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

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Abstract

Background: This experimental study examined the effect of erythropoietin (epoetin alfa) on rat model and particularly in an ovarian ischemia reperfusion (IR) protocol. The effects of that molecule were studied pathologically using mean ovarian congestion (OC) lesions.

Materials and methods: 40 rats of mean weight 247.7 g, 16-18 weeks old, were used in the study. OC lesions were evaluated after 45 min ischemia, 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: Epo administration kept non-significantly increased the OC scores without lesions (p = 0.2954). Reperfusion time kept non-significantly increased the OC scores without lesions (p = 0.8063). However, Epo administration and reperfusion time together kept non-significantly increased the OC scores without lesions (p = 0.3882).

Conclusions: Epo administration, reperfusion time and their interaction kept non-significantly increased the OC scores. Epo short-term restored the congestive lesions from significant to non-significant level.

Keywords

Ischemia, Erythropoietin, Ovarian congestion, Reperfusion

Introduction

Permanent or transient damage with serious implications on adjacent organs and certainly on patients' health may be due to tissue ischemia and reperfusion (IR). Important progress has been made regarding the usage of erythropoietin (Epo) Epoetin alfa [EPREX (151118) API] of Janssen-Cilag Pty Ltd with CAS Registry Number: 113427-24-0 which is human erythropoietin produced in cell culture using recombinant DNA technology, authorized by the European Medicines Agency on 28 August 2007. Although Epo managed 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 at what 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 18 published seric variables, coming from the same experimental setting, tried to provide a numeric evaluation of the Epo efficacy at the same endpoints (Table 1). Furthermore, several 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 kind of effects that molecule provokes, were studied by evaluating mean ovarian congestion (OC) lesions. Kim J et al. experienced [1] an extremely rare case of acute abdomen pain induced by OC triggered by the fallopian tube accompanying a paratubal cyst coiling around the utero-ovarian ligament. Kaido Y et al. revealed [2] that an elongated right fallopian tube accompanied by a paratubal cyst coiling tightly 2.5 times round the right ovary, caused apparent congestion and enlargement of the right ovary in a pregnant woman for her right lower abdominal pain at 30 weeks of gestation. Soon after the congested right ovary was released from the coiling of the fallopian tube, the congestion subsided. Yassin S et al.. associated [3] OC changes with injectable contraceptives and related them with cycle control.

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Table 1: The erythropoietin (Epo) influence (± SD) on the levels of some seric [1] variables concerning reperfusion (rep) time .

Variable	1 h rep	p-value	1.5h rep	p-value	2 h rep	p-value	interaction of Epo and rep	p-value
White BC	+ 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
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
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
ALT[2]	+ 18.89% ± 12.42%	0.1372	+ 7.63% ± 18.94%	0.6396	- 3.63% ± 25.19%	0.8617	+ 8.03% ± 11.36%	0.4698
γGT[3]	- 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
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
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
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	+ 2.39% ± 10.96%	0.6131	+ 3.11% ± 10.01%	0.3210	+ 3.82% ± 11.86%	0.2897	+ 2.48% ± 6.25%	0.3696

SD: Standard Deviation; rep: Reperfusion

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

Groups	Variable	Mean	Std. Dev
A	Weight	243 g	45.77724 g
A	OC	moderate lesions 1.6	1.074968
B	Weight	262 g	31.10913 g
B	OC	moderate lesions 1.9	0.9944289
C	Weight	242.8 g	29.33636 g
C	OC	moderate lesions 2.1	0.5676462
D	Weight	243 g	32.84644 g
D	OC	moderate lesions 2	0.8164966

Std. Dev: Standard Deviation

Table 3: Statistical significance of mean values difference for groups 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
A-B	OC	without lesions -0.3	0.6009
A-C	Weight	0.2 g	0.9900
A-C	OC	mild -0.5	0.3183
A-D	Weight	0 g	1.0000
A-D	OC	without lesions -0.4	0.4974
B-C	Weight	19.2 g	0.2598
B-C	OC	without lesions -0.2	0.5632
B-D	Weight	19 g	0.1011
B-D	OC	without lesions -0.1	0.6310
C-D	Weight	-0.2 g	0.9883
C-D	OC	without lesions 0.1	0.6547

DG: difference for groups

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. Everything needed for the study including consumables, equipment and substances were a courtesy of Experimental 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 ad libitum diet. Post-experimental awakening and preservation of the rodents was not permitted, even if euthanasia was needed. 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, diluted in 2 ml water of injection.

The detailed preceded pre narcotic and general anesthesiologic techniques of animals are described in related references [4-6]. Oxygen supply, electrocardiogram and acidiometry were continuously provided during whole experiment performance.

The protocol of IR was followed. Ischemia was caused by laparotomic forceps clamping inferior aorta over renal arteries for 45 min. Reperfusion was induced by removing the clamp and reestablishment of inferior aorta patency. The molecules were administered at the time of reperfusion, through catheterized inferior vena cava. The OC evaluations were performed at 60 min of reperfusion (for groups A and C) and at 120 min of reperfusion (for groups B and D). The mean weight of the forty (40) female Wistar albino rats used was 247.7 g [Std. Dev: 34.99172 g], with min weight ≥ 165 g and max weight ≤ 320 g. Rats' weight could be potentially a confusing factor, e.g. the more obese rats to have higher OC score lesions (Table 2). This suspicion was investigated. Also, detailed pathological [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) mild lesions, (1.5 -2.499) moderate lesions and (2.5-3) serious lesions damage, because the study concerns score ranges rather than point scores.

Model of ischemia-reperfusion injury

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

Group A: Reperfusion lasted for 60 min (n = 10 controls rats) mean mass 243 g [Std. Dev: 45.77724 g], mean moderate OC lesions score 1.6 [Std. Dev: 1.074968] (Table 2).

