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The Effect of Erythropoietin on Oviductal Congestion during Ischemia Reperfusion Injury in Rats

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Abstract

Introduction: This experimental study examined the effect of erythropoietin on a rat model and particularly in an oviductal ischemia reperfusion (IR) protocol. The effects of that molecule were studied pathologically using mean oviductal congestion (OC) lesions. Materials and methods: 40 rats of mean weight 247.7g were used in the study. OC lesions were evaluated at 60 min (groups A and C) and at 120min (groups B and D) of reperfusion. Erythropoietin was administered only in groups C and D. Results: Epo administration significantly decreased the OC scores by 0.3 without lesions [-0.6200848 - 0.0200848] (p= 0.0496). Reperfusion kept non-significantly increased the OC scores by 0.25 without lesions [-0.4759001 - 0.1759001] (P=0.2831). Together, the Epo administration and reperfusion time non significantly decreased the OC scores by 0.1090909 without lesions [-0.3078424 - 0.0896606] (p=0.2735).

Conclusion: Epo administration significantly decreased the OC score lesions in oviducts, however, within the same grade class. A longer study time or a higher Epo dose may reveal more significant results.

Keywords: Ischemia; Erythropoietin; Oviductal congestion; Reperfusion

Introduction

Erythropoietin (Epo) is generally one of the more well studied growth factors. Epo implicates over 29001 known biomedical studies at present. 3.42% at least of these studies concern tissue ischemia-reperfusion (IR) experiments. Certainly, important progress has been made concerning the Epo usage in reversing the IR kind of transient or permanent injuries including adjacent organs and certainly patients' health. Nevertheless, satisfactory answers have not been provided yet to basic questions, as, its action velocity, the administration timing and the dosage. The concept is to forward the knowledge away from the original action of Epo in stem blood cells recovery. However, just few related reports were found, not covering completely more specific matters. A numeric evaluation of the Epo efficacy was yielded by a meta-analysis of 31 published seric variables, based on the same experimental setting, at the same endpoints (Table 1). The special aim of this experimental work was to study the effect of Epo on a rat model and mainly in an oviductal IR protocol. The effect of Epo molecule was tested by evaluating the mean oviductal congestion (OC) 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 consumables, equipment and substances, were a courtesy of Experimental Research Centre of ELPEN Pharmaceuticals Co. Inc. S.A. at Pikermi, Attiki. Appropriate humanistic care was adopted for Albino female Wistar rats. Normal housing in laboratory 7 days before the experiment included ad libitum diet. Post-experimental euthanasia did not permit awakening and preservation of the rodents. They were randomly delivered to four experimental groups by 10 animals in each one. Ischemia for 45min followed by reperfusion for 60min (group A). Ischemia for 45min followed by reperfusion for

120min (group B). Ischemia for 45min followed by immediate Epo intravenous (IV) administration and reperfusion for 60min (group C). Ischemia for 45min followed by immediate Epo IV administration and reperfusion for 120min (group D). The molecule Epo dosage was 10mg/Kg body weight of animals. Prenarcosis of animals proceeded of nonstop intra-experimental general anesthesia, oxygen supply, electrocardiogram and acidometry [1-6]. The protocol of IR was followed. Ischemia was caused by laparotomic forceps clamping inferior aorta over renal arteries for 45min. The clamp removal restored the inferior aorta patency and reperfusion. The molecules were administered at the time of reperfusion, through catheterized inferior vena cava. The OC evaluations were performed at 60min of reperfusion (for groups A and C) and at 120min of reperfusion (for groups B and D). The mean weight of the forty (40) female Wistar albino rats used was 247.7g [Standard Deviation (SD): 34.99172g], with min weight ≥165g and max weight <320g. Rats' weight could be potentially a confusing factor, e.g. the more obese rats to have greater OC scores lesions. This assumption was investigated. Also, detailed pathologic [7] study and grading of OC findings was performed by scores, this is: 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) 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. OC scores were measured by 1st Department of Pathology at Department of Clinical-Laboratory studies in Faculty of Medicine of Athens University.

