The Co-Evaluation of Ovarian Epithelium Edema and Congestion after the Erythropoietin effect on Ovarian Ischemia Reperfusion Injury

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ABSTRACT

Aim: This study co-evaluated the 2 quoted histological variables after the erythropoietin (Epo) administration. The calculation was based on the results of 2 preliminary studies, each one evaluating a respective histological variable of ovarian epithelium edema (OE) or congestion (OC) in an induced ischemia reperfusion animal experiment.

Materials and methods: The 2 main experimental endpoints at which the OE and OC scores were evaluated was the 60th reperfusion min (for the groups A and C) and the 120th reperfusion min (for the groups B and D). Specially, the groups A and B were processed without drugs, whereas the groups C and D after Epo administration.

Results: The first preliminary study showed that Epo non significantly recessed the ovarian epithelium edema (OE) within the “without lesions alterations” grade by 0.1272727 ± 0.4530022 (p-value=0.4339). However, the second preliminary study showed that Epo non significantly enhanced the ovarian congestion (OC) within the “without lesions alterations” grade by 0.1454545 ± 0.4827978 (p-value=0.3882). These 2 studies were co-evaluated since they came from the same experimental setting. This study investigated the combined diagnostic value of both variables together.

Conclusions: Epo has a hardly deteriorating potency of these histologic parameters within the “without lesions alterations” grade by 0.0090909 ± 0.2586031 (p-value=0.9456) since they were co-evaluated together.

Keywords: ischemia, ovarian epitheliumedema, congestion, erythropoietin, reperfusion

INTRODUCTION

Erythropoietin (Epo) was investigated whether having antioxidant capacities. 2 histological variables in an ovarian ischemia reperfusion (OIR) experiment was tested for this purpose. The one variable was that of ovarian epithelium edema (OE), which was non significantly recessed within the “without lesions alterations”
grade by 0.1272272 [-0.4530022 - +0.1984567] (p-value=0.4339)1. The other variable was that of ovarian congestion (OC) but was significantly enhanced within the “without lesions alterations” grade by 0.1454545 [-0.1918887 - +0.4827978] (p-value=0.3882)2. Although Epo is met in over 30,606 published biomedical studies, only a 3.57% of them negotiate its antioxidant capacities. The present experimental work tried to co-evaluate these OE and OC variables together and to compare its outcome with each one separately, from the same rat induced OIR protocol.

MATERIALS AND METHODS

Animal Preparation

This study received 2 ethics committee approvals under the 3693/12-11- 2010 & 14/10-1-2012 numbers fully following the tenants of the Declaration of Helsinki. The granting company, the experiment location and the Pathology Department are mentioned in preliminary references1,2. The human animal care of Albino female Wistar rats, the 7 days pre-experimental ad libitum diet, the non-stop intra-experimental anesthesiologic techniques, the acidometry, the electrocardiogram and the oxygen supply and post-experimental euthanasia are also described in preliminary references. Rats were 16 – 18 weeks old. They were randomly assigned to four (4) groups consisted in N=10.

The stage of 45 min ischemia was common for all 4 groups. Afterwards, reperfusion of 60 min was followed in group A: reperfusion of 120 min in group B; immediate Epo intravenous (IV) administration and reperfusion of 60 min in group C; immediate EpoIV administration and reperfusion of 120 min in group D. The dose height assessment was described at preliminary studies as 10 mg/Kg body mass. Ischemia was caused by laparotomic clamping the inferior aorta over renal arteries with forceps for 45 min. The clamp removal was restoring the inferior vena cava catheter. The OE and OC scores were determined at 60th min of reperfusion (for A and C groups) and at 120th min of reperfusion (for B and D groups). Relation was raised between animals’ mass with neither OE scores (p-value=0.8726); nor with OC ones (p-values=0.7816). The pathologic score grading was maintained the same as in preliminary studies: (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.

MODEL OF ISCHEMIA-REPERFUSION INJURY

Control Groups

The 20 control rats were the same for preliminaries and this study.

Group A

Reperfusion which lasted 60 min concerned 10 controls rats of combined OE and OC (OE&OC) score as the mean of OE score and OC one (Table 1).