Group B: Reperfusion lasted for 120 min (n = 10 controls rats) mean mass 262 g [Std. Dev: 31.10913 g], mean moderate OC lesions score 1.9 [Std. Dev: 0.9944289] (Table 2).

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

Group C: Reperfusion lasted for 60 min (n = 10 Epo rats) mean

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

Increase	95% c. in.	Reperfusion time	Wilcoxon sign rank	glm
mild 0.5	-0.3076338 - 1.307634	1 h	0.3183	0.2098
without lesions 0.3	-0.256345 - 0.856345	1.5 h	0.3090	0.2819
without lesions 0.1	-0.7548341 - 0.9548341	2 h	0.6310	0.8086
without lesions 0.1	-0.4640457 - 0.6640457	reperfusion time	0.8909	0.7217
without lesions 0.1454545	-0.1918887 - 0.4827978	interaction	-	0.3882

c. in: Confidence intervals

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

Increase	95% c. in.	Reperfusion time	p-values
mild 0.5	-0.3076338 - 1.307634	1 h	0.2640
without lesions 0.3	-0.256345 - 0.856345	1.5 h	0.2954
without lesions 0.1	-0.7548341 - 0.9548341	2 h	0.7198
without lesions 0.1	-0.4640457 - 0.6640457	reperfusion time	0.8063
without lesions 0.1454545	-0.1918887 - 0.4827978	interaction	0.3882

c. in: Confidence intervals

mass 242.8 g [Std. Dev: 29.33636 g], mean moderate OC lesions score 2.1 [Std. Dev: 0.5676462] (Table 2).

Group D: Reperfusion lasted for 120 min (n = 10 Epo rats) mean mass 243 g [Std. Dev: 32.84644 g], mean moderate OC lesions score 2 [Std. Dev: 0.8164966] (Table 2).

Statistical analysis

Weight comparison of everyone from 4 rats groups initially was performed with each other from 3 remained groups applying statistical paired t-test (Table 3). Any emerging significant difference among OC scores lesions was investigated whether owed in the above probable significant weight correlations. OC scores lesions comparison of everyone from 4 rats groups initially was performed with each other from 3 remained groups applying Wilcoxon signed-rank test (Table 3). The application of generalized linear models (glm) with dependant variable the OC scores lesions and independent variables the Epo administration or no, the reperfusion time and their interaction were followed. Inserting the rats weight as independent variable at glm, a non significant relation turned on with OC scores lesions (p = 0.7774), so as to further investigation was not needed.

Results

The glm resulted in: Epo administration kept non-significantly increased the OC scores by 0.3 without lesions [-0.256345 - 0.856345] (p = 0.2819). This finding was in accordance with the results of Wilcoxon signed-rank test (p = 0.3090). Reperfusion time kept non-significantly increased the OC scores by 0.1 without lesions [-0.4640457 - 0.6640457] (P = 0.7217), also in accordance with the Wilcoxon signed-rank test result (P = 0.8909). However, Epo administration and reperfusion time together kept non-significantly increased the OC scores by 0.1454545 without lesions [-0.1918887 - 0.4827978] (p = 0.3882). Reviewing the above and table 3, table 4 and table 5 sum up concerning the alteration influence of Epo in connection with reperfusion time.

Discussion

The following situations show the association between ischemia and congestion in ovaries. Akdemir A et al. found [8] congestion among histopathological findings in torsion groups after induced ovarian IR injury in rats. Sapmaz-Metin M et al. assessed [9] congestion as apparent among histopathological changes following IR injury in rat ovaries. Aslan MK et al. examined [10] ovarian and periovarian congestion after IR injury. Aran T et al. evaluated [11] high vascular congestion scores after ovarian torsion in female adult Sprague-Dawley rats. Coskun A et al. indicated [12] a gradually increased congestion correlated with respective increased ischemic times for tied ovaries in rats. Kart A et al. observed [13] severe congestion on ovary IR injury in rabbits. Cigremis Y et al. observed [14] severe congestion in the torsion-detorsion female rabbits group. Smorgick N et al. imaged [15] the pathological series of increased ovarian congestion by ultrasound at twisted IR ovaries and necrosis in menstrually cycling women. Kazez A et al. assessed congestion [16] in both ovaries after delayed ovarian torsion detorsion in female Wistar albino rats. Uguralp S et al. showed [17] congestion of contralateral ovaries in different degrees after unilateral ovarian IR in albino Wistar rats. Taskin O et al. showed [18] prominent congestion on all sections 36 h after detorsion of IR adnexa in cycling female rats. Cavender JL et al. paralleled [19] congestion chronologically by evidence of ischemia during ovulation in ewe.

The following situations show the association between Epo and ischemic ovaries. Mahmoodi M et al. found [20] that Epo reduced IR injury and free radical production, increasing follicle survival and function in transplanted ovarian tissue. Sayyah-Melli M et al. determined [21] that Epo 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 [22] the Epo administration as effective in reversing tissue damage induced by IR in ovaries of adult female rats. Suzuki H et al. demonstrated [23] that administration of a sialo Epo could effectively enhance the survival of the follicles of transplanted cryopreserved ovaries in frozen-thawed canine ovarian xenotransplantation. However, David RB et al. did not detect [24] expression of Epo mRNA in porcine ovaries. Kristiansson B et al. concluded [25] 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 [26] a transgenic calf from in vitro produced bovine embryos microinjected with a gene construct consisting of genomic sequences encoding human Epo. Kamiński M claimed [27] 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 , reperfusion time and their interaction kept non-significantly increased the OC scores. Epo short-term restored the congestive lesions from significant to non-significant level. This may contribute to relief in subsided congestion at situations as decoiled paratubal cysts or side effects of contraceptives.

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