ECRONICON OPEN ACCESS

EC PROTEOMICS AND BIOINFORMATICS
Research Article

The Co-Evaluation of Endometrial Karyorrhexis and Uterus Congestion after the "U-74389G" Effect on Uterine Ischemia Reperfusion Injury

Constantinos Tsompos[1]*, Constantinos Panoulis[2], Konstantinos Toutouzas[3], Aggeliki Triantafyllou[4], George C Zografos[5], Kalliopi Tsarea[6], Maria Karamperi[6] and Apostolos Papalois[7]

[1]*Consultant A, Department of Gynecology, General Hospital of Thessaloniki "St. Dimitrios" Thessaloniki, Hellas, Greece*

[2]*Assistant Professor, Department of Obstetrics and Gynecology, Aretaieion Hospital, Athens University, Athens, Attiki, Hellas, Greece*

[3]*Assistant Professor, Department of Surgery, Ippokrateion General Hospital, Athens University, Athens, Attiki, Hellas, Greece*

[4]*Associate Professor, Department of Biologic Chemistry, Athens University, Athens, Attiki, Hellas, Greece*

[5]*Professor, Department of Surgery, Ippokrateion General Hospital, Athens University, Athens, Attiki, Hellas, Greece*

[6]*Researcher, Experimental Research Centre ELPEN Pharmaceuticals, S.A. Inc., Co., Pikermi, Attiki, Hellas, Greece*

[7]*Director, Experimental Research Centre ELPEN Pharmaceuticals, S.A. Inc., Co., Pikermi, Attiki, Hellas, Greece*

***Corresponding Author:** Constantinos Tsompos, Consultant A, Department of Gynecology, General Hospital of Thessaloniki "St. Dimitrios" Thessaloniki, Hellas, Greece.

Received: July 24, 2018; **Publisded:** October 29, 2019

Abstract

Aim: This study examined 2 histologic variables after process with the antioxidant lazaroid agent "U-74389G" (L). The concept was based on the co-evaluation of the results of 2 preliminary studies. Each preliminary one evaluated a respective histologic variable either this of endometrial karyorrhexis (EK) or that of uterus congestion (UC) in an induced uterine ischemia reperfusion (IR) animal experiment.

Materials and Methods: 2 main experimental endpoints were set at which the EK and UC scores were evaluated. They were the 60th reperfusion min (for both A and C groups) and the 120th reperfusion min (for both B and D groups). Certainly, the groups A and B were placebo processed, whereas the rest groups C and D were processed with L.

Results: The result of the first preliminary study was that L non-significantly reduced the EK scores within the grade "without lesions" 0.0727273 ± 0.16741255 (p-value = 0.6562). The result of the second preliminary study was that L significantly reduced the UC scores again within the grade "without lesions" 0.1779784 ± 0.04812883 (p-value = 0.0005). The common setting of both studies permitted their co-evaluation. A combined result was outcome by both variables together.

Conclusions: L has a non-significant recessing potency for these histologic parameters at the "without lesions" grade 0.1253529 ± 0.08529668 (p-values = 0.1373) since they were co-evaluated together.

Keywords: *Ischemia; U-74389G; Endometrial Karyorrhexis; Uterus Congestion; Reperfusion*

Introduction

U-74389G is a novel antioxidant agent implicating just only 258 published studies. The special ischemia reperfusion (IR) type of experiments is estimated in 18.60% of these studies. U-74389G has tissue protective capacities in these IR studies. The chemical form of U-74389G is 21-[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-pregna-1,4,9(11)-triene-3,20-dione maleate salt. It prevents the lipid peroxidation either iron-dependent, or arachidonic acid-induced one as an antioxidant complex. Animal liver, kidney, heart models

and brain microvascular endothelial cells monolayers are protected by U-74389G after IR injury. The U-74389G presents anti-shock property, attenuates the leukocytes, protects the endothelium, down-regulates the proinflammatory gene, enhances the mononuclear immunity, treats the endotoxin shock and produces cytokines. The 2 histologic variables were tested in an uterine ischemia reperfusion (UIR) experiment. The one variable was that of endometrial karyorrhexis (EK) which was recessed by the grade "without lesions" 0.0727273 ± 0.16741255 (p-value = 0.6562) [1]. The other histologic variable was the uterus congestion (UC). This was restored within the grade "without lesions alterations" 0.1779784 ± 0.04812883 (p-value = 0.0005) [2]. This complex experimental work co-evaluated both EK and UC variables together comparing its outcome with each one variable separately.

