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The Effect of Erythropoietin on Endometrial Karyorrhexis during Ischemia Reperfusion Injury in Rats

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Abstract

Aim: This experiment evaluated the influence of erythropoietin in an animal model of uterine ischemia reperfusion using the quoting established protocol. The effects of erythropoietin treatment were evaluated by mean Endometrial Karyorrhexis (EK) lesions.

Materials and methods: EK lesions were determined at the 60th reperfusion min (for groups A and C) and at the 120th reperfusion min (for groups B and D). Groups A and B received no drugs, whereas rats from groups C and D were administered with erythropoietin. 40 rats of mean mass 247.7 g were employed for the study.

Results: Epo administration non-significantly decreased the EK scores by [without lesions] 0.4 [-0.9640457 - 0.1640457] (p = 0.1242). Reperfusion time non-significantly decreased the EK scores by [without lesions] 0.1 [-0.678208 - 0.478208] (P = 0.6729). However, Epo administration and reperfusion time together non-significantly decreased the EK scores by [without lesions] 0.2727273 [-0.6102818 - 0.0648273] (p = 0.1102).

Conclusions: Epo administration whether it interacted or not with reperfusion time, non-significantly decreased the EK scores in a short-term context of 2 hours.

Keywords: Ischemia; Erythropoietin; Endometrial karyorrhexis; Reperfusion

Introduction

Erythropoietin (Epo) is considered from the more well studied drugs. Epo implicates over 28,517 known biomedical studies at present. Over than 3.4% of these studies include ischemia-reperfusion (IR) injury experiments. Epo usage prevails thus in trials of reverse the transient or permanent type IR injuries including the ischemic or adjacent organs and certainly patients' health.

The investigation is focused mainly to basic questions, such as, action velocity, administration timing and dosage height; apart from the original knowledge of Epo in stem blood cells recovery. However, the more specific matters are investigated, the fewer related reports are found. Furthermore, a numeric evaluation of the Epo trends was provided by a meta-analysis of 19 published seric variables, coming from the same experimental setting, for the same endpoints (Table 1).

Table 1: The trends (+SD) of erythropoietin (Epo) on levels of some seric1 variables regarding reperfusion time (rep).

Variables	1 hour rep	p-value	1.5 hour rep	p-value	2 hour rep	p-value	interaction of Epo and reperfu	p-value
WBCC	+24.01%+13.38%	0.1012	+22.09% +9.11%	0.0351	+20.17%+12.94%	0.0902	+14.63%+5.40%	0.008
hematocrit	+0.14%+2.89%	0.9626	-0.61% +2.37%	0.8072	-1.37%+4.05%	0.7485	+0.24%+1.38%	0.8586
MCH	+0.01%+1.29%	0.9904	+0.67%+0.80%	0.3549	+1.34%+1.08%	0.1509	-0.36%+0.47%	0.443
Platelet DW	+1.60%+0.80%	0.0765	+1.36%+0.58%	0.0205	+1.13%+0.74%	0.1152	+0.37%+0.37%	0.0615

platelet-crit	-16.47%+10.40%	0.0921	-13.74%+7.01%	0.0158	-11.01%+7.34%	0.0882	-6.88%+3.69%	0.0615
urea	+21.42%+7.84%	0.0115	+20.11%+7.25%	0.0059	+18.80%+9.44%	0.0709	+15.64%+4.04%	0.0003
uric acid	+10.13%+15.10%	0.4917	+15.86%+10.21%	0.1408	+21.59%+15.45%	0.194	+9.33%+6.16%	0.1264
total protein	-0.02%+2.47%	0.9904	-1.27%+1.51%	0.3721	-2.52%+2.03%	0.1509	-0.68%+2.48%	0.443
alkaline phosphatase	+0.20%+18.57%	0.9904	+10.70%+12.78%	0.3549	+21.20%+17.11%	0.1509	+5.79%+7.72%	0.443
acid phosphatase	+0.06%+5.79%	0.9904	+3.11%+3.71%	0.3172	+6.16%+4.97%	0.1509	+1.68%+2.23%	0.443
CPK	+0.15%+14.09%	0.9904	+7.91%+9.44%	0.3549	+15.67%+12.65%	0.1509	+4.28%+5.70%	0.443
LDH	+0.08%+7.92%	0.9904	+4.48%+5.35%	0.3549	+8.89%+7.17%	0.1509	+2.42%+3.22%	0.443
sodium	+0.72%+0.74%	0.3054	+0.21%+0.63%	0.7136	-0.29%+1.09%	0.767	-0.11%+0.38%	0.7531
phosphorus	+1.92%+5.25%	0.6982	+3.95%+3.35%	0.21	+5.98%+4.81%	0.293	+2.45%+2.01%	0.2168
progesterone	-0.20%+18.65%	0.9904	-8.86%+10.58%	0.3549	-17.53%+14.15%	0.1509	-4.79%+6.39%	0.443
mean	+2.91%+9591%	0.6448	+4.39%+9.81%	0.2941	+5.88%+11.93%	0.2282	+2.93%+6.29%	0.3458

The special aim of this experimental work was to study the effect of Epo on a rat model and mainly in a uterine IR protocol. The effect of Epo molecule was tested by evaluating mean endometrial karyorrhexis (EK) lesions.

