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The Erythropoietin Effect on Uterus Congestion after Uterine Ischemia Reperfusion

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Abstract

Objective: This experiment investigated the erythropoietin (Epo) effect after uterine ischemia-reperfusion (IR) in rats. The effect of Epo was evaluated studying the mean uterine congestion (UC) lesions.

Materials and methods: The mean weight of 40 rats used in the study was 247.7 g. The UC lesions were estimated for the groups A and C on 60 min and for the groups B and D on 120 min after reperfusion. Only the groups C and D were administered by Epo.

Results: Epo administration non-significantly declined the UC lesions scores by (without lesions) 0.15 (-0.5595137-0.2595137) (p=0.4545). Reperfusion time non-significantly raised the UC lesions scores by (without lesions) 0.15 (-0.5676974-0.3676974) (P=0.5058). However, the combined Epo administration with reperfusion time non-significantly declined the UC lesions scores by (without lesions) 0.0090909 (-0.2577992-0.2396174) (p=0.9414).

Conclusions: The Epo administration presented a non-significantly declining short-term effect on UC lesions scores. Perhaps, a higher Epo dosage and/or an experimental time lasting longer than 2 hours may reveal more significant efficacies.

Keywords: Ischemia; Erythropoietin; Uterus congestion lesions; Reperfusion

Introduction

Erythropoietin (Epo) belongs to the most occupied growth factor in biomedical studies. It implicates over 29,207 such studies at present; the 3.45% at least of which concern tissue ischemia-reperfusion (IR) models. A popular aim of Epo usage is the reverse potency of IR transient injuries of organs, including their tissues and certainly patients' health. However, satisfactory responses have not yet been received concerning basic affairs, such as, the dosage height, the administration timing, and the action velocity. The knowledge must be promoted besides the original action of Epo in red blood cells production. These specific matters require more detailed management. A numeric estimation of Epo trends was revealed by a meta-analysis of 33 published studies concerning serum variables, yielded by the present experiment (**Table 1**).

Table 1 The erythropoietin (Epo) influence (±SD) on the levels of some seric [1] 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%	0.008
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

Hemoglobin	+4.09% ± 5.20%	0.335	+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%	0.443
MCV	+0.01% ± 1.08%	0.9904	+0.56% ± 0.66%	0.3549	+1.12% ± 0.91%	0.1509	+0.30% ± 0.39%	0.443
MCHC	+1.82% ± 0.56%	0.0076	+1.73% ± 0.50%	0.0016	+1.65% ± 0.92%	0.0721	+0.89% ± 0.31%	0.0061
RBC DW	-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	-7.32% ± 13.11%	0.5219	-2.14% ± 8.04%	0.7581	+3.04% ± 10.78%	0.7204	-0.16% ± 4.76%	0.9725
MPV	+3.82% ± 4.10%	0.3105	-0.12% ± 2.13%	0.9513	-4.07% ± 3.75%	0.2608	-0.27% ± 0.92%	0.7585
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
Glucose	+0.75% ± 8.11%	0.9307	+5.59% ± 6.46%	0.3208	+10.44% ± 10.99%	0.3491	+4.94% ± 3.81%	0.1892
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%	0.443
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
Albumins	-4.61% ± 4.21%	0.253	-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%	0.78	-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%	0.443
ACP	+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
CK-MB	+0.08% ± 7.90%	0.9904	+4.28% ± 5.11%	0.3721	+8.49% ± 6.85%	0.1509	+2.32% ± 3.09%	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
Potassium	-6.17% ± 4.94%	0.154	-2.21% ± 3.66%	0.5134	+1.74% ± 5.43%	0.7299	+0.18% ± 2.22%	0.9338
Calcium	0.28% ± 1.19%	0.8065	-0.56% ± 1.13%	0.5761	-1.41% ± 2.08%	0.41	-0.34% ± 0.68%	0.6095
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
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%	0.443
Mean	+2.20% ± 9.77%	0.5742	+2.58% ± 8.93%	0.3823	+2.97% ± 10.26%	0.3554	+2.18% ± 5.83%	0.4148

This experiment tried to estimate the Epo action on a rat setting of IR using the mean uterine congestion (UC) lesions scores.

