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The co-evaluation of endosalpingeal karyorrhexis and salpingitis after the erythropoietin effect on fallopian ischemia reperfusion injury

Endosalpingeal karyorrhexis ve salpingitis 'nin eritropoietin etkisinin ardından ortak değerlendirilmesi

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Abstract

Aim: This study co-evaluated the 2 quoted histologic variables after the cytokine erythropoietin (Epo) administration. The calculation was based on the results of 2 preliminary studies, each one evaluating a respective histologic variable of endosalpingeal karyorrhexis (EK) or salpingitis (S) in an induced ischemia reperfusion (IR) animal experiment.

Methods: The 2 main experimental endpoints at which the EK and S scores were evaluated, were the reperfusion 60th min (for A & C groups) and the reperfusion 120th min (for B & D groups). 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 hardly non-significantly increased the EK scores by the grade "without lesions" 0.0181818 [-0.0679319 - +0.1042955] (p-value=0.6715). The other preliminary study found that Epo did not influence the S scores (p-value=1.0000). Both studies were co-estimated since they belong to the same experimental setting. This study co-evaluated the combined diagnostic values of both variables together.

Conclusions: Epo again hardly non significantly increased both scores for these histologic parameters at the grade of "without lesions" 0.0090909 [-0.0339659 - +0.0521478] (p-value=0.6715) since they were co-evaluated together.

Keywords: Ischemia, Erythropoietin, Endosalpingeal karyorrhexis, Salpingitis, Reperfusion

Öz

Amaç: Bu çalışma sitokin eritropoietin (EPO) uygulamasından sonra 2 atfedilen histolojik değişkenleri ortaklaşa değerlendirdi. Hesaplama, her biri endosalpingeal karyorrhexis (EK) veya salpingitis (S) bir indüklenen iskemi reperfüzyonu (IR) hayvan denemesi içinde ilgili histolojik değişken değerlendiren 2 ön çalışmanın sonuçlarını temel aldı.

Yöntemler: EK ve S puanlarının değerlendirilmesi gereken 2 ana deneysel uç noktası reperfüzyon 60 dk (A & C grupları için) ve reperfüzyon 120 dk (B & D grupları için) idi. Özel olarak, A ve B grupları ilaç olmadan işlendi, oysa C ve D EPO uygulamasından sonra işlendi.

Bulgular: İlk ön çalışmada EPO çok önemli ölçüde anlamlı "lezyonlar olmadan" 0,0181818 [-0,0679319-+ 0,1042955] (p-değer = 0,6715) notu tarafından ek puanları artış gösterdi. Diğer ön çalışmada EPO, S puanlarını etkilemez bulundu (p-değer = 1,0000). Her iki çalışmada aynı deneysel ayarlara sahip olduğundan birlikte tahmin edilmiştir. Bu çalışma her iki değişkenin kombine tanı değerlerini birlikte değerlendirir.

Sonuç: EPO yine de çok önemli ölçüde bu histolojik parametreler için "lezyonlar olmadan" 0,0090909 [-0,0339659-+ 0,0521478] (p-değer = 0,6715) sınıfında birlikte değerlendirildiğinde her iki skoru artmıştır.

Anahtar kelimeler: İskemi, Eritropoietin, Endosalpingeal karyorrhexis, Salpenjit, Reperfüzyon

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Introduction

Erythropoietin (Epo) was investigated whether having antioxidant capacities. 2 histologic variables in a fallopian ischemia reperfusion (FIR) experiment were tested for this purpose. The one variable was that of endosalpingeal karyorrhexis (EK) which was recessed by "without lesions" 0.0181818+0.04393556 (p-value=0.6715) [1]. The other variable was that of salpingitis (S) but did not influence the S scores (p-value=1.0000) [2]. Although Epo is met in over 29,975 published biomedical studies, only a 3.52% of them negotiate its antioxidant capacities. The present experimental work tried to co-evaluate these EK and S variables together and to compare its outcome with each one separately, from the same rat induced FIR protocol.

Materials and methods

Animal management

The Vet No 3693/12-November-2010 & 14/10-January-2012 licenses, the auspices company, the experimental location and the Pathology Department are mentioned in preliminary references 1, 2. The human animal care of female Wistar Albino rats, the one week pre-experimental ad libitum diet, the 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 common stage of 45 min ischemia was preceded in all 4 groups. Afterwards, 60 min reperfusion was followed in group A; 120 min in group B; immediate Epo intravenous (IV) administration and 60 min reperfusion in group C; and immediate Epo IV administration 120 min in group D. The dose height was assessed at pre-experimental phase as 10 mg/Kg body mass.

Ischemia was induced by laparotomic clamping the inferior aorta upper the renal arteries level with forceps for 45 min. The forceps removal was restoring the inferior aorta blood patency and reperfusion. Epo was administered at the time of reperfusion; through an inferior vena cava catheter. The EK and S scores were determined at 60th min of reperfusion (for A and C groups) and at 120th min of reperfusion (for B and D groups). The pathologic score grading was maintained the same as in preliminary studies: (0-0.499) grade without lesions, (0.5-1.499) grade mild lesions, (1.5-2.499) grade moderate lesions and (2.5-3) grade serious lesions damage. Relation was rised between animals' mass with neither EK scores (p-value=0.7202) nor with S ones (p-values=1.0000).