Materials and Methods

Animal preparation

The preliminary references [1,2] report the required Vet licenses under 3693/12-11-2010 and 14/10-1-2012 numbers, the granting company with the experiment location and the Pathology laboratory. The preliminary references also describe the human animal care of Albino female *Wistar* rats, the 7 days pre-experimental *ad libitum* diet and the entire peri-experimental anesthesiologic techniques. The rats were selected at 16 - 18 weeks old and then were randomly assigned to [4] four groups, each consisted in N = 10. The preceded stage of 45 min ischemia was common for all 4 groups. Afterwards, reperfusion of 60 minutes was followed in group A; reperfusion of 120 minutes in group B; immediate U-74389G intravenous (IV) administration and reperfusion of 60 minutes in group C; immediate U-74389G IV administration and reperfusion of 120 min in group D. The dose height was assessed at preliminary studies for both drugs at 10 mg/Kg body mass.

Induced ischemia was by laparotomic clamping the inferior aorta upper the renal arteries with forceps lasting 45 minutes. The clamp retraction was restoring the inferior aorta patency and reperfusion. The exclusion of the blood flow engaged the protocol of UIR; iterated for each experimental group. U-74389G was administered at the commencement of reperfusion; through an inferior vena cava catheter. The EK and UC scores were assessed at 60^{th} minutes of reperfusion (A and C groups) and at 120^{th} minutes of reperfusion (B and D groups). Relation was raised between animals' mass no with EK scores (p-value = 0.8683) but with UC ones (p-values = 0.0047) and the predicted UC scores were used. The pathologic score grading was kept same as in preliminary studies: without lesions (0 - 0.499), the mild lesions (0.5 - 1.499), the moderate lesions (1.5 - 2.499) and the serious lesions (2.5 - 3) damage.

Model of ischemia-reperfusion injury

- **Control groups:** The 20 control rats were common for preliminaries and this study.
- **Group A:** Reperfusion lasted 60 minutes; concerned 10 controls rats of combined EK and predicted UC (EK and UC) score as the mean of EK score and UC one (Table 1).
- **Group B:** Reperfusion lasted 120 minutes; concerned 10 controls rats of combined EK and UC (cEK and UC) score as the mean of EK and predicted UC one (Table 1).
- **L group:** The 20 L rats were common for preliminaries and this study.
- **Group C:** Reperfusion lasted 60 minutes; concerned 10 L rats of cEK and UC score as the mean of EK score and predicted UC one (Table 1).
- **Group D:** Reperfusion lasted 120 minutes; concerned 10 L rats of cEK and UC score as the mean of EK score and predicted UC one (Table 1).

	Mean EE score ± SD	Mean predic UI score ± SD	Mean EE and UI score ± SD
Group A	Mild lesions 1 ± 0.942809	Mild lesions 0.8862579 ± 0.3549345	Mild lesions 0.943129 ± 0.5121834
Group B	Mild lesions 1.1 ± 0.875595	Mild lesions 1.033575 ± 0.241205	Mild lesions 1.066787 ± 0.3325432
Group C	Mild lesions 0.5 ± 0.5270463	Mild lesions 0.6497756 ± 0.138277	Mild lesions 0.5748878 ± 0.2454082
Group D	Mild lesions 1 ± 0.942809	Mild lesions 0.6303919 ± 0.1403745	Mild lesions 0.8151959 ± 0.509367

Table 1: *Endometrial karyorrhexis (EK), predicted uterus congestion (UC), and their mean and SD scores.*

Statistical analysis

Every cEK and UC groups score was compared with each other from 3 remained groups applying Wilcoxon signed-rank test (Table 2). Then, the generalized linear models (glm) were applied with dependent variable the cEE and UI scores, and independent variables the L administration or no, the reperfusion time and their interaction.