Materials and Methods

Animal preparation

This basic experimental research was licensed by the Veterinary Address of East Attiki Prefecture [No 3693l|/12-11-2010 &14l|/10-1-2012 decisions]. All consumables, substances and equipment used, were a grant of ELPEN Pharmaceuticals Co. Inc. S.A. at Pikermi, Attiki. Accepted standards of humane animal care were adopted for Albino female Wistar rats. 7 days pre-experimental normal housing including ad libitum diet in laboratory.

Post-experimental awakening and preservation of animals was not permitted, even if euthanasia was required. Rats were randomly delivered to four groups by 10 rodent in each one, using following protocols of IR: 45 min ischemia followed by 60 min reperfusion (group A); 45 min ischemia followed by 120 min reperfusion (group B); 45 min ischemia followed by 60 min immediate Epo (Epoetin, Recombinant Human Erythropoetin Alfa, Janssen-Cilag, Beerse, Belgium) intravenous (IV) administration and reperfusion (group C); 45 min ischemia followed by 120 min immediate Epo IV administration and reperfusion (group D). The dose of Epo was 10 mg/kg body mass of animals. Isik et al., [1] described detailed preceded prenarcotic and general anesthesiologic techniques of animals. Continuous intra-experimental oxygen supply, electrocardiogram and acidometry were provided.

The protocol of IR was followed. Ischemia was induced by laparotomic clamping inferior aorta over renal arteries with forceps for 45 min. Reperfusion was established by removing the clamp and securing inferior aorta patency. After exclusion of a blood flow, the protocol of IR was iterated for each experimental group. Epo was administered starting reperfusion through catheterized inferior vena cava. The UK lesions were determined at 60th min of reperfusion (for A and C groups) and at 120th min of reperfusion (for B and D groups).

Fourty female Wistar albino rats were used (mean mass 231.875 g [standard deviation (SD): 36.59703 g], with minimum mass 165 g and maximum mass 320 g. Rats mass could be potentially a confusing factor, e.g. more obese rats to have more or less EK lesions scores. This assumption was also investigated. Along, detailed pathological study and grading of EK findings was performed by scores: 0 lesions were not found, 1 mild lesion was found, 2 moderate lesions were found and 3 serious lesions were found. The previous grading was transformed as follows: (0 - 0.499) without lesions, (0.5 - 1.499) mild lesions, (1.5 - 2.499) moderate lesions and (2.5 - 3) serious lesions damage, because the study concerned score ranges rather than point scores. EK scores were measured by 1st Department of Pathology at Department of Clinical – Laboratory studies in Faculty of Medicine of Athens University.

Model of ischemia-reperfusion injury

Control groups

20 control rats (mean mass 252.5 g [SD:39.31988 g]) experienced ischemia lasting 45 min followed by reperfusion.

Table 2: Mass and endometrial karyorrhexis (EK) score mean levels and SD of groups.

Groups	Variable	Mean	SD
A	Mass	243 g	45.77724 g
	EK	mild 1	0.942809
B	Mass	262 g	31.10913 g
	EK	mild 1.1	0.875595
C	Mass	242.8 g	29.33636 g

	EK	mild 0.8	0.788811
D	Mass	243 g	32.84644 g
	EK	mild 0.5	0.971825

Group A

Reperfusion lasted 60 min (n = 10 controls rats) mean mass 243 g [SD: 45.77724 g], mean mild EK score 1 [SD: 0.942809] (Table 2).

Group B

Reperfusion lasted 120 min (n = 10 controls rats) mean mass 262 g [SD: 31.10913 g], mean mild EK score 1.1 [SD: 0.875595] (Table 2).

Erythropoietin group

20 Epo rats (mean mass 242.9 g [SD:30.3105 g]) experienced ischemia lasting 45 min followed by reperfusion on the commencement of which 10 mg Epo/kg body weight were IV administered.

Group C

Reperfusion lasted 60 min (n =10 Epo rats) mean mass 242.8 g [SD: 29.33636 g], mean mild EK score 0.8 [SD: 0.7888106] (Table 2).

Group D

Reperfusion lasted120 min (n = 10 Epo rats) mean mass 243 g [SD: 32.84644 g], mean mild EK score 0.5 [SD: 0.9718253] (Table 2).

