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Research Article

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

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Abstract

The aim of this experimental study was to examine the effect of erythropoietin on rat model and particularly in an ovarian ischemia reperfusion (IR) protocol. The effect of that molecule was studied pathologically using the mean ovarian epithelium karyorrhexis (OK) lesions. Materials and methods: 40 rats of mean weight 247.7 g were used in the study. OK lesions were evaluated at 60 min (groups A and C) and at 120 min (groups B and D) of reperfusion. Erythropoietin was administered only in groups C and D. Results were that Epo administration non-significantly decreased the OK scores by 0.15 without lesions [-0.371518 - 0.071518] (p= 0.1679). Reperfusion time non-significantly increased the OK scores by 0.1 without lesions [-0.27768095 – 0.14211844] (p=0.4073). However, Epo administration and reperfusion time together non-significantly decreased the OK scores by 0.0818182 without lesions [-0.2159977 - 0.0523614] (p=0.2246). Conclusions: Results of this study indicate that Epo administration interacted or not with reperfusion time non-significantly short-term decreased the OK scores. Perhaps, a longer study time than 2 hours may provide more significant effects.

Keywords: Ischemia; Erythropoietin; Ovarian epithelium karyorrhexis; Reperfusion

Introduction

Tissue Ischemia and Reperfusion (IR) remain of the main causes of permanent or transient damage with serious implications on adjacent organs and certainly on patients' health. Although important progress has been made regarding the usage of erythropoietin (Epo) in managing of this kind of damages, satisfactory answers have not been given yet to fundamental questions, as, by what velocity this factor acts, when it should be administered, and in which dosage. The particularly satisfactory action of Epo in stem blood cells recovery has been noted in several performed experiments. However, just few relative reports were found concerning Epo trial in IR experiments, not covering completely this particular matter. A meta-analysis of 13 published [1] seric variables, coming from the same experimental setting, tried to provide a numeric evaluation of the Epo efficacy for the same endpoints. (Table 1). Also, a lot of publications addressed trials of other similar molecules of growth factors to which the studied molecule also belongs to. The aim of this experimental study was to examine the effect of Epo on rat model and particularly in an ovarian IR protocol. The effect of that molecule was studied by evaluating mean ovarian Epithelium Karyorrhexis (OK) lesions.

Materials and Methods

Animal Preparation

This experimental study was licensed by Veterinary Address of East Attiki Prefecture under 3693/12-11-2010 & 14/10-1-2012 decisions. All settings needed for the study including consumables, equipment and substances used, were a courtesy of Exprerimental Research Center of ELPEN Pharmaceuticals Co. Inc. S.A. at Pikermi, Attiki. Accepted standards of humane animal care were adopted for Albino female Wistar rats. Normal housing in laboratory 7 days before the experiment included continuous access to water and food. The experiment was acute, that means that awakening and preservation of the rodents was not following the experiment. They were randomly delivered to four experimental groups by 10 animals in each one. Ischemia for 45 min followed by reperfusion for 60 min (group A). Ischemia for 45 min followed by reperfusion for 120 min (group B). Ischemia for 45 min followed by immediate Epo intravenous (IV) administration and reperfusion for 60 min (group C). Ischemia for 45 min followed by immediate Epo IV administration and reperfusion for 120 min (group D). The molecule Epo dosage was 10 mg/Kg body weight of animals. At first, the animals were submitted into prenarcosis followed by general anaesthesia. The detailed anesthesiologic technique is described in related references [1,2]. Oxygen supply, electrocardiogram and acidometry were continuously provided during whole experiment performance. The protocol of IR was followed. Ischemia was caused by forceps clamping inferior aorta over renal arteries for 45 min after laparotomic access had been achieved. Reperfusion was induced by removing the clamp and reestablishment of inferior aorta patency. The molecules were administered at the time of reperfusion, through inferior vena cava after catheterization had been achieved. The OK lesions evaluations were performed at 60 min of reperfusion (for groups A and C) and at 120 min of reperfusion (for groups B and D). Forty (40) female Wistar albino rats were used of mean weight 231.875 g [Std. Dev: 36.59703 g], with min weight ≥ 165 g and max weight < 320 g. Rats' weight could be potentially a confusing factor, e.g. fatter rats to have more or less OK lesions scores. This suspicion was also investigated. Also, detailed histopathological [3] study (pathology) and grading of OK findings was performed by scores, this is: 0 when lesions were not found, 1 when mild lesions were found, 2 when moderate lesions were found and 3 when serious lesions were found. The previous grading