Group B

Reperfusion which lasted 120 min concerned 10 controls rats of combined OE&OC (cOE &OC) score as the mean of OE and OC one (Table 1).

Epo group

The 20 Epo rats were the same for preliminaries and this study.

Group C

Reperfusion which lasted 60 min concerned 10 Epo rats of cOE &OC score as the mean of OE score and OC one (Table 1).

Group D

Reperfusion which lasted 120 min concerned 10 L rats of cOE &OC score as the mean of OE score and OC one (Table 1).

Table1. Ovarian epithelium edema(OE), ovarian congestion (OC) and their mean and SD scores

<table>
<thead>
<tr>
<th>Group</th>
<th>Mean OE score +SD</th>
<th>Mean OC score +SD</th>
<th>Mean OE&amp;OC score +SD</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>mild lesions 0.7±0.8232726</td>
<td>moderate lesions 1.6±1.074968</td>
<td>mild lesions 1.15±0.7835106</td>
</tr>
<tr>
<td>B</td>
<td>mild lesions 1.1±0.9944289</td>
<td>moderate lesions 1.9±0.9944289</td>
<td>mild lesions 1.5±0.8164966</td>
</tr>
<tr>
<td>C</td>
<td>mild lesions 0.5±0.7071068</td>
<td>moderate lesions 2.1±0.5676462</td>
<td>mild lesions 1.3±0.5868939</td>
</tr>
<tr>
<td>D</td>
<td>mild lesions 0.7±0.8232726</td>
<td>moderate lesions 2±0.8164966</td>
<td>mild lesions 1.35±0.5797509</td>
</tr>
</tbody>
</table>

Statistical Analysis

CoE & OC groups score was compared with each other from 3 remained groups applying Wilcoxon on signed-rank test (Table 2). Then, the
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generalized linear models (glm) were applied with dependent variables the cOE & OC scores, and independent variables the Epo administration or no, the reperfusion time and their and the interaction.

Table2. The values difference for groups (DG) after Wilcoxon signed-rank test for mean OE&OC scores.

<table>
<thead>
<tr>
<th>DG</th>
<th>Difference</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-B</td>
<td>+0.35</td>
<td>0.3513</td>
</tr>
<tr>
<td>A-C</td>
<td>+0.15</td>
<td>0.6787</td>
</tr>
<tr>
<td>A-D</td>
<td>+0.2</td>
<td>0.5009</td>
</tr>
<tr>
<td>B-C</td>
<td>-0.2</td>
<td>0.4914</td>
</tr>
<tr>
<td>B-D</td>
<td>-0.15</td>
<td>0.6009</td>
</tr>
<tr>
<td>C-D</td>
<td>+0.05</td>
<td>0.5994</td>
</tr>
</tbody>
</table>

Table3. The alteration influence of erythropoietin in connection with reperfusion time p-values

<table>
<thead>
<tr>
<th>Reperfusion time</th>
<th>95% c. in.</th>
<th>Wilcoxon</th>
<th>Glm</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1.5h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>2h</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>3h</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

RESULTS

Epo administration hardly non significantly deteriorated the cOE &OC scores within the “without lesions alterations” by0.025 [-0.42442325 - +0.47442325] (p=0.8958) after co-calulation by the same methods. However, Epo administration and reperfusion time together also hardly deteriorated the cOE &OC scores within the “without lesions alterations” by 0.02 [-0.63224825 - +0.2767849] (p=0.3034) after co-calulation by the same methods. However, Epo administration and reperfusion time together also hardly deteriorated the cOE &OC scores within the “without lesions alterations” grade by 0.0090909 [-0.2586031 - +0.2767849] (p-value=0.9456) since they were co-evaluated together. A concise form of the above findings is depicted at table 4.

