscience*. Just Accepted publication April. 30, 2018.

Background: Brain ischemic reperfusion injury (IRI) caused the activation of different pathophysiological processes and changes of physiological conditions such as blood sugar (BS). An increase in BS after stroke is associated with poor clinical outcomes. Erythropoietin has been shown to be effective on both reduce inflammation and BS. Therefore, in this study the erythropoietin pre-treatment effect on BS and its relationship to inflammatory markers after brain IRI was targeted.

Methods: Thirty adult male Wistar rats randomly divided into 5 groups: sham, control and 3 pretreatment groups: single-dose, double dose and triple-time dose that received 1000 U/Kg of Erythropoietin before stroke induction in different times intraperitoneally. A rat model of IRI was established by middle cerebral artery occlusion (MCAO) for 60 minutes. Infarct volume, neurological defects, IL-1α and IL-6 serum levels was evaluated 24 hours after reperfusion. Also BS was measured in 1, 6 & 24 hours later .

Results: single dose of erythropoietin significantly decreased infarct volume and improved neurological defects which was associated with decreased serum level of IL-1α and IL-6 but higher doses of erythropoietin administration had adverse effects on histological, neurological and inflammatory results. Also, erythropoietin significantly increased BS in a dose depended manner.

Conclusion: Erythropoietin could reduce brain IRI by reducing inflammation and BS stabilization . The results of the present study demonstrated a relationship between inflammatory factors and hyperglycemia after IRI and investigated that erythropoietin may be useful for preventing brain IRI, but its higher doses should be used with caution due to possible side effects.

Keyword: Erythropoietin, Pretreatment, Blood glucose, Interleukin -1α & 6, Brain ischemia

Introduction

Ischemic stroke is the main cause of death in the world that imposes high costs on individuals, families and communities (Yuen et al., 2011). Different conditions such as heart bypass surgery, transplantations, sickle cell anemia and heart attack are threatening to cerebral ischemia (M. R. Bigdeli & Mohagheghi, 2014). Despite enormous advances in medicine, but there is still no safe and effective treatment for stroke (Yip et al., 2011). The only effective treatment is now thrombolytic which has golden time and limitations for use (Yuen et al., 2011). Recently, research strategies have been concentrated on the preconditioning mechanisms, such as pharmacological preconditioning which is used to increase tolerance to ischemia (Gholamzadeh, Eskandari, Mostafavi, & Bigdeli, 2016) Cerebral ischemia leads to the activation of inflammatory processes, metabolic and electrophysiological disorders (M. R. Bigdeli & Mohagheghi, 2014; Shah). Reperfusion after ischemic injury is associated with greater harm named ischemic-reperfusion injury (IRI) (M. R. Bigdeli & Mohagheghi, 2014; Paschos, Lykissas, & Beris, 2008; S.-K. Wu et al., 2014). In addition, body physiological conditions change after cerebral ischemia such as blood oxygen saturation, blood sugar, temperature and blood pressure (Radermecker & Scheen, 2010). Hyperglycemia is common in the early phase of stroke and has been observed in two-thirds of all types of ischemic stroke (Lindsberg & Roine, 2004). Studies have shown that it is associated with worse outcomes and enhancement in infarct volume and higher mortality risk after stroke (Bhalla, Wolfe, & Rudd, 2001; Lindsberg & Roine, 2004; Mehta, 2003). Oxidative metabolism of glucose is perturbed following ischemia that causes lactic acidosis and vasogenic edema (Bhalla et al., 2001). Hyperglycemia leads to an increased production of lactic acid and develops neuronal damage (Bhalla et al., 2001; Mehta, 2003). Also, both hyperglycemia and hypoglycemia lead to an increased production of free radicals and inflammatory factors such as IL-1 and IL-6 (Shukla, Shakya, Perez-Pinzon, & Dave, 2017). A neuroendocrine stress and inflammatory response may contribute to the increased blood sugar after a stroke. Thus, maintaining physiological homeostasis and reduction of inflammation after stroke has an important role in healing and mortality reduction (Wong & Read, 2008). Erythropoietin is a glycoprotein hormone. It is secreted from liver in fetal and kidney in adults (Chen et al., 2015; S.-K. Wu et al., 2014). Erythropoietin is used first time to treat anemia (Yao et al., 2016). Recently, protective effect of erythropoietin preconditioning on reducing the IRI and inflammatory responses has been investigated on the brain (Yu, Fan, Sun, Yao, & Chai,