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Table 1: Meta-analysis of the erythropoietin (Epo) influence (±SD) on the levels of some seric variables concerning reperfusion (rep) time coming from the same experimental setting.

Variable	1h rep	p-value	1.5h rep	p-value	2h rep	p-value	interaction of Epo and rep	p-value
white blood cells	+24.01%±13.38%	0.1012	+22.09%±9.11%	0.0351	+20.17%±12.94%	0.0902	+14.63%±5.40%	0.0080
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
mean corpuscular hemoglobin	+0.01%±1.29%	0.9904	+0.67%±0.80%	0.3549	+1.34%±1.08%	0.1509	-0.36%±0.47%	0.4430
platelet distribution width	+1.60%±0.80%	0.0765	+1.36%±0.58%	0.0205	+1.13%±0.74%	0.1152	+0.37%±0.37%	0.0615
plateletcrit	-16.47%±10.40%	0.0921	13.74%±7.01%	0.0158	-11.01%±7.34%	0.0882	-6.88%±3.69%	0.0615
uric acid	+10.13%±15.10%	0.4917	+15.86%±10.21%	0.1408	+21.59%±15.45%	0.1940	+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%	0.4430
alkaline phosphatase	+0.20%±18.57	0.9904	+10.70%±12.78%	0.3549	+21.20%±17.11%	0.1509	+5.79%±7.72%	0.4430
acid phosphatase	+0.06%±5.79%	0.9904	+3.11%±3.71%	0.3172	+6.16%±4.97%	0.1509	+1.68%±2.23%	0.4430
CPK	+0.15%±14.09%	0.9904	+7.91%±9.44%	0.3549	+15.67%±12.65%	0.1509	+4.28%±5.70%	0.4430
LDH	+0.08%±7.92%	0.9904	+4.48%±5.35%	0.3549	+8.89%±7.17%	0.1509	+2.42%±3.22%	0.4430
sodium	+0.72%±0.74%	0.3054	+0.21%±0.63%	0.7136	-0.29%±1.09%	0.7670	-0.11%±0.38%	0.7531
progesterone	0.20%±18.65%	0.9904	-8.86%±10.58%	0.3549	-17.53%±14.15%	0.1509	-4.79%±6.39%	0.4430
mean	+1.57%±8.76%	0.6894	+3.22%±9.49%	0.3228	+4.87%±12.29%	0.2353	1.99%±5.63%	0.3823

Table 2: Weight and ovarian epithelium karyorrhexis (OK) score mean levels and Std. Dev. of groups.

Groups	Variable	Mean	Std. Dev
A	Weight	243 g	45.77724 g
	OK	without lesions 0.1	0.3162278
B	Weight	262 g	31.10913 g
	OK	without lesions 0.2	0.6324555
C	Weight	242.8 g	29.33636 g
	OK	without lesions 0	0
D	Weight	243 g	32.84644 g
	OK	without lesions 0	0

is transformed as follows: (0-0.499) without lesions, (0.5-1.499) the mild lesions, (1.5 -2.499) the moderate lesions and (2.5-3) the serious lesions damage, because the study concerns score ranges rather than point scores.

Model of Ischemia-Reperfusion Injury

Control groups: 20 control rats of mean weight 252.5 g [Std. Dev: 39.31988 g] suffered by ischemia for 45 min followed by reperfusion.

