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 = 0.2695). 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.

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) on rat model 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 arising from the uterus-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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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 ELLEN 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 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 acidimetry were continuously provided during whole experiment performance.

The protocol of IR was followed. Ischemia was caused by laparotomy 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 confounding 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 1.6 [Std. Dev: 31.10913 g] (Table 2).

Erythropoietin group: 20 Epo rats (mean mass 242.9 g [Std. Dev: 45.77724 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 mass 242.9 g [Std. Dev: 45.77724 g], mean moderate OC lesions score 1.6 [Std. Dev: 31.10913 g] (Table 2).

Group D: Erythropoietin group (n = 10 Epo rats) experienced ischemia for 45 min followed by reperfusion for 120 min of reperfusion (for groups A and C) and at 120 min of reperfusion (for Group 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 confounding 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.
Table 4: The restoring influence of erythropoietin in connection with reperfusion time.

<table>
<thead>
<tr>
<th>Increase</th>
<th>95% c. in.</th>
<th>Reperfusion time</th>
<th>Wilcoxon sign rank</th>
<th>glm</th>
</tr>
</thead>
<tbody>
<tr>
<td>mild 0.5</td>
<td>-0.3076338 - 1.307634</td>
<td>1 h</td>
<td>0.3183</td>
<td>0.2998</td>
</tr>
<tr>
<td>without lesions 0.3</td>
<td>-0.256345 - 0.856345</td>
<td>1.5 h</td>
<td>0.3090</td>
<td>0.2819</td>
</tr>
<tr>
<td>without lesions 0.1</td>
<td>-0.7548341 - 0.9548341</td>
<td>2 h</td>
<td>0.6310</td>
<td>0.8086</td>
</tr>
<tr>
<td>without lesions 0.1</td>
<td>-0.4640457 - 0.6640457</td>
<td>reperfusion time</td>
<td>0.8086</td>
<td>0.2819</td>
</tr>
<tr>
<td>without lesions 0.1454545</td>
<td>-0.1918887 - 0.427978</td>
<td>interaction</td>
<td>-</td>
<td>0.3882</td>
</tr>
</tbody>
</table>

c. i.n: Confidence intervals

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

<table>
<thead>
<tr>
<th>Increase</th>
<th>95% c. in.</th>
<th>Reperfusion time</th>
<th>p-values</th>
</tr>
</thead>
<tbody>
<tr>
<td>mild 0.5</td>
<td>-0.3076338 - 1.307634</td>
<td>1 h</td>
<td>0.2640</td>
</tr>
<tr>
<td>without lesions 0.3</td>
<td>-0.256345 - 0.856345</td>
<td>1.5 h</td>
<td>0.2954</td>
</tr>
<tr>
<td>without lesions 0.1</td>
<td>-0.7548341 - 0.9548341</td>
<td>2 h</td>
<td>0.7198</td>
</tr>
<tr>
<td>without lesions 0.1</td>
<td>-0.4640457 - 0.6640457</td>
<td>reperfusion time</td>
<td>0.8063</td>
</tr>
<tr>
<td>without lesions 0.1454545</td>
<td>-0.1918887 - 0.427978</td>
<td>interaction</td>
<td>0.3882</td>
</tr>
</tbody>
</table>

c. i.n: Confidence intervals


The following situations show the association between Epo and ischemic ovaries. Mahmooodi 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 sielo 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 miRNA 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 microinjectected 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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References