Statistical analysis

Every mass group was compared with each other by statistical standard T-test and every EK lesions scores group with each other by statistical Wilcoxon signed-rank test (Table 3). Any probable significant difference among EK scores was investigated about potent significant mass correlations. The application of generalized linear models (glm) with dependant variable the EK scores was followed. The 3 independent variables were the Epo or no drug, the reperfusion time and both variables in combination. Inserting the rats weight as independent variable at glm, a non-significant relation turned on with EK scores (p = 0.0692), so as to further investigation was not needed. The statistical analysis was performed by Stata 6.0 software [Stata 6.0, Stata Corp LP, Texas, USA].

Table 3: Mean values differences for groups (DG) after statistical standard t test application for mass and Wilcoxon signed-rank test for scores and their statistical significance.

DG	Variable	Difference	p-value
A-B	Weight	-19 g	0.2423
	EK	without lesions -0.1	0.9581
A-C	Weight	0.2 g	0.99
	EK	without lesions 0.2	0.6371
A-D	Weight	0 g	1
	EK	mild 0.5	0.2337
B-C	Weight	19.2 g	0.2598
	EK	without lesions 0.3	0.4233
B-D	Weight	19 g	0.1011
	EK	mild 0.6	0.0265
C-D	Weight	-0.2 g	0.9883
	EK	without lesions 0.3	0.4233

Results

The application of glm resulted in: Epo administration non-significantly decreased the EK scores by [without lesions] 0.4 [-0.9640457 - 0.1640457] (p = 0.1593). This finding was in accordance with the results of Wilcoxon signed-rank test (p = 0.0891). Reperfusion time non-significantly decreased the EK scores by [without lesions] 0.1 [-0.678208 - 0.478208] (p = 0.7282), also in accordance with Wilcoxon signed-rank test (p = 0.6177). However, Epo administration and reperfusion time together non-significantly decreased the EK scores by [without lesions] 0.2727273 [-0.6102818 - 0.0648273] (p = 0.1102). Reviewing the above Tables 3 and 4 sums up concerning the decreasing influence of Epo in connection with reperfusion time.

Table 4: The declining trend of Epo regarding reperfusion time.

Decrease	95% c. in. Reperfusion	time	p-values	
			Wilcoxon	glm
without lesions 0.2	-1.016692-0.6166917	1 h	0.6371	0.6132
without lesions 0.4	-0.9640457-0.1640457	1.5 h	0.0891	0.1593
mild 0.6	-1.469059-0.2690586	2 h	0.0265	0.1641
without lesions 0.1	-0.678208-0.478208	reperfusion time	0.6177	0.7282
without lesions 0.2727273	-0.6102818-0.0648273	interaction	-	0.1102

Discussion

Ischemia may be associated with EK lesions. Isik S et al., [1] found less karyorrhexis 3 lesions by local antithrombin treatment in hepatic IR injury of Wistar rats. Takizawa Y et al., [2] considered that oxidative stress significantly induces DNA peroxidation, apoptotic neuronal death and karyorrhexis 24-72 h after neonatal hypoxic-ischemic (HI) encephalopathy. Sun L et al., [3] found eosinophilic neurons (Ens) with minimally abnormal nuclei and swollen cell bodies at 3 h in the ischemic core and at 12 h in the periphery of post-ischemic gerbils brain. In the ischemic periphery, ENs had slightly atrophic cytoplasm and sequentially developed pyknosis, karyorrhexis and karyolysis over 1 week. Folkerth RD et al., [4] observed nuclear karyorrhexis and/or karyopyknosis with cytoplasmic hypereosinophilia in neurons of the arcuate nucleus in consecutive stillbirth brains 22 - 41 gestational weeks old, considering HI lesions such as white matter and brainstem gliosis the cause in part for unexplained stillbirth. Takizawa Y et al., [5] closely associated pontosubicular neuron necrosis and its pathological peculiarity neuronal apoptosis as one of perinatal HI brain injury with presence of karyorrhexis. Hargitai B et al., [6] associated preterm birth with HI encephalopathy including neuronal karyorrhexis mostly at diencephalon and brain stem. Hallak M et al., [7] associated brain injury featured by shrinkage of cells and karyorrhexis at hippocampus and thalamus ($P < 0.05$) with hypoxia and decreased maternal oxygen tension and pH in fetal rats. Tan S et al., [8] induced HI which resulted in significant increase of nitrogen oxides, lipid peroxidation and protein oxidation, with a concomitant decrease of total antioxidant capacity in premature fetal brains rabbit model of acute placental insufficiency in utero. Fetuses delivered 24 h post IR had increased hippocampal nuclear karyorrhexis on histology than controls. Meng SZ et al., [9] manifestedneuronal karyorrhexis more predominant in preterm infants with HI basal ganglia necrosis. Fortuna S et al., [10] observed neuronal degeneration and necrosis with nuclear pyknosis and karyorrhexis in a model of mildly HI brain injury. Khera KS et al., [11] noted a pleiotropic karyorrhexis in third or embryonic phase of embryotoxic pathogenesis, appeared aggravated, presumably by the preceding second or labyrinthine degeneration of the placental phase in rats embryos. Squier M et al., [12] considered the reactive astrocytosis, macrophage infiltration, karyorrhexis and endothelial swelling or reduplication as criteria for white matter ischemia in early neonatal brains who were stillborn or died due to cerebral palsy. Kalimo H et al., [13] found karyorrhexis and cytorrhexis and removal of their remnants subsequently by macrophages in the great majority of medium-sized neurons of caudate nucleus and putamen15 after 2-3 days IR injury [14,15].