Group A: Reperfusion which lasted 60 min concerned 10 controls rats of mean weight 243 g [Std. Dev: 45.77724 g], mean without OK lesions score 0.1 [Std. Dev: 0.3162278] (Table 2).

Group B: Reperfusion which lasted 120 min concerned 10 controls rats of mean weight 262 g [Std. Dev: 31.10913 g], mean without OK lesions score 0.2 [Std. Dev: 0.6324555] (Table 2).

Erythropoietin group: 20 rats of mean weight 242.9 g [Std. Dev: 30.3105 g] suffered by ischemia for 45 min followed by reperfusion in the beginning of which 10 mg Epo/kg body weight were IV administered.

Group C: Reperfusion which lasted 60 min concerned 10 Epo rats of mean weight 242.8 g [Std. Dev: 29.33636 g], mean without OE lesions score 0 [Std. Dev: 0] (Table 2).

Table 3: Statistical significance of mean values difference for groups (DG) after statistical paired t test application for weight and Wilcoxon signed-rank test for scores.

DG	Variable	Difference	p-value
A-B	Weight	-19 g	0.2423
	OK	without lesions -0.1	0.9407
A-C	Weight	0.2 g	0.9900
	OK	without lesions 0.1	0.3173
A-D	Weight	0 g	1.0000
	OK	without lesions 0.	0.3173
B-C	Weight	19.2 g	0.2598
	OK	without lesions 0.2	0.3173
B-D	Weight	19 g	0.1011
	OK	without lesions 0.2	0.3173
C-D	Weight	-0.2 g	0.9883
	OK	without lesions 0	1.0000

Group D: Reperfusion which lasted 120 min concerned 10 Epo rats of mean weight 243 g [Std. Dev: 32.84644 g], mean without OK lesions score 0 [Std. Dev: 0] (Table 2).

Results

Initially, everyone from 4 rats weight groups was compared with each other from 3 remained groups applying statistical paired t-test (Table 3). Any emerging significant difference among OK scores was investigated whether owed in the above mentioned significant weight correlations. Also, everyone from 4 rats OK scores groups was compared with each other from 3 remained groups applying statistical Wilcoxon signed-rank test. (Table 3). Applying generalized linear models (glm) with dependant variable the OK scores and independent variables the Epo administration or no, the reperfusion time and their interaction, resulted in: Epo administration non-significantly decreased the OK scores by 0.15 without lesions

Table 4: The decreasing influence of erythropoietin in connection with reperfusion time.

		p-values		
Decrease	**95% c. in**	**Reperfusion time**	**Wilcoxon**	**glm**
without lesions 0.1	-0.3100922 - 0.1100922	1h	0.3173	0.3306
without lesions 0.15	-0.371518 - 0.071518	1.5h	0.1574	0.1785
without lesions 0.2	-0.6201844 - 0.2201844	2h	0.3173	0.3306
without lesions -0.05	-0.1763341 – 0.2763341	reperfusion time	0	0.6573
without lesions -0.15	-0.3790278 - 0.0790278	reperfusion time	0.1574	0
without lesions 0.0818182	-0.2159977 – 0.0523614	interaction	0	0.2246

Table 5: Concise presence of the decreasing influence of erythropoietin in connection with reperfusion time.

Decrease	**95% c. in**	**Reperfusion time**	**p-values**
without lesions -0.1	-0.3100922 - 0.1100922	1h	0.3239
without lesions -0.15	-0.371518 - 0.071518	1.5h	0.1679
without lesions -0.2	-0.6201844 - 0.2201844	2h	0.3239
without lesions +0.1	-0.27768095 – 0.14211844	reperfusion time	0.4073
without lesions -0.0818182	-0.2159977 – 0.0523614	interaction	0.2246

