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The Rat Uterus after Erythropoietin Process

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Abstract

Aim: This study co-evaluated the 4 quoted histologic variables after Erythropoietin (Epo) administration. The calculation was based on the results of 2 preliminary studies, each one evaluating two respective histologic variables of Endometrial Edema (EE) and Uterus Inflammation (UI) or Endometrial Karyorrhexis (EK) and Uterus Congestion (UC); in an induced ischemia reperfusion animal experiment.

Materials and methods: The 2 main experimental endpoints at which the EE, UI and EK, UC 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 has a hardly enhancing potency for EE and UI together (p-values=0.7381) within the "without lesions" alterations 0.05 [-0.2504947 - 0.3504947]. The second preliminary study showed that Epo has a non-significant recessing potency for EK and UC also within the "without lesions" grade 0.1409091±0.12249148 (p-values=0.2421). These 2 studies were co-evaluated since they came from the same experimental setting. This study co-evaluated the combined diagnostic values of the four variables together.

Conclusions: Epo has a borderline significant recessing potency for all these histologic parameters within the "without lesions" grade alterations 0.2363636 [-0.4814257 - 0.0086984] (p-values=0.0583).

Keywords: Erythropoietin, Endometrial Edema, Endometrial Karyorrhexis, Ischemia, Reperfusion Uterus Congestion, Uterus Inflammation

Introduction

Erythropoietin (Epo) was investigated whether having antioxidant capacities. 4 histologic variables in a uterine ischemia reperfusion (UIR) experiment was tested for this purpose. The two variables were those of endometrial edema (EE) and uterus inflammation (UI) which were hardly enhanced (p-values=0.7381) within the "without lesions" alterations 0.05 [-0.2504947 - 0.3504947]1. The other variables were those of endometrial karyorrhexis (EK) and uterus congestion (UC) which were non-significantly recessed also within the "without lesions" grade 0.1409091±0.12249148 (p-values=0.2421)2. Although Epo is met in over 31,019 published

biomedical studies, only a 3.64% of them negotiate its antioxidant capacities. The present experimental work tried to co-evaluate these EE, UI, EK and UC variables together and to end up to their outcome totally, from the same rat induced UIR protocol.

Materials and methods

Animal preparation

The 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 references [1,2]. 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 electrocardiograms, the oxygen supply and the 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 Epo IV 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 aorta patency and reperfusion. After the blood flow interruption, the protocol of UIR were applied, as described above for each experimental group. Epo was administered at the time of reperfusion; through inferior vena cava catheter. The EE, UI, EK and UC 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 EE scores (p-value=0.0861), nor with EK scores (p-value=0.0692), nor with UC ones (p-values=0.5769), nor with UI ones (p-values=0.7954). The pathologic score grading was maintained the same as in preliminary studies: (0-0.499) without lesions, (0.5-1.499) for mild lesions, (1.5 -2.499) for moderate lesions and (2.5-3) for 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 EE, UI, EK and UC uterine score (cUS) as the mean of EE&UI scores and the EK&UC ones (Table 1).

Group B

Reperfusion which lasted 120 min concerned 10 controls rats of combined EE, UI, EK and UC uterine score (cUS) as the mean of EE&UI scores and the EK&UC ones (Table 1).

Erythropoietin 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 combined EE, UI, EK and UC uterine score (cUS) as the mean of EE&UI scores and the EK&UC ones (Table 1).

Group D

Reperfusion which lasted 120 min concerned 10 Epo rats of combined EE, UI, EK and UC uterine score (cUS) as the mean of EE&UI scores and the EK&UC ones (Table 1).

	Mean EE&UI score +SD	Mean EK&UC score +SD	Mean EE&EK&UC&UI score +SD
Group A	mild lesions 1.35 +0.6687468	mild lesions 1.2 +0.4830459	Mildlesions1.275+0.5329426
Group B	moderate lesions 1.55+0.831665	mild lesions 1.1+0.4594683	mildlesions1.325+0.6129392
Group C	mild lesions 1.45+0.8959787	mild lesions 0.85+0.6258328	mild lesions 1.15+0.7187953
Group D	mild lesions 1.4+0.7745967	mild lesions 0.9+0.875595	mild lesions 1.15+0.8181958

Table 1: Endometrial Edema (EE), Endometrial Karyorrhexis (EK), Uterus Congestion (UC) and Uterus Inflammation (UI) And Their Mean and SD Scores

Statistical analysis

Every cUS groups score was compared with each other from 3 remained groups applying Wilcoxon signed-rank test (Table 2).

DG	Difference	p-value
A-B	+0.05	0.9181
A-C	-0.125	0.6447
A-D	-0.125	0.8370
B-C	-0.175	0.4425
B-D	-0.175	0.4102
C-D	0	1.0000

Table 2: The values difference for groups (DG) after Wilcoxon signed-rank test for all histologic variables mean scores.

Then, the Generalized Linear Models (glm) were applied with dependent variable the cUS scores, and independent variables the Epo administration or no, the reperfusion time and their interaction.