Table4. Concise form of the table 3

<table>
<thead>
<tr>
<th>Reperfusion time</th>
<th>95% c. in.</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1h</td>
<td></td>
<td>0.4151</td>
</tr>
<tr>
<td>1.5h</td>
<td></td>
<td>0.8958</td>
</tr>
<tr>
<td>2h</td>
<td></td>
<td>0.7375</td>
</tr>
<tr>
<td>3h</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

DISCUSSION

Kolusari A et al improved3 the survival of follicles, determined significantly higher levels of E2 in ovarian grafts most likely by reducing ischemic injury, by improving neangiogenesis, and by its antioxidant effects. Follicle counts in the EPO group were significantly higher than those in the untreated group (P ≤ 0.05) after condensated Epo administration in auto transplanted rat ovaries. Mahmoodi M et al found the mean total volume of ovary, cortex, medulla, the number of follicles, the follicle survival and function and the concentration of E2 increased whereas, apoptosis rate and the concentration of MDA decreased significantly in the auto grafted EPO-treated group than in the auto grafted placebo one (P<0.01) reducing the IR injury in grafted ovaries of Naval Medical Research Institute mice. Ma YS et al found the number of apoptosis cells decreased in rhEPO treated group (P < 0.01) than I/R group. rhEPO showed effects to inhibit the apoptosis of fetal neural cells and the expression of Caspase-3 protein due to intrauterine hypoxic-ischemic brain tissue injury. Ma YS et al found6 the expression of caspase-3, the death rate of fetal rats and the number of fetal rat brain cells apoptosis decreased in rhEPO treated groups (P < 0.05) than the I/R group in an intrauterine hypoxic-ischemic injury. Task in MI et al evaluated7 the tissue and serum TOS levels and OSI levels
markedly decreased. The ovarian protective effect of 2-APB appears to be mediated through its antiapoptotic and antioxidative effects in experimental I/R injury in rat ovaries.

Stanley JA et al have shown8 that edaravone mitigated or inhibited the effects of CrVI on follicle atresia, pubertal onset retardation, steroidogenesis hormone levels and AOX enzyme activity, as well as the expression of Bcl2 and Bcl21 in the ovary; whereas increased E2 restored CrVI-induced depletion of glutathione peroxidase 1, catalase, thioredoxin 2, and peroxiredoxin 3 in the ovary of female Sprague Dawley rats. Yapca OE et al found9 that etoricoxib [a selective cyclooxygenase (COX)-2 inhibitor] prevented oxidative damage induced with I/R that may arise with reperfusion by detorsion in rat ovarian tissue. Yapca OE et al10 suggested that thiamine pyrophosphate may be useful in the prevention of IR-related infertility in diabetic rats. Celik M et al ameliorated11 I/R injury by sildenafil treatment in an ovarian tissue rat model. Gungor AN et al observed that omeagven improved12 the detrimental effects of ovarian IR in torsioned - detorsioned ovaries. Kurt RK et al revealed13 that colchicine significantly reduced catalase activities and thus ovarian ischemia-reperfusion injury in experimental rat ovarian torsion model up to 5 days. Dokuyucu R et al found14 the numbers of primordial follicles (p=0.006) and primary follicles (p=0.036) increased whereas the mean levels of (Total Oxidant Status) TOS and (Oxidative Stress Index) decreased in groups that received erdosteine and/or alpha lipoic acid ALA than the detorsion group in an experimental rat ovarian IR torsion model injury. Keskin Kurt R et al revealed that zofenopril attenuated injury in an experimental model of ovarian IR torsion in rats. Guven S et al observed16 that the elevated serum ischemia-modified albumin IMA levels with high sensitivity-specificity values in women with ovarian torsion seem to have a potential role as a serum marker in the preoperative diagnosis of ovarian torsion in emergency settings and significantly distinguished patients with or without ovarian torsion. Yurtcu E et al found17 statistically significant dose-dependent decreased edema and follicle degeneration, with vascular congestion, hemorrhage and follicle degeneration in vardenafil treatment groups attenuating ischemia-reperfusion induced ovary injury in a rat model. Türk E et al considered18 hypothermia as effective in inhibiting inflammatory responses and also ischemia/reperfusion injury perhaps by inhibiting the production of oxidative stress in ovaries subjected to torsion/detorsion injury. Yıldırım Ş et al reduced19 hemorrhage, edema and vascular dilatation after proanthocyanidin administration known as free radical scavenger, antioxidant and protective against tissue damage induced by IR in rat ovaries. Mete Ural Ü et al reversed20 the biochemical, histopathological and immune histochemical alterations, alleviated the injury and attenuated ovarian ischemia and ischemia/reperfusion injury after thymoquinone administration in rats. Aksak Karamese S et al normalized21 values after beta-carotene treatment which is a potent antioxidant in an experimental ischemia-reperfusion groups model. Sayar I et al suggested22 that ozone (O) and ellagic acid (EA) are effective against an ovarian torsion-detorsion I/R injury. Eser A et al showed23 that curcumin exerted no major significant protective effect on ischemia-reperfusion injury in the rat ovary female Wistar albino rats. Bayır Y et al concluded24 that aliskiren [a direct renin inhibitor] treatment is effective in reversing IR induced ovary damage via the improvement of cytokine and oxidative stress, reduction of inflammation and suppression of the renin-angiotensin aldosteron system in rat ovaries. Esteban-Zubero E et al proved25 melatonin as a potentially useful therapeutic tool in the reduction of graft rejection. Its benefits are based on its direct actions as a free radical scavenger as well as its indirect antioxidative actions in the stimulation of the cellular antioxidant defense system. Moreover, it has significant anti-inflammatory activity. Melatonin has been found to improve the beneficial effects of preservation fluids when they are enriched with the indoleamine.