DG	Difference	p-value
A-B	-0.1236583	0.7213
A-C	+0.3682412	0.0593
A-D	+0.1279331	0.5751
B-C	+0.4918995	0.0069
B-D	+0.2515914	0.0926
C-D	-0.2403081	0.1530

Table 2: *The values difference for groups (DG) after Wilcoxon signed-rank test for mean EK and UC scores.*

Results

L administration non-significantly recessed the cEK and UC scores by the "without alterations" grade 0.3099163 [-0.5766808 -0.0431518] (p = 0.0182) by both Wilcoxon signed-rank and glm test. Reperfusion time non-significantly deteriorated the cEK and UC scores by the "without alterations" grade 0.12907565 [-0.13593205 - 0.39408335] (p = 0.4045) by both tests too. However, L administration and reperfusion time together also non-significantly recessed the cEK and UC scores by the "without alterations" grade 0.1253529 [-0.2925344 - 0.0418286] (p = 0.1373). A concise description of the above results is depicted at table 4.

Alteration	95% c. in.	Reperfusion time	Wilkoxon	glm
Without alterations -0.3682412	-0.0216744 0.7581568	1h	0.0593	-
Without alterations 0.1236583	-0.2820519 0.5293685	1h	-	0.5300
Without alterations -0.3099163	-0.5766808 -0.0431518	1.5h	0.0124	0.0240
Without alterations 0.2403081	-0.1353282 0.6159444	2h	-	0.1956
Without alterations -0.2515914	-0.0927753 0.5959582	2h	0.0926	
Without alterations 0.0761681	-0.1746518 0.326988	Reperfusion	0.6142	-
Without alterations 0.1819832	-0.0972123 0.4611787	Reperfusion	-	0.1949
Without alterations -0.1253529	-0.2925344 0.0418286	Interaction		0.1373

Table 3: *The alteration influence of U-74389G in connection with reperfusion time.*

Decrease	95% c. in.	Reperfusion time	p-value
Without alterations 0.2445829	-0.15186315 0.64376265	1h	0.2946
Without alterations 0.3099163	-0.5766808 -0.0431518	1.5h	0.0182
Without alterations 0.00564165	-0.11405175 0.6059513	2h	0.2882
Without alteration -0.12907565	-0.13593205 0.39408335	reperfusion	0.4045
Without alterations 0.1253529	-0.2925344 0.0418286	interaction	0.1373

Table 4: Concise form of the table 3.