Table 1: The erythropoietin (Epo) influence (+-SD) on the levels of some seric1 variables concerning reperfusion (rep) time.

Variable	1h rep	p-value	1.5h rep	p-value	2h rep	p-value	interaction of Epo and rep	p-value
White BCC	+24.01%±13.38%	0.1012	+22.09%±9.11%	0.0163	+20.17%±12.94%	0.0902	+14.63%±5.40%	0.0080
Red BCC	+1.45%±3.31%	0.6589	+0.37%±3.02%	0.9048	-0.70%±4.68%	0.8844	+0.81%±1.79%	0.6446
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
Hemoglobi [6]	+4.09%±5.20%	0.3350	+2.15%±2.63	0.4527	+0.20%±5.08%	0.9584	+1.31%±1.59%	0.3984
MCH	+0.01%±1.29%	0.9904	+0.67%±0.80%	0.3549	+1.34%±1.08%	0.1509	-0.36%±0.47%	0.4430
MCV [5]	+0.01%±1.08%	0.9904	+0.56%±0.66%	0.3549	+1.12%±0.91%	0.1509	+0.30%±0.39%	0.4430
MCHC [3]	+1.82%±0.56%	0.0076	+1.73%±0.50%	0.0016	+1.65%±0.92%	0.0721	+0.89%±0.31%	0.0061
RbcDW	-1.85%±4.24%	0.6703	-1.64%±2.53%	0.5159	-1.43%±3.34%	0.6078	-1.06%±1.43%	0.4733
Plt C [2]	-7.32%±13.11%	0.5219	-2.14%±8.04%	0.7581	+3.04%±10.78%	0.7204	-0.16%±4.76%	0.9725
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
Creatinine	-0.10%±9.78%	0.9904	-4.84%±5.78%	0.3721	-9.59%±7.74%	0.1509	-2.62%±3.49%	0.4430
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 protei	-0.02%±2.47%	0.9904	-1.27%±1.51%	0.3721	-2.52%±2.03%	0.1509	-0.68%±2.48%	0.4430
Albumins	-4.61%±4.21%	0.2530	-9.28%±3.20%	0.0054	-13.96%±5.03%	0.0095	-5.37%±2.73%	0.0072
ALT	+18.89%±12.42%	0.1372	+7.63%±18.94%	0.6396	-3.63%±25.19%	0.8617	+8.03%±11.36%	0.4698
AST	+29.53%±9.72%	0.0096	+26.71%±13.17%	0.0235	+23.89%±21.59%	0.1709	+19.73%±7.70%	0.0119
γGT	-19.35%±18.58%	0.2362	-12.70%±13.11%	0.3541	-6.06%±19.96%	0.7800	-4.62%±7.97%	0.5534
ALP	+0.20%±18.57%	0.9904	+10.70%±12.78%	0.3549	+21.20%±17.11%	0.1509	+5.79%±7.72%	0.4430
ACP	+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
CK-MB [4]	+0.08%±7.90%	0.9904	+4.28%±5.11%	0.3721	+8.49%±6.85%	0.1509	+2.32%±3.09%	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
Potassium	-6.17%±4.94%	0.1540	-2.21%±3.66%	0.5134	+1.74%±5.43%	0.7299	+0.18%±2.22%	0.9338

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Calcium	0.28%±1.19%	0.8065	-0.56%±1.13%	0.5761	-1.41%±2.08%	0.4100	-0.34%±0.68%	0.6095
Phosphorus	+1.92%±5.25%	0.6982	+3.95%±3.35%	0.2100	+5.98%±4.81%	0.2930	+2.45%±2.01%	0.2168
Magnesium	+1%±6.20%	0.8596	-1.09%±3.34%	0.7248	-3.19%±3.90%	0.3729	-0.19%±1.93%	0.9197
Amylase	+6.50%±9.15%	0.4161	+5.04%±6.12%	0.3831	+3.59%±8.42%	0.6649	+4.36%±3.65%	0.2258
Progesteron	-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	+2.20%±10.08%	0.5712	+2.58%±9.20%	0.3660	+2.96%±10.43%	0.3586	+2.18%±5.98%	0.4110

Model of ischemia-reperfusion injury

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

Table 2: Weight and oviductal congestion (OC) score mean levels and Std. Dev. of groups.