2016), heart (Rong & Xijun, 2015), kidneys (Elshiekh, Kadkhodaee, Seifi, Ranjbaran, & Ahghari, 2015; Liao, Li, Wang, & Xie, 2016), intestines (Kai-Lan & Si, 2015), liver (Liu et al., 2015) and lung (H. Wu et al., 2006). Erythropoietin could reduce infarct size and improve neurological deficits (Yuen et al., 2011). As well as several studies have determined effects of erythropoietin on blood sugar reduction and an increased glucose tolerance in diabetic rats (Chen et al., 2015; Meng, Zhu, Bi, Yang, & Wang, 2013; Niu, Chang, Niu, Cheng, & Lee, 2016). Already, no study has been conducted on the erythropoietin pretreatment effects on blood glucose in non-diabetic patients after stroke and its relationship with inflammatory factors and outcomes has not been investigated. Therefore in the present study, we investigated if erythropoietin can improve IRI by reducing blood sugar and inflammatory factors.

Material and Methodes

Experimental groups

In this study, according to diagram Fig.1 30 adult male Wistar rats (200 to 300 g) were maintained at standard conditions (with 12 h light/dark cycle and controlled temperature in the range of 24±2 °C) and then randomly divided into 5 groups (6 rat in each group): sham (receiving surgical stress), control (MCAO model receiving salin 0/9%) and 3 pretreatment groups: single-dose pretreatment group (0.5 hours before the stroke induction), double dose (0.5 and 48 hours before), triple-time dose (0.5, 48 and 96 hours before) that received 1000 U/Kg of Recombinant Human Erythropoietin-alfa (ampoule Eprex 4000 unit, code:1228048326 Prepared of POUYESH drug company) intraperitoneally before the stroke induction (Fig .1) . The animals were anesthetized 0.5 hours after the last injection by chloral hydrate (Merck, Germany, Code 102425). Brain ischemia was induced by MCAO model for 60 minutes. Then the animals were evaluated in terms of stroke volume, blood sugar, inflammatory factors (IL1-α & IL6) and motor neurological defects at 24 hours after the stroke induction.

Fig. 1: Schematic of the experimental groups: stroke induced by middle central artery occlusion model and blood flow established after 1 hour. Tissue and blood parameters assessed 24 hours after reperfusion.

To close the middle cerebral artery (MCA) and the induction of ischemia, the weighed animals were anesthetized with chloral hydrate (400 mg / kg) and were placed in the supine position on the surgical pad. Under cardiac monitoring, MCAO surgery model was performed in accordance with Longa and his colleague's method. Briefly, the surgical site was sterilized with 70% alcohol. A linear incision with a length of 2 cm was created on the right side of the neck along the spine of the animal. Under microscopic surgery by pushing the salivary tissue and esternohid muscle, common carotid artery (CCA) was appeared and separated from the vagus nerve. Then a 3-0 silicone coated nylon suture with a round tip was entered to the external carotid artery (ECA) and was pushed through internal carotid artery (ICA) to reach to the middle cerebral artery. A slight resistance against suture passing after 20-22 mm of suture length indicated that it is placed in the correct location. After 60 minutes, blood flow is restored. During the surgery, rectal body temperature was measured and maintained about 37 ° C (M. Bigdeli, 2008; Chiang, Messing, & Chou, 2011).

Measurement of stroke volume

For measuring infarct volume, 24 hours after induction of ischemia, animals sacrificed by chloral hydrate (800 mg/kg) and decapitated. Then their brain was removed immediately and placed in cool saline (4 ° C) for 10 min. Seven brain coronal sections were cut in thicknesses of 2 mm according to a rat brain matrix from frontal to temporal. Tissue sections were immersed in

Triphenyltetrazolium chloride (sigma, USA, cod: 1.38380) solution and incubated in an incubator at 37 ° C for 10 minutes. Then images were taken from sections using a digital camera and after transferring to the PC were analyzed by means of the image J software. Stroke volume as shown in Fig.2 in areas including core, penumbra and sub-cortex was calculated according to the following formula (M. Bigdeli, 2008; Yuen et al., 2011).

$$\text{brain infarct volume} = \frac{\text{left hemisphere volume-(right hemisphere volume- infarct volume)}}{\text{left hemisphere volume}}$$

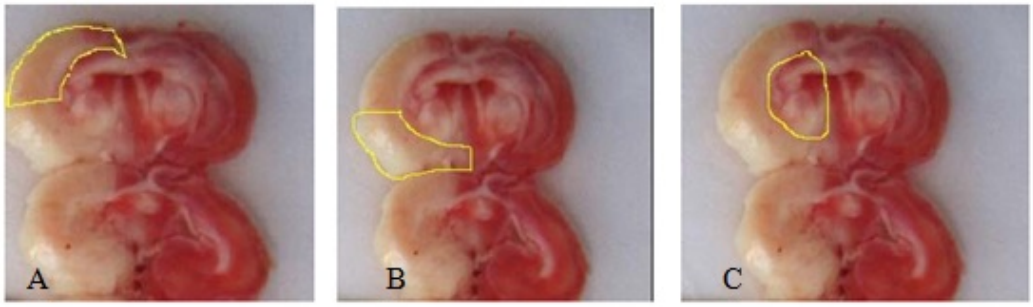


Fig. 2: separating of penumbra (A), core (B) and subcortex (c) with Image J software.