Results

Epo administration non-significantly recessed the 4 histologic variables within the "without lesions alterations" score 0.275[-0.713354 - 0.163354] (p-value=0.2398), after co-calculation by both Wilcoxon signed-rank test and glm methods. Similarly, reperfusion time non-significantly recessed the 4 histologic variables within the "without lesions alterations" score 0.15 [-0.60359645 - 0.30359645] (p-value=0.4438), after co-calculation by the same methods. Totally, Epo administration and reperfusion time together borderline significantly recessed the 4 histologic variables within the "without lesions alterations" score 0.2363636 [-0.4814257 - 0.0086984] (p-value=0.0583). A concise form of the above findings is depicted at tables 3,4.

Restore	**95% c. in.**	**Reperfusion time**	**P-values**	
			Wilcoxon	**Glm**
without lesions alterations -0.125	-0.8265626 0.5765626	1h	0.6447	
without lesions alterations -0.075	-.6715472 .5215472	1h		0.7947
without lesions alterations -0.275		1.5h	0.2902	0.1894
without lesions alterations -0.175	-0.652277 0.302277	2h	0.4102	
without lesions alterations -0.1	-0.8003073 0.6003073	2h		0.7676
without lesions alterations -0.15	-0.60359645 0.30359645	reperfusion	0.4099	0.4777
Without lesions alterations -0.2363636	-0.4814257 0.0086984	interaction		0.0583

Table 3: The restoring influence of erythropoietin in connection with reperfusion time.

Restore	**95% c. in.**	**Reperfusion time**	**p-value**
without lesions alterations -0.1	-0.7490549 0.5490549	1h	0.7197
without lesions alterations -0.275	-0.713354 0.163354	1.5h	0.2398
without lesions alterations -0.1375	-0.72629215 0.45129215	2h	0.5889
without lesions alterations -0.15	-0.60359645 0.30359645	reperfusion	0.4438
without lesions alterations -0.2363636	-0.4814257 0.0086984	interaction	0.0583

Table 4: Concise form of the table 3.

Discussion

Tsuji M et al. (2018) [3] consider intrauterine hypo-perfusion/ischemia as one of the major causes of intrauterine/fetal growth restriction, preterm birth, and low birth weight. The offspring of their Mild Intrauterine Hypo Perfusion (MIUH) model clearly demonstrates long-lasting alterations in neurological, neuroanatomical and behavioral test results. Ugurlu T et al. (2018) [4] showed that antioxidant acetyl L-carnitine that added to the organ preservation solution (Histidine-Tryptophan-Ketoglutarate) HTK, has prevented the formation of free radicals and mitochondrial damage, thus protects the uterus that was stored in short and long cold storage periods in female rats. Ischemia-reperfusion is a complex pathophysiological process involved in hypoxia and/or reoxygenation, ionic imbalance-induced oedema and acidosis, oxidative stress, mitochondrial uncoupling, coagulation and endothelium activation. VEGFR-2 plays an important role in angiogenesis, chemotaxis, proliferation and migration of endothelial cells. Sholapurkar SL et al. (2018) [5] proposed an "ischemia and mal-apposition hypothesis for cesarean scar (CS) niche", stating that the surgical technique of uterine incision closure is the most important determinant of CS defect formation. Single-layer technique may be best reserved for thin myometrial edges especially during repeat cesareans. Alotaibi M (2018) [6] suggested that a mechanism of uterine tolerance (preconditioning) is confined to uterine tissues very close to labour and it is a protective phenomenon to improve the uterine activity despite the long-lasting paradoxical metabolic challenges that occur during the repeated strong labour contractions in rat uterine term-pregnant tissues. Ren Z et al. (2018) [7] showed that correcting soluble fms-like tyrosine kinase-1 (sFlt-1)/Placental Growth Factor (PlGF) Imbalance by Infusing PlGF reverses the decreases in vascular and uteroplacental Matrix Metalloproteinase (MMP)-2 and MMP-9 and the increases in MMP-1, MMP-7, and collagen types I and IV induced by placental ischemia and antiangiogenic sFlt-1 in hypertension of pregnancy. Angiogenic factors and MMP modulators could rectify changes in vascular and uteroplacental MMPs and collagen content and ameliorate hypertension and intrauterine growth restriction in preeclampsia. Clayton AM et al. (2018) [8] claims that placental ischemia, induced by Reducing Uterine Perfusion Pressure (RUPP), leads to cerebral edema and increased Blood-Brain Barrier (BBB) permeability and thus increased mortality risk from Alzheimer's disease, stroke, and cerebrovascular complications in women with a history of preeclampsia in pregnancy. Astrocyte number was increased in both regions but area covered by astrocytes increased only in posterior cortex following RUPP. Posterior cortical occludin was decreased. These results suggest that 2 months postpartum, neuro-inflammation, along with decreased occludin expression, may partly explain posterior cortical edema in rats with history of placental ischemia. Simoni M et al. (2018) [9] considered insufficient stem cell recruitment to adequately repair the uterus resulting in conditions such as Asherman syndrome, endometriosis and other endometrial receptivity defects. In contrast, excessive recruitment of stem cells underlies endometriosis. Further, the normal endometrium is a rich source of multipotent stem cells that can be used for numerous applications in regenerative medicine beyond reproduction. Stem-cell mobilization inhibiting may also be helpful in endometriosis therapy. Almohanna AM et al. (2018) [10] suggested what may be general mechanisms of conditioning occurring in all smooth muscles and tabulated tissue-specific mechanistic findings. Kisu I et al. (2018) [11] achieved the first delivery after autologous uterus transplantation (UTx) in primates and the first periodic recovery of menstruation after allogeneic UTx in nonhuman primate models. In addition, more validation in nonhuman primate models is needed for resolution of medical issues and further development of UTx in humans, despite clinical application of UTx in several countries. Vaka VR et al. (2018) [12] Mitochondrial ROS was significantly elevated in endothelial cells incubated with Reduced Uterine Perfusion Pressure (RUPP) serum compared with normal ones. Impaired mitochondrial function and vascular, placental, and renal mitochondrial ROS play an important role in hypertension and reduced fetal weight in response to placental ischemia during pregnancy in female pregnant Sprague Dawley rats. Koizumi N et al. (2019) [13] submitted a 41-year-old woman.in an elective needlescopic operation using 2- and 3-mm instruments after bowel decompression out of right broad ligament hernia. The defect in the right broad ligament was closed with sutures and she was discharged 2 days after the operation. In the treatment of broad ligament hernia without bowel ischemia, neither an abdominal incision nor any energy devices are required. Needlescopic operation seems to be a promising approach among minimally invasive operations. Kopko J et al. (2019) [14] found intraoperatively acute appendicitis and levorotation of the pregnant uterus at 19th week of gestation by about 100 degrees. As the signs of ischemia were absent, the uterus was returned into its normal position. The patient underwent cesarean section at 36 weeks of pregnancy due to early leakage of amniotic fluid and failure to progress during first stage of labor. Padma AM et al. (2019) [15] distinguished reperfusion injury-related differences associated with organ preservation; that may lead to improved human uterus transplantation protocols curing women with uterine factor infertility. A much faster and severe reperfusion damage of all uterine layers15 during the reperfusion experiment was got evident following 48 hours of cold ischemia. This was indicated by major accumulation of extracellular fluid, presence of apoptotic-labeled glandular epithelial layer and vascular endothelium. A significant accumulation of lactate was measured in the perfusate with a subsequent decrease in pH in a novel ex vivo sheep uterus model. Chen C et al. (2019) [16] assessed the safety and efficacy of Transcatheter Arterial Embolization (TAE) of the Inferior Mesenteric Artery (IMA) for the management of Post-