Groups	Variable	Mean	Std. Dev
A	Weight	243g	45.77724g
	OC	mild 0.5	0.5270463
B	Weight	262g	31.10913g
	OC	without lesions 0.4	0.6992059
C	Weight	242.8g	29.33636g
	OC	without lesions 0	0
D	Weight	243g	32.84644g
	OC	without lesions 0.3	0.4830459

Group A: Reperfusion lasted for 60min (n=10 controls rats) mean mass 243g [SD: 45.77724 g], mean mild OC score 0.5 [SD: 0.5270463] (Table 2).

Group B: Reperfusion lasted for 120 min (n=10 controls rats) mean mass 262g [SD: 31.10913 g], mean without lesions

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

Group C: Reperfusion lasted for 60min (n=10 Epo rats) mean mass 242.8g [SD: 29.33636g], mean without OC score 0 [SD: 0] (Table 2).

Group D: Reperfusion lasted for 120min (n=10 Epo rats) mean mass 243g [SD: 32.84644g], mean without lesions OC score 0.3 [SD: 0.4830459] (Table 2).

Statistical analysis

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

DG	Variable	Difference	p-value
A-B	Weight	-19g	0.2423
	OC	without lesions 0.1	0.7055
A-C	Weight	0.2g	0.9900
	OC	mild 0.5	0.0253
A-D	Weight	0g	1.0000
	OC	without lesions 0.2	0.3173
B-C	Weight	19.2g	0.2598
	OC	without lesions 0.4	0.0842
B-D	Weight	19g	0.1011
	OC	without lesions 0.1	0.5637
C-D	Weight	-0.2g	0.9883
	OC	without lesions -0.3	0.0833

Every weight and OC lesions score group was compared with each other from 3 remained groups applying statistical standard t-tests and Wilcoxon signed-rank tests respectively (Table 3). If any probable significant difference among OC lesions score was raised, it would be investigated whether owed in any respective probable significant mass one (Table 3). Then, the application of generalized linear models (glm) was followed. It included as dependant variable the OC lesions scores. The 3 independent variables were the Epo administration or no, the reperfusion time and their interaction. Inserting the rats' weight as independent variable at glm, a non significant relation turned on with OC scores lesions (p=0.0585), so as to further investigation was not needed. The statistical analysis was performed by Stata 6.0 software [Stata 6.0, StataCorp LP, Texas, USA] [5].

Results

The glm resulted in: Epo administration significantly decreased the OC scores by 0.3 [-0.6200848 - 0.0200848] (p= 0.0654). This finding was crepant with the results of Wilcoxon signed-rank test (p= 0.0339). Reperfusion time kept non-significantly increased the OC scores by 0.1 [-0.2332897 - 0.4332897] (P=0.5472), approximately in accordance with the Wilcoxon signed-rank test result increased by 0.4 [-0.7185105 - -0.0814895] (P=0.0190). However, Epo administration and reperfusion time in combination non-significantly decreased the OC scores by 0.1090909 [-0.3078424 - 0.0896606] (p= 0.2735). The above and Table 3 are summed in Tables 4 & 5 [6,7].

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

Decrease	95% c. in.	p-values Reperfusion time	Wilcoxon	glm
mild 0.5	-0.850153-0.1498463	1h	0.0253	0.0077
without lesions 0.3	-0.6200848 - 0.0200848	1.5h	0.0339	0.0654
without lesions 0.1	-0.6646058 - 0.4646058	2h	0.5637	0.7142

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without lesions -0.1	-0.2332897 - 0.4332897	reperfusion time		0.5472
without lesions -0.4	-0.7185105 -0.0814895	reperfusion time	0.0190	
Without lesions 0.1090909	-0.3078424 - 0.0896606	interaction		0.2735

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

Decrease	95% C. In.	Reperfusion Time	P-Values
mild 0.5	-0.8501537 -0.1498463	1h	0.0165
without lesions 0.3	-0.6200848 - 0.0200848	1.5h	0.0496
without lesions 0.1	-0.6646058 - 0.4646058	2h	0.6389
without lesions -0.25	-0.4759001 - 0.1759001	reperfusion time	0.2831
without lesions 0.1090909	-0.3078424 - 0.0896606	interaction	0.2735