Blood sugar measurement

The level of blood glucoses were controlled at 1, 6 and 24 hours after the stroke induction by using a glucometer (ACCU-CHEK, performa) through the animal tail vein (Chen et al., 2015).

Inflammatory factors of IL-1α and IL-6

2 cc of blood samples were taken from the rats to measure IL-1α and IL-6, 24 hours after induction of stroke. Blood samples were transferred to sterile tubes and centrifuged with 4000 rpm, at 4 ° C. serum samples were stocked in micro-tubes in the refrigerator at 70° C. Serum levels of IL-1α and IL-6 were measured by ELISA method according to Diaclone France protocol kit (Gul, Yasim, & Aral, 2009).

Assessment of motor neurological defects

Animals' neurological defects were evaluated by standard Bederson criteria at 24 hours after the induction of ischemia and then the animals' neurological status were scored.

Score (0): any neurologic deficit was not observed.

Score (1): complete failure at the end of the front paws (mild focal neurological deficit).

Score (2): turning to the left (moderate focal neurological deficits).

Score (3): falling to the left (severe focal deficits).

Score (4): animals could not walk spontaneously and had a low level of consciousness.

Score (5): death (M. Bigdeli, 2008).

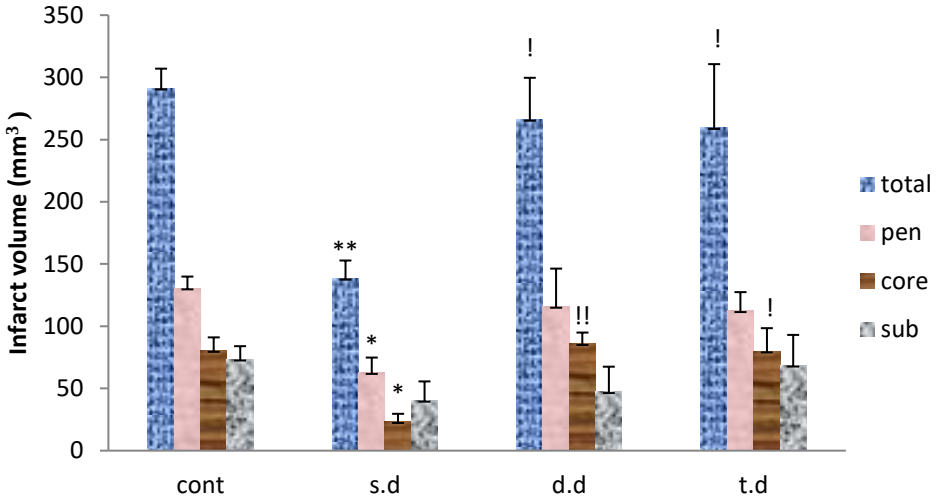
Statistical analysis

The results were analyzed by spss software (version 16) with one-way analysis of variances (ANOVA) and LSD backup test. Also neurological defects analyzed with nonparametric test of Maan-Whitney U. All results are expressed as mean ±SEM and P values <0.05 was considered statistically significant.

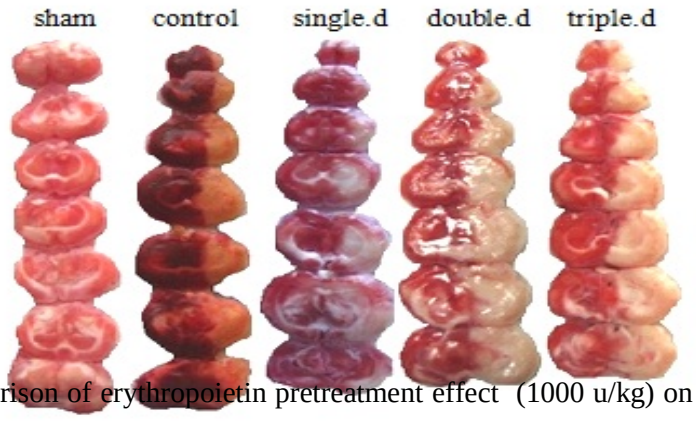
Results

stroke volume and Motor neurological defects

A single dose of erythropoietin pretreatment significantly reduced stroke volume of total, core and penumbra area. Also it improved neurological deficits score compared with control group ($P<0.05$). Double and triple doses of erythropoietin pretreatment registered for the groups had no effect on stroke volume and neurological deficit scores. Other than, significant differences were observed between pretreatment groups in infarct volume and neurologica deficits ($p<0.05$). (Fig. 3 and Table 1)