Yao D et al described carthamus tinctorius26 in prescriptions and composite to promote blood circulation, remove blood stasis, regulate menstruation, alleviate pain, significantly promote ovarian granulosa cell proliferation with the effects of ant oxidation. Tuncer AA et al evaluated27 the combination of alpha-lipoic acid and coenzyme Q10 having beneficial effects on oxidative stress induced by ischemia-reperfusion injury related with rat model of ovarian torsion. Naykı UA et al significantly decreased28 severe hemorrhage, degeneration, inflammatory signs in the follicular cells and markedly ameliorated increased apoptosis, caused by IR in rats ovarian tissue. Ugurel V et al significantly retained29 severe acute inflammation, polynuclear leukocytes, macrophages, stromal edema, hemorrhage, degenerative changes in the ovary PCNA (+) cell numbers; decreasing lipid per oxidation products and leukocytes aggregation after treatment with erdosteine in adnexal torsion of ovarian IR injury in rats. Pınar N et al found
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catalase levels significantly increased whereas MDA levels significantly lower in the I/R + tempol.p group. Tempol can be used for reducing ovarian I/R injury in female Wistar albino rats. Güleç Başer B et al found vascular congestion, hemorrhage, polymorph nuclear neutrophils interstitial edema and the number of apoptotic cells lower in PG group. Preoperative PG treatment might exert protective effects in ovarian IR injury through its anti-apoptotic and antioxidant properties. Melekoglu R et al evaluated the serum follicle-stimulating hormone levels significantly reduced, the serum anti-Müllerian hormone levels significantly increased and the histopathological scores ameliorated in rats treated with Chrysin and Glycyrrhetinic Acid preventing I/R injury in rat adnexal torsion detorsion procedure. A numeric evaluation of the erythropoietin efficacies was provided by a meta-analysis of 35 seric variables of complete blood count and blood chemistry tests versus reperfusion time coming from the same experimental setting (table 5).

Table 5. The erythropoietin influence (+SD) on the levels of 35 seric variables of complete blood count and blood chemistry tests versus reperfusion (rep) time

<table>
<thead>
<tr>
<th>Variables</th>
<th>1h rep</th>
<th>p-value</th>
<th>1.5h rep</th>
<th>p-value</th>
<th>2h rep</th>
<th>p-value</th>
<th>interaction of Epo and rep</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>+3.39%±12.15%</td>
<td>0.5636</td>
<td>+4.44%±14.50%</td>
<td>0.3711</td>
<td>+5.49%±18.55%</td>
<td>0.3496</td>
<td>+2.83%±7.13%</td>
<td>0.4045</td>
</tr>
</tbody>
</table>

CONCLUSION

Epo has a slight deteriorating potency for ovarian epithelium edema and congestion together (p-values=0.9456) discouraging for beneficial usage in situations such as the survival of follicles in ovarian grafts, the follicle atresia, the pubertal onset retardation, the steroidogenesis hormone levels, the follicle degeneration and inflammatory responses inhibition and the adnexal torsion detorsion procedure.

ACKNOWLEDGEMENT

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REFERENCES


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