Table 1: Endosalpingeal karyorrhexis (EK), salpingitis (S) and their mean and SD scores

	Mean EK score ±SD	Mean S score ±SD	Mean EK&S score ±SD
Group A	without lesions 0±0.00	without lesions 0±0.00	without lesions 0±0.00
Group B	without lesions 0±0.00	without lesions 0±0.00	without lesions 0±0.00
Group C	without lesions 0.2±0.421637	without lesions 0±0.00	without lesions 0.1±0.2108185
Group D	without lesions 0±0.00	without lesions 0±0.00	without lesions 0±0.00

The ischemia-reperfusion injury model

Placebo groups

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

Group A

60 min reperfusion concerned 10 placebo rats of combined EK and S (EK&S) score as the mean of EK score and S one (Table 1).

Group B

120 min reperfusion concerned 10 placebo rats of combined EK&S (cEE&S) score as the mean of EK and S one (Table 1).

Epo group

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

Group C

60 min reperfusion concerned 10 Epo rats of cEK&S score as the mean of EK score and S one (Table 1).

Group D

120 min reperfusion concerned 10 Epo rats of cEK&S score as the mean of EK score and S one (Table 1).

Statistical analysis

Successive comparisons among the 4 cEK&S groups were performed applying Wilcoxon signed-rank test (Table 2). Then, the generalized linear models (glm) were applied with dependant variable the cEK&S scores. Epo administration or no, the reperfusion time and their interaction were used as independent variables.

Table 2: The values difference for groups (DG) after Wilcoxon signed-rank test for mean EK&S scores

DG	Difference	p-value
A-B	0	1.0000
A-C	-0.1	0.1573
A-D	0	1.0000
B-C	-0.1	0.1573
B-D	0	1.0000
C-D	0.1	0.1573

Results

Epo administration hardly non-significantly increased the cEK&S scores by without alterations 0.05 [-0.02084505 - 0.12084505] (p=0.1558) by both Wilcoxon signed-rank test and glm methods respectively. Reperfusion time did not influence the cEK&S scores by "without alterations" 0.00 [-0.07084505 - 0.07084505] (p=0.1558) by similar methodology. Finally, Epo administration and reperfusion time together also hardly non significantly increased the cEK&S scores by "without alterations" 0.0090909 [-0.0339659 - 0.0521478] (p-valus=0.6715) (table 3). A concise form of the above findings is depicted at table 4.

Table 3: The alteration influence of Epo in connection with reperfusion time

Alteration	95% c. in.	Reperfusion time	wilkoxon	glm p
without alterations 0.1	-0.0508105 0.2508105	1h	0.1573	
without alterations 0.1	-0.0400615 0.2400615	1h		0.1510
without alterations 0.05	-0.0196642 0.1196642	1.5h		0.1544
without alterations 0.05	-0.0220259 0.1220259	1.5h	0.1573	
without alterations 0.00	0.00 - 0.00	2h	1.0000	
without alterations 0.1	-0.0400615 0.2400615	2h		0.1510
without alterations -0.05	-0.1196642 0.0196642	reperfusion		0.1544
without alterations 0.05	-0.0220259 0.1220259	reperfusion	0.1573	
without alterations 0.0090909	-0.0339659 0.0521478	interaction		0.6715

Table 4: Concise form of the table 3

Increase	95% c. in.	Reperfusion time	p-value
without alterations 0.1	-0.045436 0.245436	1h	0.1541
without alterations 0.05	-0.02084505 0.12084505	1.5h	0.1558
without alterations 0.05	-0.02003075 0.12003075	2h	0.5755
without alterations 0.00	-0.07084505 0.07084505	reperfusion	0.1558
without alterations 0.0090909	-0.0339659 0.0521478	interaction	0.6715