(A)



(B)

Figure 3: Comparison of erythropoietin pretreatment effect (1000 u/kg) on tissue volume damage in the treatment groups: single dose (s.d) , double dose (d.d), triple dose (t.d) with control group in penumbra, core, subcortex and total volume. A: $p<0.05$، $^{*}P<0.01^{**}$ significant differences with control. $P<0.05$، $^{!}P<0.007^{!!}$ significant differences with single dose. B: sections of brain tissue in different groups were stained with the characteristics of red in safe areas and white in the affected areas.

Table 1: Compare neurological deficits between the control and experimental groups with bederson criteria

Groups	Median	Mean	Neurological deficits' grading						Total	Significant differences
			0	1	2	3	4	5		
1. Cont	4	4/066	0	0	2	3	2	8	15	
2. s.d	3	2/866	1	1	5	2	3	3	15	$p<0.05^{*}$
3. d.d	4	4/266	0	0	2	0	5	8	15	$p<0.05^{\#}$
4. t.d	4	3/666	0	1	3	2	3	6	15	

s.d: single dose, d.d: double dose, t.d: triple dose. $p<0.05$ * the symbol is used to show the significant difference with control group. $p<0.05$ $^{\#}$ is used to show the significant difference with double-dose group.

Blood sugar

The control group blood glucose investigated at 1, 6 and 24 hours after brain ischemia had no significant difference compared to the sham group. Erythropoietin pretreatment increased blood glucose after stroke that this enhancement in double and triple-dose erythropoietin pretreatment groups was significant compared to control and single dose groups; especially in the first and sixth hours after the stroke ($p<0.05$). Also all experimental groups showed a reduction in blood glucose within 24 hours after stroke.

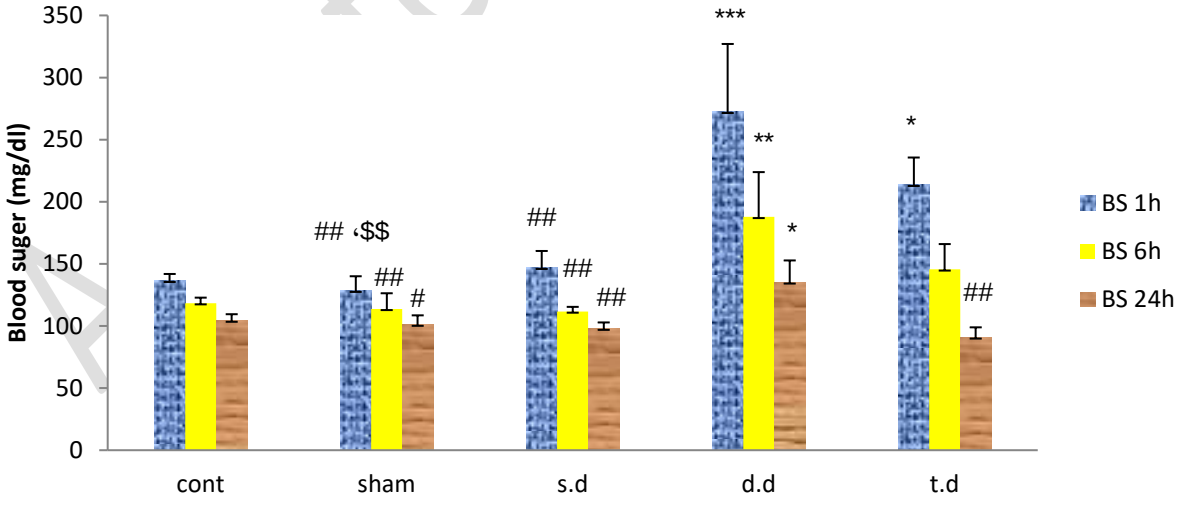


Figure 2: Comparison of blood sugar at 1, 6 and 24 hours after stroke induction in sham, control and experimental groups (sd: single dose, d.d: double dose, t.d: triple dose). $p<0.05$ * $p=0.003$ ** $p<0.000$ ***

significant difference with control. $p<0.05$ [#] significant difference with double dose, $p<0.01$ [$$،##] significant difference with double dose (#) and triple dose ($).