Discussion

The following situations show the association between ischemia and congestion in oviducts. Ajayi OL et al. [8] observed severe congestion, hyperemia, edema, dilatation and devitalization in the affected portion of an oviductal 360 degrees volvulus clockwise around the dorsal ligament during routine postmortem examination in an 11 months old chicken (Gallus gallus domesticus). Gordts S et al. [9] elucidated the process of human ovum retrieval by fimbriae. The fimbriae on the ovulatory side appeared congested, tumescent and showed pulsatile movements synchronous with the heartbeat. Vascular congestion causing erection and pulsatile movements of the fimbriae play a role in the retrieval of the ovum. Tuffrey M et al. [10] suggested that severe mucus congestion accompanied by tubal edema and loss of ciliated epithelia play a major role in the aetiology of chlamydial-induced tubal damage. Kleinstein J et al. [11] supposed that the oviduct damage caused only by the mechanical influence of the secretion congestion is the reason for the unfavorable pregnancy rate after salpingoneostomy of a chronic atrophied hydrosalpinx.

Thus, congestion is associated with Epo not only in oviducts but also in different tissues. Rashed FK et al. [12] showed short-term protective efficacy of Epo in rat testicular IR injury although vascular congestion [12], edema, hemorrhage and acute inflammation were observed in some groups. McMurray JJ et al. [13] described the long duration heart failure in patients treated by α-darbepoetin who had more signs of congestion. Lagarto A et al. [14] showed weak edema and vascular congestion in the right nostril of all control and treated Wistar rats groups, after 15μl Epo administration, similar to that produced in the brain during hypoxia. Zheng L et al. [15] might improve aortic stenosis induced pulmonary congestion in patients treating pre-operative aortic valve replacement with rhEpo in a mouse model. Piloto N et al. [16] supposed heart failure as sudden death cause in died rat's tissues presented [16] with brain vascular congestion. Naito Y et al. [17] investigated the mechanisms of cardiac remodeling induced by 20 weeks iron deficiency anemia promoting lung congestion, with decreased serum Epo concentration. Kiris I et al. [18] showed that Epo significantly decreased the focal glomerular necrosis, dilation of Bowman's capsule, degeneration and necrosis of tubular epithelium, interstitial inflammatory infiltration and congestion of blood vessels induced by aortic IR in rats. Minamishima YA et al. [19] demonstrated premature mortality associated with marked venous congestion in mice lacking enzyme PHD2. Lee TH et al. [20] noted splenomegaly caused by the congestion of red pulp in a mouse model deficient in peroxiredoxins II-/-, although healthy in appearance and fertility. Ruschitzka FT et al. [21] treated polyglobulic transgenic mice over expressing hEpo by NO synthase inhibitor N(G)-nitro-L-arginine methyl ester (L-NAME) and revealed acute left ventricular dilatation and vascular engorgement associated with pulmonary congestion. Gentz EJ et al. [22] revealed pulmonary congestion resulting from polycythemia 74% due to high serum Epo concentration in a llama.

Specially, oviducts and Epo are associated in the following situations. Lappin T [23] supposed that the beneficial effects of hEpo may extend to organs such as ovaries, oviducts, uterus which has Epo receptors. Sasaki R et al. [24] claimed that Epo is both estrogen inducible and produced in oviducts. Masuda S et al. [25] found E2 and hypoxia induced, transient, rapidly down-regulated stimulation of Epo mRNA in oviductal ampulla and isthmus regions. The E2 action is probably mediated through the E2 receptor and de novo protein synthesis is not required for E2 induced Epo mRNA. Ochiai H et al. [26] attained the synthesis of hEpo protein attempting localized *in-vivo* plasmid DNA gene transfer in laying chicken oviducts.

Conclusion

Epo administration decreased the OC score lesions in oviducts, however, within the same grade class. A longer study time or a higher Epo dose may reveal more significant results.

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