Discussion

Thaete LG., *et al.* [3] used Pep-1 (inhibits low-molecular-weight hyaluronan (LMW-HA) due to binding to toll-like receptor 4 [TLR4]). TLR4 was shown to have a regulatory role for two anti-inflammatory cytokines: interferon-B1 decreased only in wild-type mice ($P < 0.01$) and interleukin-10 increased only in TLR4-deficient mice ($P < 0.001$), in response to UIR. Pep-1 completely prevented the UIR induced fetal growth restriction (FGR) ($P < 0.001$), indicating a potential role for the endogenous TLR4 ligand LMW-HA in UIR induced FGR. FGR depends on TLR4 and on endogenous ligand(s) as breakdown products of HA. TLR4 also may prevent pregnancy loss after UIR. Reiter RJ., *et al.* [4] described placenta, in particular, often as a site of excessive free radical production. It is due to IR because of suboptimal adhesion to the uterine wall. This may cause pre-eclampsia and other disorders which often complicate pregnancy. Melatonin has improved free radical damage to the animal placenta and to their fetus. Optimal maternal circadian rhythmicity is important since it programs the developing fetal master oscillator. Disturbed maternal circadian rhythms, known as chronodisruption and perturbed melatonin cycles retard the maturing fetal oscillators, which may lead to behavioral and psychological neonatal impairments. Melatonin, of both pineal and placental origin, has essential functions in fetal maturation and placenta/uterine homeostasis. Circadian clock genes, which are components of all cells including those in the peripheral reproductive organs, have important roles in reproductive and organismal (fetal and maternal) physiology. Indoleamine may have utility in the treatment of pre-eclampsia, intrauterine growth restriction, placental and fetal IR due to the potent antioxidant actions of melatonin, coupled with its virtual absence of toxicity. The propensity for parturition to occur at night may relate with the synergism between the nocturnal increase in melatonin and oxytocin. Sahin S., *et al.* [5] indicated that immunosuppressant tacrolimus reduces oxidative damage in rat UIR Histologic evaluation revealed that tacrolimus attenuates the inflammatory response and protects the tissue damage induced by UIR in rats. Alawadhi F., *et al.* [6] ameliorated fertility after bone marrow derived stem cells transplant in Asherman's Syndrome mice. Trifonova EA., *et al.* [7] studied a cluster of 63 differentially expressed genes (DEG) whose expression level is increased in patients with preeclampsia includes not only the known candidate genes that have been identified in many other genome-wide studies (e.g. LEP, BHLHB2, SIGLEC6, RDH13, BCL6), but also new genes (ANKRD37, SYDE1, CYBA, ITGB2, etc.), as new biological markers of preeclampsia. The results of a functional annotation of DEG show that the development of preeclampsia may be related with an immune processes, stress response, intracellular signaling cascades, the regulation of cell-cell interactions, etc. Iran-Nejad A., *et al.* found uterus weight increased by estradiol ($P < 0.05$) after renal IR injury in female rats. Drobyshevsky A., *et al.* [9] showed a significant 3.72-fold decrease in maternal placental perfusion in reperfusion-reoxygenation phase in the saline than the antioxidant group dynamic contrast enhanced (DCE) MRI, relative to pre-occlusion values correspondingly. 31% systematic underestimation of true perfusion in placenta by steepest slope DCE MRI is significant in a rabbit model of fetal antenatal hypoxia-ischemia. Vafapour M., *et al.* [10] found uterus weight decreased significantly in female rats treated with GABA. Atalay YO., *et al.* [11] found remifentanil to protect

the UIR and can be used safely in uterus transplantation in exposed rats. Talebi N., *et al.* [12] found the uterus weight increased significantly after estradiol administration ($P < 0.05$) in ovariectomized rats. Tang Y., *et al*. [13] indicated that soy isoflavone (SI), a soy-derived phytoestrogen, which has similar chemical structure to endogenous estrogen-estradiol; protects myocardial IR injury in ovariectomized rats through increasing PI3K/Akt/eNOS signal pathway and decreasing oxidative stress. Ingles J., *et al.* [14] defined the preconditioning as "the preparation for a subsequent action". The cellular stress response of unfolded protein response (UPR) is controlled at the endoplasmic reticulum level. However, in the context of remote preconditioning, activation of these intracellular molecular pathways must result in the extracellular transmission of adaptive signals to remote targets. The activation of the UPR in the pregnant uterine myocyte may be associated with increased uterine myocyte quiescence and normal gestational length. A gestational stress-induced uterine paracrine secretome - for example, glucose-regulated protein 78, with preconditioning-like properties - acts to promote both local and systemic tolerance to the ensuing gestational insults, allowing for the maintenance of uterine quiescence. In this context, preterm labor may be the result of a pregnant uterus experiencing a stress it cannot accommodate or when it is unable to host an appropriate UPR resulting in insufficient preconditioning and a diminished local and systemic capacity to tolerate pregnancy-dependent increases in normal gestational stress; in order to prolong uterine quiescence in pregnancy. Tricard J., *et al.* [15] revealed a moderate inflammation of the endometrium and serosa at 90 minutes following reperfusion in the 3-h group and severe inflammation in the 24-h group. These first macroscopic and histological results suggest that the uterus is an organ with a good tolerance to extended cold ischemic storage before transplantation in ewes. Aslan M., *et al.* [16] found the cellular damage of uterus reduced in oxytocin and kisspeptin administered IR group than only kisspeptin injected IR group and IR group. The present results suggest that exogenous application of oxytocin and kisspeptin can have antioxidant effects on the uterus. Similar [17] L efficacies are provided by a meta-analysis of 35 complete blood count and blood chemistry tests variables versus reperfusion time also originated from the same experimental setting (Table 5).