Inflammatory factors IL-1α and IL-6

Serum inflammatory factors interleukin 1-α and 6 were increased after stroke in all groups compared to the sham group that this raise was significant about IL-1α. A single dose of erythropoietin pretreatment reduced serum levels of IL-1α and IL-6 compared to control group, but this reduction was not significant. Double and triple-dose erythropoietin pretreatment groups had no effect on IL-1α and IL-6 serum levels (Fig.3 & 4).

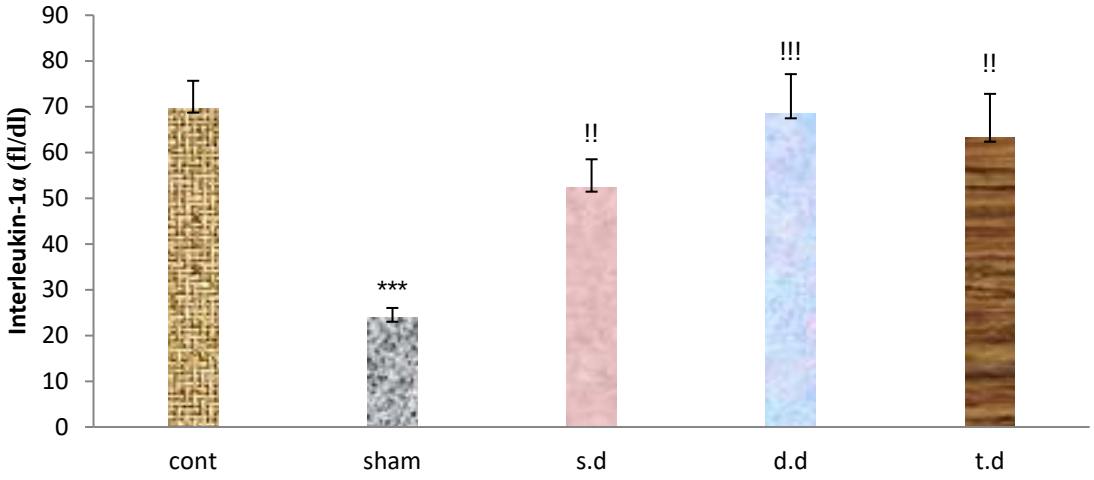


Figure 3: comparison of interleukin-1 alpha serum levels 24 hours after stroke induction in sham, control and experimental groups. $p<0.000$ [***] shows significant difference with control. $p<0.01$، [!!] $p<0.000$ [!!!] show significant difference with sham.

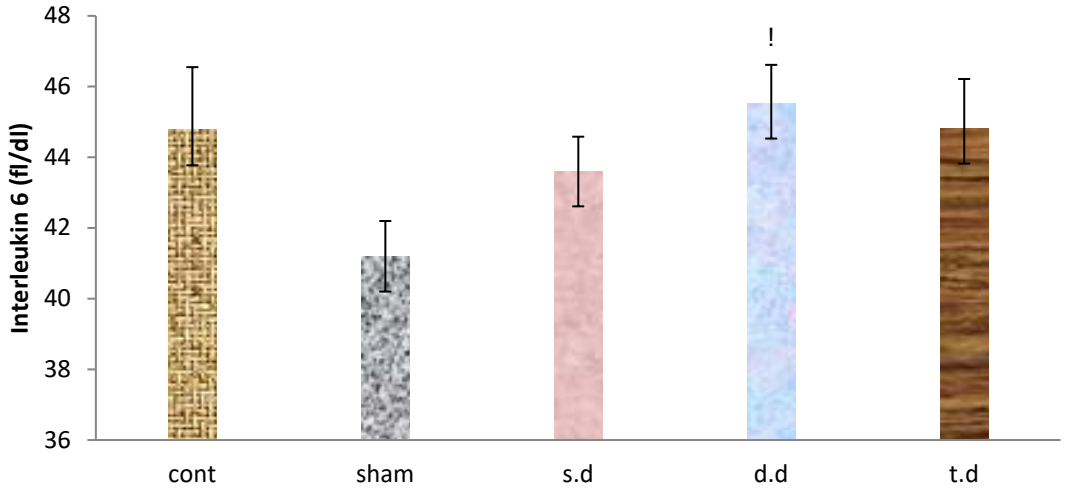


Figure 4: comparison of interleukin-6 serum level 24 hours after stroke induction in sham, control and experimental groups. $p<0.05$ [!]shows significant difference with sham.