Materials and Methods

Animal preparation

This biomedical research received the 3693/12 November 2010 and 14/10 January 2012 licenses by the East Attiki Prefecture Vet Address. Elpen Pharmaceuticals Co Inc SA

granted all consumables, facilities and equipment at Pikermi, Attiki. Pure humanistic care was provided for Albino female Wistar rats. Pre-experimental normal housing included continuous ad libitum feeding in laboratory. Euthanasia excluded the post-experimental survival and preservation of the animals. The 40 rats were randomly assigned to four equal groups. The quoting protocols of IR were used: 45 min ischemia and then 60 min reperfusion for group A; 45 min ischemia and then 120 min reperfusion for group B; 45 min ischemia and then 60 min concurrent Epo (Epoetin, rhEpo$_{\alpha}$, Janssen-Cilag, Beerse, Belgium) intravenous (IV) administration with reperfusion for group C; 45 min ischemia and then 120 min concurrent Epo IV administration with reperfusion for group D. The Epo dosage was assessed at 10 mg/Kg [1], mass per animal. Prenarcosis, general anesthesia, non-stop intra-experimental oxygen supply, electrocardiogram and acidometry are also confirmed in related references. Laparotomic clamping with forceps of inferior abdominal aorta over the renal arteries level, induced ischemia for 45 min. The forceps removal was restoring the inferior aorta reperfusion patency. Blood flow exclusions were iterated for every animal. Epo was administered starting reperfusion via inferior vena cava catheter. The UC lesions scores were estimated at 60th min of reperfusion for A and C groups groups and at 120th min of reperfusion for B and D groups. 40 female Wistar brand albino rats with mean body mass (M:) of 247.7 g (Std. Dev[SD]: 36.59703 g) were used. The mass range was fluctuated between 165 g and 320 g. Rats' body mass could be practically a confusing factor, e.g. the more obese rats were supposed to have more pronounced UC lesions scores. This assumption was statistically investigated with grading of UC lesions findings. Detailed pathologic classification [2] was performed by scores: 0 without lesions, 1 mild ones, 2 moderate ones and 3 serious ones. The previous classification was transformed as: (0-0.499) without lesions, (0.5-1.499) the mild ones, (1.5-2.499) the moderate ones and (2.5-3) the serious one's scores since non-integer estimations were appeared. UC lesions scores were estimated by the 1st Pathology Department of Clinical-Laboratory Sector at Faculty of Medicine in Athens University.

The Ischemia-Reperfusion Injury Model

Control groups

The 20 control rats with M: 252.5 g (SD: 39.31988 g) were submitted into ischemia lasting 45 min and then into reperfusion.

A group: Reperfusion lasting 60 min featured 10 control (placebo) rats of M: 243 g [SD: 45.77724 g] and mean mild UC score 1.4 (SD: 0.5163978) (**Table 2**).

Table 2 Weight and uterus congestion (UC) score mean levels and Std. Dev. of groups.

Groups	Variable	Mean	Std. Dev
A	Weight	243 g	45.77724 g
	UC	mild 1.4	0.516398
B	Weight	262 g	31.10913 g
	UC	mild 1.1	0.316228
C	Weight	242.8 g	29.33636 g
	UC	mild 0.9	0.567646
D	Weight	243 g	32.84644 g
	UC	mild 1.3	0.948683

B group: Reperfusion lasting 120 min featured 10 control (placebo) rats of M: 262 g (SD: 31.10913 g) and mean mild UC score 1.1 (SD: 0.3162278) (**Table 2**).

Erythropoietin group

The 20 Epo rats with mean mass 242.9 g (SD: 30.3105 g) were submitted into ischemia lasting 45 min and then into reperfusion on its beginning 10 mg Epol/kg body mass were IV provided.

C group: Reperfusion lasting 60 min featured 10 Epo rats of M: 242.8 g (SD: 29.33636 g) and mean mild UC score 0.9 (Std. Dev: 0.5676462) (**Table 2**).

D group: Reperfusion lasting 120 min featured 10 Epo rats of M: 243 g (SD: 32.84644 g) and mean mild UC score 1.3 (SD: 0.9486833) (**Table 2**).

Statistic Analysis

The bodies mass and UC lesions scores columns were compared each other by the statistic standard t-test and by the statistic Wilcoxon signed-rank test respectively (**Table 3**).

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	-19 g	0.2423
	UC	without lesions 0.3	0.0833
A-C	Weight	0.2 g	0.99
	UC	mild 0.5	0.0951
A-D	Weight	0 g	1
	UC	without lesions 0.1	0.6547
B-C	Weight	19.2 g	0.2598
	UC	without lesions 0.2	0.3173
B-D	Weight	19 g	0.1011
	UC	without lesions-0.2	0.5948
C-D	Weight	-0.2 g	0.9883

	UC	without lesions-0.4	0.3632

Any raised significant difference among UC scores, was investigated whether being due to any significant mass one. The generalized linear models (GLM) test with dependent variable the UC scores and independent variables, first the drug Epo or no drug administration, second the reperfusion time and third both the interacted variables were applied. The statistic calculations were performed by the Stata 6.0 software (Stata 6.0, StataCorp LP SA, Texas, USA).