Discussion

Adamyan LV et al [3] considered the principal advantage of fibrin glue anastomoses than microsurgical anastomoses to reduce surgical trauma to oviduct stumps and absence of tissue ischemia. These features promote reparative regeneration and decrease adhesion formation, resulting in complete recanalization of fallopian tubes. Castadot RG [4] protected against salpingitis, other pelvic infections and against tubal pregnancies after combined oral contraceptives administration. Estrogens are clearly responsible for some of the complications, apparently due to a weakening of the fibrinolytic systems, but progestagens or estrogen-progestagen combinations are also implicated. Guennoun A et al [5] reported the case of a pregnant presenting with acute lateropelvic pain. Normal adnexal torsion is rare during pregnancy. Çılgın H et al [6] indicated that plasma heat shock protein 70 level could be used as a serum marker in the early detection of adnexal torsion since its significant increase in the study group was 1.50-fold and 1.47-fold respectively (P = 0.001) than that in the laparotomy and control groups, following 12 h of adnexal torsion. Ayachi A et al [7] reported two cases of adnexal torsion during the second trimester of pregnancy; presenting with appendix syndrome the one and acute left iliac fossa pain the other. Early treatment could avoid irreversible damages due to ischemia which could be fertility-threatening. Laparotomy revealed the torsion of a hydatid of Morgagni whose necrotic appearance due to twisting required hydatid ablation. Sukkong K et al [8] evaluated clinical risk factors predictive of torsion with gangrenous adnexa estimated at ~ 46.2%. Adnexal torsion results in ischemia of structures distal to twisted pedicle and acute onset of pain is responsible for about 3% of all gynecologic emergencies especially in young nulliparous women. Lee MH et al [9] reviewed all computed tomography signs of adnexal torsion with the exception of deviation of the uterus to the twisted side. However, for a twisted vascular pedicle, there was moderate agreement in patients with a mass and no agreement for patients without a mass. Damasceno RW et al [10] concluded a decrease in elastic fibers with ultrastructural abnormalities and an overexpression of elastin-degrading enzymes as the consequence of local ischemia, inflammation, and/or chronic mechanical stress. Aging with progressive loss of tone and laxity may affect the adnexal tissues, resulting in different clinical symptoms and signs. Spinelli C et al [11] described the conservative treatment for adnexal torsion, consisting of detorsion, as the best surgical approach to guarantee the future reproductive capacity of patients. Tunç SY et al [12] observed degeneration of epithelium, loss of cilia, dilation of blood vessels, and hemorrhages in sections of the ischemic group in the fallopian tube structure following ovarian torsion. The studied fallopian section revealed a significant decrease in density of desmin in the torsion group. Moreover, strong positive cytoplasmic CD68 expression was observed in the torsion group. Türk E et al [13] found that adnexal torsion and detorsion significantly increased the tissue level of malondialdehyde, superoxide dismutase and reduced glutathione, whereas hypothermia inhibited their production as well the histopathological changes in rats. Calis P et al [14] found only the loss of cohesion to be significantly different by 1.28-fold than control sides (p=0.017) in terms of the means of total tissue damage. Significantly lower PCNA counts were revealed in the 16-hour torsion group only in a rat model with adnexal torsion. PCNA confirms the viability of the counted follicles and appears to be a more precise approach necessary for demonstrating the functional status than net mean primordial+primary follicle count which were comparable in twisted and control sides. Navve D et al [15] associated the lateral whirlpool sign with enlarged masses the mean volume of which among cases was significantly greater by 2.81-fold than those with the medial whirlpool sign (P = 0.035). Sánez HA et al [16] described that adnexal torsion over its pedicle produces lymphatic and venous stasis, later it develops into ischemia and necrosis, when is not treated. Hirth D et al [17] identified cell necrosis by high mobility group box 1 protein and apoptosis by Caspase 3a staining of tissue samples taken at 3 endpoints postburn. Furthermore, endothelial cell necrosis was deeper than interstitial cell necrosis at 1 hour ($p < 0.001$). Endothelial cell necrosis at 1 hour divided the zone of injury progression (Jackson's zone of stasis) into an upper subzone with necrotic endothelial cells and initially viable adnexal and interstitial cells at 1 hour that progressed to necrosis by 24 hours and a lower zone with initially viable endothelial cells at 1 hour but necrosis and apoptosis of all cell types by 24 hours in a validated porcine model of vertical burn injury progression. Ozler A et al [18] found the mean number of preantral and small antral follicles lower and only AMH levels significantly decreased following the 3-hour IR ($P < 0.05$) in detorsion group than those of the sham group ($P < 0.01$). After torsion, anti-Müllerian hormone (AMH), estradiol, and inhibin B levels were decreased significantly than preoperative and postoperative periods (P = 0.032).

Table 5: The Epo influence (±SD) on the levels of 35 seric variables of complete blood count and blood chemistry tests versus reperfusion (rep) time [19]

35 Variables	1h rep	p-value	1.5h rep	p-value	2h rep	p-value	interaction of Epo and rep	p-value
Mean	+3.39%±12.15%	0.5636	+4.44%±14.50%	0.3711	+5.49%±18.55%	0.3496	+2.83%±7.13%	0.4045

A numeric evaluation of the Epo 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) [19].

Conclusion

Epo hardly non significantly increased the cEK&S scores by "without alterations" (p-values=0.6715) creating a suspicion for beneficial usage in situations such as tubal pregnancies, fertility, elastic and desmin ultrastructure, aging, tone, laxity and cohesion, regeneration of epithelium, conservation of cilia, blood vessel diameter regulation and lymphatic and venous stasis, cytoplasmic CD68, antioxidant markers, PCNA counts, mobility group box 1 protein, caspase 3a staining, anti-Müllerian hormone, estradiol and inhibin B presence or absence, ischemia, cell necrosis and apoptosis.

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