Discussion

Blood flow maintenance in the ischemic area is one of the main goals of treatment in stroke, but on the other hand, restoration of blood flow to the damaged area may lead to aggravation of the injury. Also after the stroke change different physiological conditions of the body such as blood glucose, blood saturation, temperature which could increase severity of the lesion. So prevention of further damage after reperfusion is important. Stabilization of physiological conditions as well as lowering of inflammation can reduce the injury caused by the stroke and reperfusion (Gentile, Seftchick, Huynh, Kruus, & Gaughan, 2006; Wong & Read, 2008; Yu et al., 2016). In one study it was demonstrated that erythropoietin pretreatment administration has a satisfying effect on the infarct volume and neurological deficits (Yu et al., 2016). The results of our study showed that single dose of erythropoietin pretreatment (1000 U/kg) 30 minutes before the stroke induction, significantly reduced infarct volume damage and improved neurological defects that these results are consistent with previous studies. However, higher doses of erythropoietin showed some reversed results than other previous experimental studies. These different results may be due to different time and dose of erythropoietin administration. We demonstrated that the pretreatment with a lower single dose of erythropoietin can also have positive effects on IRI. In comparison with the other recent researches, it seems that receiving a single dose of erythropoietin has better effects on IRI than administration of multiple doses likely due to fewer side effects. In another

study, we showed that erythropoietin pre-treatment for 48 and 96 hours were associated with a significant increase in hemoglobin, hematocrit and brain edema that may explain the adverse consequences due to side effects (M. Bigdeli, Mostafavi, Gholamzadeh, & Eskandari, 2016). Also in the acute phase of stroke, a moderate hyperglycemia was observed in 40% of individuals without diabetes (Quinn, Dawson, & Walters, 2011). It has been proven that hyperglycemic people to nurmoglycemic have more severe neurological defects and poor clinical outcomes (Bhalla et al., 2001; Dietrich, Alonso, & Busto, 1993; Radermecker & Scheen, 2010). Our research showed that erythropoietin increased blood sugar levels in all groups, especially in the acute phase of stroke. The increase in blood glucose was approximately dose-dependent and higher blood glucose values was conformed with poor clinical and histological sequel. Although unlike the other carried out studies in diabetic rats erythropoietin increased blood sugar. Several studies have reported a possible link between erythropoietin level and hypoglycemia (Chen et al., 2015). It is found that hypoglycemia exacerbates stress responses. Stress response causes a further increase in blood sugar in hypoglycemia compared to normoglycemia condition (Ceriello et al., 2012). These results may suggest a hypothesis which indicates erythropoietin pre-treatment possibly decreased blood sugar in this no diabetic rats before stroke induction and exacerbated hyperglycemia resulted from stress response after IRI. It is likely that lack of weight gaining of the treated animals with higher doses of erythropoietin confirms this hypothesis. As was shown in another study, the decreased glucose levels in the following of treatment with erythropoietin in high fat diet rats for two weeks was associated with their weight loss (Meng et al., 2013). Also, lowering blood sugar over time 24 hours in all groups can be confirmed the presence of a stress response. In addition, Previous studies have shown that there is a likely connection between blood sugar levels, inflammatory markers and the activation of the stress response after stroke (Meng et al., 2013; Shukla et al., 2017). Also several studies have demonstrated ischemic-reperfusion is associated with enhancement of inflammatory factors such as IL-1, IL-1β, TNF-α and IL-6 and worse histological outcomes which erythropoietin pretreatment protected tissue from IRI by reducing these factors (Liao et al., 2016; Liu et al., 2015; Nandra et al., 2013; Rong & Xijun, 2015; H. Wu et al., 2006). In connection with inflammatory markers, we showed that single dose of erythropoietin pretreatment decreased levels of IL-1α and IL-6 compared to the control group and the groups with higher levels of inflammatory markers had worsen tissue results and also our results identified an association

between inflammatory markers and blood glucose level. Ran Meng and colleagues showed that in their study on high-fat diet-fed Mice along with the increment of blood glucose, IL-6 and TNF-α increased (Meng et al., 2013). Based on previous studies, the main determinants of blood glucose concentration were cortisol, glucagon and insulin (O'neill, Davies, Fullerton, & Bennett, 1991). Ramirez showed that treatment of anemia in dialysis patients with recombinant human erythropoietin hormone could affect the pituitary-hypothalamic-adrenal axis and increase responses of adrenocorticotropin hormone (ACTH) to corticotropin- releasing hormone (CRH) .However, to confirm the hypothesis of hyperglycemia after hypoglycemia and stress response occurrence in brain ischemia following erythropoietin pretreatment, it is necessary to measurement blood glucose before stroke induction and cortisol level in the early hours after stroke that evaluation of these parameters is recommended for further studies (Ramirez, Bittle, Sanders, Rabb, & Bercu, 1994).