Results

The Epo administration non-significantly declined the UC scores by (without lesions) 0.15 (-0.5595137-0.2595137) (p=0.4629). This result was accordant with the one of Wilcoxon signed-rank test (p=0.4461). The reperfusion time variable non-significantly augmented the UC scores by (without lesions) 0.05 (-0.3621388-0.4621388) (P=0.8073), nearly in accordance with one of Wilcoxon signed-rank test 0.25 (-0.773256-0.273256) (P=0.2043). However, the interaction of Epo administration with reperfusion time non-significantly declined the UC scores by (without lesions) 0.0090909 (-0.2577992-0.2396174) (p=0.9414). The co-evaluation of the above results and **Table 3**, yields the **Tables 4 and 5** regarding the declining influence of Epo vs reperfusion time.

Considering the rats' weight as a more independent variable of GLM, a non-significant correlation appeared (p=0.5769).

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

			p-values	
Alteration	**95% c. in.**	**Reperfusion time**	**Wilcoxon**	**GLM**
mild 0.5	-1.01966- 0.0098314	1h	0.0951	0.0541
without lesions 0.15	-0.5595137-0.2595137	1.5h	0.4461	0.4629
without lesions-0.2	-0.4643699-0.8643699	2h	0.5948	0.535
without lesions +0.05	-0.3621388-0.4621388	reperfusion time	-	0.8073
without lesions +0.25	-0.773256-0.273256	reperfusion time	0.2043	-
without lesions-0.0090909	-0.2577992-0.2396174	interaction	-	0.9414

Table 5 Synoptic presence of the alteration influence of erythropoietin in connection with reperfusion time.

Alteration	**95% c. in.**	**Reperfusion time**	**p-values**
mild 0.5	-1.009831-0.0098314	1h	0.0746
without lesions 0.15	-0.5595137-0.2595137	1.5h	0.4545
without lesions-0.2	-0.4643699-0.8643699	2h	0.5649
without lesions +0.15	-0.5676974-0.3676974	reperfusion time	0.5058
without lesions-0.0090909	-0.2577992-0.2396174	interaction	0.9414

Discussion

The contribution of ischemia in UC is investigated. Salas postulated [3] that secondary compressing of cerebral congestion by the large uterus, diverts blood to the brain, causing eclamptic convulsions. Surcel et al. showed that uterus fibroma has always been accompanied by pelvic congestion inducing [4] experimentally estrogen tumors in animals. Douglas observed liver and renal glomerular congestion both in pregnant and non-pregnant rats producing [5] hypertension, however, only in pregnant ones. Thus, tissue congestion is associated with Epo in different tissues besides uterus. Rashed et al. proved short-term protective efficacy of Epo after vascular congestion [6] among other findings in rat testicular IR injury. McMurray et al. presented [7] the baseline characteristics of patients with α-darbepoetin, long-term heart failure and signs of marked congestion. Lagarto et al. showed [8] signs of a minimal irritation consisting of weak edema with vascular congestion into the right nostril, after 15 μl Epo administration; alike the one induced in Wistar rats brain during hypoxia. Zheng et al. got on [9] improving aortic stenosis patients' cardiac hypertrophy, pulmonary congestion and left ventricular dysfunction treating pre-operative aortic valve replacement with rhEpo administration in a mouse model. Piloto et al. implicated the heart failure as cause of sudden death when was present [10] with brain vascular congestion; left ventricular hypertrophy and elevated hematocrit in rats. Naito et al. implicated decreased serum Epo concentration for [11] the cardiac remodeling mechanisms induced by iron deficiency anemia promoting cardiac fibrosis and lung congestion. Kiris et al. proved [12] that Epo significantly decreased (P=0.05 versus aortic IR) the focal renoglomerular necrosis, the Bowman's capsule dilatation, the tubular epithelium degeneration, the tubular epithelium necrosis, interstitial tissues inflammatory cells infiltration and the blood vessels congestion upon aortic IR in rats. Minamishima et al. associated [13] the premature mortality with pronounced venous congestion and dilated cardiomyopathy in enzyme PHD2 lack mice. Lee et al.

implicated [14] the red pulp congestion for splenomegaly in peroxiredoxins II-/- deficient mice, although healthy in appearance and fertility. Ruschitzka et al. treated [15] the acute left ventricular dilatation, vascular engorgement, pulmonary congestion and hemorrhage in polyglobulic transgenic mice overexpressing human Epo by NO synthase inhibitor. Gentz et al. implicated polycythemia 74% for [16] pulmonary congestion due to high serum Epo concentration in a llama.

Conclusion

Epo administration generally short-term non-significantly declines the UC lesions scores. Perhaps, a higher Epo dose and/or an experimental time lasting longer than 2 hours may reveal more significant efficacies.

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