[-0.371518 - 0.071518] (p= 0.1785). This finding was in accordance with the results of Wilcoxon signed-rank test (p=0.1574). Reperfusion time non-significantly increased the OK scores by 0.05 without lesions [-0.1763341 - 0.2763341] (P=0.6573), approximately in accordance with Wilcoxon signed-rank test increased result by 0.15 without lesions [-0.3790278 - 0.0790278] (p=0.1574). However, Epo administration and reperfusion time together non-significantly decreased the OK scores by 0.0818182 without lesions [-0.2159977 - 0.0523614] (p=0.2246). Reviewing the above and table 3, the tables 4 and 5 sum up concerning the decreasing influence of Epo in connection with reperfusion time. Inserting the rats weight as independent variable at glm, a non significant relation turns on (p=0.5797), so as to further investigation is not needed.

Discussion

The following clinical situations show the association between ischemia and ok lesions. Isik S et al. found less karyorrhexis [4] lesions by local antithrombin treatment in hepatic IR injury Wistar rats. Takizawa Y et al. considered [5] 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. found[6] 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. observed [7] nuclear karyorrhexis and/or karyopyknosis with cytoplasmic hypereosinophilia in neurons of the actuate 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. closely associated [8] pontosubicular neuron necrosis and its pathological peculiarity neuronal apoptosis as one of perinatal HI brain injury with presence of karyorrhexis. Hargitai B et al. associated [9] preterm birth with HI encephalopathy including neuronal karyorrhexis mostly at diencephalon and brain stem. Hallak M et al. associated [10] 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. induced[11] 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-ischemia had increased hippocampal nuclear karyorrhexis on histology than controls. Meng SZ et al. manifested [12] neuronal karyorrhexis more predominant in preterm infants with HI basal ganglia necrosis. Fortuna S et al. observed [13] neuronal degeneration and necrosis with nuclear pyknosis and karyorrhexis in a model of mildly HI brain injury. Khera KS et al. noted a pleiotropic karyorrhexis in third or embryonic phase of embryo toxic pathogenesis [14], appeared aggravated, presumably by the preceding second or labyrinthine degeneration of the placental phase in rats embryos. Squier M et al. considered the reactive astrocytosis, macrophage infiltration, karyorrhexis and endothelial swelling or reduplication as criteria [15] for white matter ischemia in early neonatal brains who were stillborn or died due to cerebral palsy. Kalimo H et al. found karyorrhexis and cytorrhexis and removal of their remnants subsequently by macrophages in the great majority of medium-sized neurons of caudate nucleus and putamen [16] after 2-3 days IR injury.

The following situations show the association between Epo and ischemic ovaries. Mahmoodi M et al. found [17] that Epo reduced IR injury and free radical production, increasing follicle survival and function in transplanted ovarian tissue. Sayyah-Melli M et al. determined[18] that rEpo was effective in reducing the oxidative damage of ovarian torsion in operated patients, 18-35 years old, with signs and symptoms of ovarian torsion. Karaca M et al. evaluated [19] the Epo administration as effective in reversing tissue damage induced by IR in ovaries of adult female rats. Suzuki H et al. demonstrated [20] that administration of asialo Epo could effectively enhance the survival of the follicles of transplanted crypreserved ovaries in frozen-thawed canine ovarian xenotransplantation. However, David RB et

al. did not detect [21] expression of Epo mRNA in porcine ovaries. Kristiansson B et al. concluded [22] that females with carbohydrate-deficient glycoprotein syndrome type I have primary ovarian failure, but the syndrome does not affect the terminal charged carbohydrate portion in Epo. Hyttinen JM et al. generated [23] a transgenic calf from in vitro produced bovine embryos microinjected with a gene construct consisting of genomic sequences encoding human Epo. Kamiński M claimed [24] that apoptosis regulates the atrophy of completely developed organs, e.g. thymus, and the hormonal restructuring of ovaries and others but on the other hand, the development of apoptosis is arrested by so called "survival factors" as Epo.

Conclusion

Epo administration interacted or not with reperfusion time non-significantly short-term decreased the OK scores. Perhaps, a longer study time than 2 hours or a greater Epo dosage may provide more significant effects.

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