In conclusion, our results showed an existence of communication between hyperglycemia, levels of inflammatory factors and severity of injury after the IRI by MCAO model in the rat and erythropoietin pre-treatment able to decreased IRI by reducing of inflammatory factors, the establishment of stable physiological conditions, amelioration of infarct volume and neurological deficits. It appears that its effects are dose-dependent. Our results recommend that administration of higher erythropoietin doses should be taken with caution due to its possible side effects. However, determination of more detail effects of erythropoietin on physiological conditions and stress response over activation after IRI in the brain needs more studies.

Conflict of interest

This research has been done with the agreement and interest of all the authors.

Acknowledgments

All authors would like to appreciate from Doctor Abdolreza EsmaeilZadeh in immunology department of zanjan university of medical science and sepideh Khaksari in shahid behshti university for their scientific cooperation.

Bhalla, A., Wolfe, C., & Rudd, A. (2001). Management of acute physiological parameters after stroke. *Qjm, 94*(3), 167-172.

Bigdeli, M. (2008). The threshold assessment of ischemic tolerance induced by normobaric hyperoxia in rat stroke model. *Journal of Reserch in medical sience*, ۳۳(۲) , 95-۱۰۳ .

Bigdeli, M., Mostafavi, H., Gholamzadeh, R., & Eskandari, M. (2016). Pretreatment effect of erythropoietin on brain tissuewater content after brain ischemia induction by middle cerebral artery occlusion (MCAO) in male wistar rats. *International Journal of Medical Reviewes,3*(1), 389-400.

Bigdeli, M. R., & Mohagheghi, F. (2014). The pathophysiology of brain ischemia and ischemic preconditioning. *Zahedan Journal of Research in Medical Sciences, 16*(2), 1-5.

Ceriello, A., Novials, A., Ortega, E., La Sala, L., Pujadas, G., Testa, R., . . . Giugliano, D. (2012). Evidence that hyperglycemia after recovery from hypoglycemia worsens endothelial function and increases oxidative stress and inflammation in healthy control subjects and subjects with type 1 diabetes. *Diabetes, 61*(11), 2993-2997.

Chen, L. N., Sun, Q., Liu, S. Q., Hu, H., Lv, J., Ji, W. J., . . . Zhou, J. (2015). Erythropoietin improves glucose metabolism and pancreatic β-cell damage in experimental diabetic rats. *Molecular medicine reports, 12*(4), 5391-5398.

Chiang, T., Messing, R. O., & Chou, W.-H. (2011). Mouse model of middle cerebral artery occlusion. *JoVE (Journal of Visualized Experiments)*(48), e2761-e2761.

Dietrich, W. D., Alonso, O., & Busto, R. (1993). Moderate hyperglycemia worsens acute blood-brain barrier injury after forebrain ischemia in rats. *Stroke, 24*(1), 111-116.

Elshiekh, M., Kadkhodaee, M., Seifi, B., Ranjbaran, M., & Ahghari, P. (2015). Ameliorative effect of recombinant human erythropoietin and ischemic preconditioning on renal ischemia reperfusion injury in rats. *Nephro-urology monthly, 7*(6).

Gentile, N. T., Seftchick, M. W., Huynh, T., Kruus, L. K., & Gaughan, J. (2006). Decreased mortality by normalizing blood glucose after acute ischemic stroke. *Academic emergency medicine, 13*(2), 174-180.

Gholamzadeh, R., Eskandari, M., Mostafavi, H., & Bigdeli, M. R. (2016). A review on the pretreatment effect of EPO on ischemic tolerance in different tissues with an approach to the tissue protection mechanisms. *International Journal of Medical Reviews, 3*(1), 389-400.

Gul, M., Yasim, A., & Aral, M. (2009). The levels of cytokines in rats following the use of prophylactic agents in vascular graft infection. *Bratislavske lekarske listy, 111*(6), 316-320.

Kai-Lan, W., & Si, Z. (2015). *Pretreatment with erythropoietin attenuates intestinal ischemia reperfusion injury by further promoting PI3K/Akt signaling activation.* Paper presented at the Transplantation proceedings.

Liao, J.-G., Li, M.-Y., Wang, X.-H., & Xie, Q. (2016). The protective effect of erythropoietin pretreatment on ischemic acute renal failure in rats. *Journal of Acute Disease, 5*(5), 408-412.

Lindsberg, P. J., & Roine, R. O. (2004). Hyperglycemia in acute stroke. *Stroke, 35*(2), 363-364.

Liu, Q.-S., Cheng, Z.-W., Xiong, J.-G., Cheng, S., He, X.-F., & Li, X.-C. (2015). *Erythropoietin pretreatment exerts anti-inflammatory effects in hepatic ischemia/reperfusion-injured rats via suppression of the TLR2/NF-κB pathway.* Paper presented at the Transplantation proceedings.

Mehta, S. (2003). The glucose paradox of cerebral ischaemia. *Journal of postgraduate medicine, 49*(4), 299.