Partum Hemorrhage (PPH). Bleeding from the IMA should be suspected when there is persistent vaginal bleeding after sufficient embolization of bleeders from the bilateral iliac arteries. Saat N et al. (2019) [17] indicated that melatonin improved fertility and reduced uterine torsion related tissue damage and that its application during torsion was more effective than application following removal of torsion in pregnant rats. Tardieu A et al. (2019) [18] demonstrated hypoxia-associated degradation of the organ by the significantly higher lactate levels, accompanied by cell lysis and significantly higher levels of creatine kinase activity ($p < 0.05$). The metabolic results indicate a significant degradation of the uterus during 24 h of Cold Ischemia (CI) before transplantation in explanted ewes' uteri. Tardieu A et al. (2019) [19] calculated the mean Cold Ischemia (CI) time in studies of births from uteri obtained from live donors between 2 h 47 min and 6 h 20 min from a deceased donor; with only one birth in this case in women. Muscle contractions have also been demonstrated in myometrial samples from women, after six or more hours of CI. The uterus seems to be able to tolerate a prolonged period of CI, of at least six hours; for the development of Uterus Transplantation (UTx), particularly for procedures using grafts from deceased donors. A numeric evaluation [20] 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).

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

Table 5: The erythropoietin (Epo) influence (+SD) on the levels of 35 seric variables of complete blood count and blood chemistry tests versus reperfusion (rep) time[20].

Conclusion

Epo has a borderline significantly recessing capacity for the 4 histologic variables within the "without lesions alterations" score 0.2363636 [-0.4814257 - 0.0086984] (p-value=0.0583), encouraging for beneficial usage in obstetric situations such as intrauterine/fetal growth restriction, preterm birth, hypertension, low/reduced fetal weight due to placental ischemia, uterine activity during the repeated strong labour contractions, preeclampsia, after bowel decompression out of right broad ligament hernia or correction of a rotated pregnant uterus, uterine torsion, post-partum hemorrhage and cesarean scar niche formation. Many gynecologic situations also could be benefited such as endometriosis therapy, Asherman syndrome, Alzheimer's disease, stroke, posterior cortical edema and cerebrovascular complications in women with a history of preeclampsia, angiogenesis, chemotaxis, proliferation and migration of uterine endothelial cells in regenerative medicine beyond reproduction and development of autologous or allogeneic stored uterus transplantation protocols curing women with uterine factor infertility.

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