Conclusion

Epo administration whether it interacted or not with reperfusion time, non-significantly decreased the EK scores in the short-term context time of 2 hours. Perhaps, a longer study time than 2 hours or a higher Epo dose may provide more significant effects.

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References

1. Isik S, Tuncyurek P, Zengin NI, Demirbag AE, Atalay F, et al. (2012) Antithrombin prevents apoptosis by regulating inflammation in the liver in a model of cold ischemia/warm reperfusion injury. Hepatogastroenterology 59: 453-457.
2. Takizawa Y, Miyazawa T, Nonoyama S, Goto Y, Itoh M (2009) Edaravone inhibits DNA peroxidation and neuronal cell death in neonatal hypoxic-ischemic encephalopathy model rat. Pediatr Res 65: 636-641.
3. Sun L, Kuroiwa T, Ishibashi S, Miki K, Li S, et al. (2009) Two region-dependent pathways of eosinophilic neuronal death after transient cerebral ischemia. Neuropathology 29: 45-54.
4. Folkerth RD, Zanoni S, Andiman SE, Billiards SS (2008) Neuronal cell death in the arcuate nucleus of the medulla oblongata in stillbirth. Int J Dev Neurosci 26: 133-40.
5. Takizawa Y, Takashima S, Itoh M (2006) A histopathological study of premature and mature infants with pontosubicular neuron necrosis: neuronal cell death in perinatal brain damage. Brain Res 1095: 200-206.
6. Hargitai B, Szabo V, Cziniel M, Hajdu J, Papp Z, et al. (2004) Human brain of preterm infants after hypoxic-ischaemic injuries: No evidence of a substantial role for apoptosis by using a fine-tuned ultrasound-guided neuropathological analysis. Brain Dev 26: 30-36.
7. Hallak M, Hotra JW, Kupsky WJ (2000) Magnesium sulfate protection of fetal rat brain from severe maternal hypoxia. Obstet Gynecol 96: 124-128.
8. Tsompos C, Panoulis C , Toutouzas V, Zografos G, Papalois A (2015) The Effect of Erythropoietin on Urea Levels during Ischemia Reperfusion Injury in Rats Literati. Journal of Pharmaceutical Drug Delivery Technologies 1: 61-64.
9. Meng SZ, Isumi H, Takashima S (1998) Neuropathological characteristics and alteration of the dopamine D2 receptor in hypoxic-ischemic basal ganglia necrosis. Brain Dev 20: 98-104.
10. Fortuna S, Pestalozza S, Lorenzini P, Bisso GM, Morelli L, et al. (1997) Transient global brain hypoxia-ischemia in adult rats: Neuronal damage, glial proliferation, and alterations in inositol phospholipid hydrolysis. Neurochem Int 31: 563-569.
11. Khera KS (1992) A morphologic basis postulated for valproic acid's embryotoxic action in rats. Teratog Carcinog Mutagen 12: 277-89.
12. Squier M, Keeling JW (1991) The incidence of prenatal brain injury. Neuropathol Appl Neurobiol 17: 29-38.
13. Kalimo H, Auer RN, Siesjo BK (1985) The temporal evolution of hypoglycemic brain damage. III. Light and electron microscopic findings in the rat caudoputamen. Acta Neuropathol 67: 37-50.
14. Osmanagaoglu MA, Kesim M, Yulug E, Mentese A, Karahan SC (2012) Ovarian-protective effects of clotrimazole on ovarian ischemia/reperfusion injury in a rat ovarian-torsion model. Gynecol Obstet Invest 74: 125-30.

15. Tan S, Zhou F, Nielsen VG, Wang Z, Gladson CL, et al. (1998) Sustained hypoxia-ischemia results in reactive nitrogen and oxygen species production and injury in the premature fetal rabbit brain. J Neuropathol Exp Neurol 57: 544-53.