Meng, R., Zhu, D., Bi, Y., Yang, D., & Wang, Y. (2013). Erythropoietin inhibits gluconeogenesis and inflammation in the liver and improves glucose intolerance in high-fat diet-fed mice. *PloS one, 8*(1), e53557.

Nandra, K. K., Collino, M., Rogazzo, M., Fantozzi, R., Patel, N. S., & Thiemermann, C. (2013). Pharmacological preconditioning with erythropoietin attenuates the organ injury and dysfunction induced in a rat model of hemorrhagic shock. *Disease models & mechanisms, 6*(3), 701-709.

Niu, H.-S., Chang, C.-H., Niu, C.-S., Cheng, J.-T., & Lee, K.-S. (2016). Erythropoietin ameliorates hyperglycemia in type 1-like diabetic rats. *Drug design, development and therapy, 10*, 1877.

O'neill, P., Davies, I., Fullerton, K., & Bennett, D. (1991). Stress hormone and blood glucose response following acute stroke in the elderly. *Stroke, 22*(7), 842-847.

Paschos, N., Lykissas, M. G., & Beris, A. E. (2008). The role of erythropoietin as an inhibitor of tissue ischemia. *Int J Biol Sci, 4*(3), 161-168.

Quinn, T., Dawson, J., & Walters, M. (2011). Sugar and stroke: cerebrovascular disease and blood glucose control. *Cardiovascular therapeutics, 29*(6), e31-e42.

Radermecker, R. P., & Scheen, A. J. (2010). Management of blood glucose in patients with stroke. *Diabetes & metabolism, 36*, S94-S99.

Ramirez, G., Bittle, P. A., Sanders, H., Rabb, H., & Bercu, B. B. (1994). The effects of corticotropin and growth hormone releasing hormones on their respective secretory axes in chronic hemodialysis patients before and after correction of anemia with recombinant human erythropoietin. *The Journal of Clinical Endocrinology & Metabolism, 78*(1), 63-69.

Rong, R., & Xijun, X. (2015). Erythropoietin pretreatment suppresses inflammation by activating the PI3K/Akt signaling pathway in myocardial ischemia-reperfusion injury. *Experimental and therapeutic medicine, 10*(2), 413-418.

Shah, S. Stroke Pathophysiology. Foundation for Education and Research in Neurological Emergencies. 2000.

Shukla, V., Shakya, A. K., Perez-Pinzon, M. A., & Dave, K. R. (2017). Cerebral ischemic damage in diabetes: an inflammatory perspective. *Journal of neuroinflammation, 14*(1), 21.

Wong, A. A., & Read, S. J. (2008). Early changes in physiological variables after stroke. *Annals of Indian Academy of Neurology, 11*(4), 207.

Wu, H., Ren, B., Zhu, J., Dong, G., Xu, B., Wang, C., . . . Jing, H. (2006). Pretreatment with recombined human erythropoietin attenuates ischemia–reperfusion-induced lung injury in rats. *European journal of cardio-thoracic surgery, 29*(6), 902-907.

Wu, S.-K., Yang, M.-T., Kang, K.-H., Liou, H.-C., Lu, D.-H., Fu, W.-M., & Lin, W.-L. (2014). Targeted delivery of erythropoietin by transcranial focused ultrasound for neuroprotection against ischemia/reperfusion-induced neuronal injury: a long-term and short-term study. *PloS one, 9*(2), e90107.

Yao, M., Ni, J., Zhou, L., Peng, B., Zhu, Y., & Cui, L. (2016). Elevated Fasting Blood Glucose Is Predictive of Poor Outcome in Non-Diabetic Stroke Patients: A Sub-Group Analysis of SMART. *PLoS One, 11*(8), e0160674.

Yip, H.-K., Tsai, T.-H., Lin, H.-S., Chen, S.-F., Sun, C.-K., Leu, S., . . . Liou, C.-W. (2011). Effect of erythropoietin on level of circulating endothelial progenitor cells and outcome in patients after acute ischemic stroke. *Critical Care, 15*(1), R40.

Yu, D., Fan, Y., Sun, X., Yao, L., & Chai, W. (2016). Effects of erythropoietin preconditioning on rat cerebral ischemia‐reperfusion injury and the GLT‐1/GLAST pathway. *Experimental and therapeutic medicine, 11*(2), 513-518.

Yuen, C.-M., Sun, C.-K., Lin, Y.-C., Chang, L.-T., Kao, Y.-H., Yen, C.-H., . . . Shao, P.-L. (2011). Combination of cyclosporine and erythropoietin improves brain infarct size and neurological function in rats after ischemic stroke. *Journal of translational medicine, 9*(1), 141.