35 Variables	1h rep	p-value	1.5h rep	p-value	2h rep	p-value	Interaction of U-74389G and rep	p-value
Mean	2.03% ± 27.26%	0.2168	0.19% ± 29.41%	0.1836	-1.63% ± 33.15%	0.2389	-0.33% ± 16.23%	0.2016

Table 5: *The U-74389G influence (±SD) on the levels of 35 seric variables of complete blood count and blood chemistry tests versus reperfusion (rep) time.*

Conclusion

L has a non-significant recessing potency for EK and UC together (p-values = 0.1373) creating a suspicion for beneficial usage in situations such as fetal growth restriction, pregnancy loss, pre-eclampsia, intrauterine growth restriction, placental and fetal IR, fertility, Asherman's syndrome, uterus transplantation, preterm labor, endometritis.

Acknowledgement

Acknowledged in preliminary studies.

Bibliography

1. C Tsompos., *et al*. "The effect of the antioxidant drug "U-74389G" on endometrial karyorrhexis during ischemia reperfusion injury in rats". *Annals of the Romanian Society for Cell Biology* 19.3 (2015): 47-53.

2. Tsompos C., *et al*. "The Effect of the Antioxidant Drug "U-74389g" on Uterus Congestion During Ischemia Reperfusion Injury in Rats". *Journal of Reproductive Medicine, Gynaecology, and Obstetrics* 1 (2016): 001.

3. Thaete LG., *et al*. "Impact of toll-like receptor 4 deficiency on the response to uterine ischemia/reperfusion in mice". *Reproduction* 145.5 (2013): 517-526.

4. Reiter RJ., *et al*. "Melatonin and stable circadian rhythms optimize maternal, placental and fetal physiology". *Human Reproduction Update* 20.2 (2014): 293-307.

5. Sahin S., *et al*. "The protective effects of tacrolimus on rat uteri exposed to ischemia-reperfusion injury: a biochemical and histopathologic evaluation". *Fertility and Sterility* 101.4 (2014): 1176-1182.

6. Alawadhi F., *et al*. "Bone Marrow-Derived Stem Cell (BMDSC) transplantation improves fertility in a murine model of Asherman's syndrome". *PLoS One* 9.5 (2014): e96662.

7. Trifonova EA., *et al*. "Analysis of the placental tissue transcriptome of normal and preeclampsia complicated pregnancies". *Acta Naturae* 6.2 (2014): 71-83.

8. Iran-Nejad A., *et al*. "Preventive role of estradiol on kidney injury induced by renal ischemia-reperfusion in male and female rats". *International Journal of Preventive Medicine* 6 (2015): 22.

9. Drobyshevsky A and Prasad PV. "Placental perfusion in uterine ischemia model as evaluated by dynamic contrast enhanced MRI". *Journal of Magnetic Resonance Imaging* 42.3 (2015): 666-672.

10. Vafapour M., *et al*. "Effect of Γ-aminobutyric acid on kidney injury induced by renal ischemia-reperfusion in male and female rats: Gender-related difference". *Advanced Biomedical Research* 4 (2015): 158.

11. Atalay YO., *et al*. "Remifentanil protects uterus against ischemia-reperfusion injury in rats". *Acta Cirúrgica Brasileira* 30.11 (2015): 756-761.

12. Talebi N., *et al*. "The Protective Effect of γ-aminobutyric Acid on Kidney Injury Induced by Renal Ischemia-reperfusion in Ovariectomized Estradiol-treated Rats". *International Journal of Preventive Medicine* 7 (2016): 6.

13. Tang Y., *et al*. "Soy Isoflavone Protects Myocardial Ischemia/Reperfusion Injury through Increasing Endothelial Nitric Oxide Synthase and Decreasing Oxidative Stress in Ovariectomized Rats". *Oxidative Medicine and Cellular Longevity* (2016): 5057405.

14. Ingles J., *et al*. "Thinking Outside the Box". *Journal of Cardiovascular Pharmacology and Therapeutics* 22.4 (2017): 337-346.

15. Tricard J., *et al*. "Uterus tolerance to extended cold ischemic storage after auto-transplantation in ewes". *European Journal of Obstetrics and Gynecology and Reproductive Biology* 214 (2017): 162-167.

16. Aslan M., *et al*. "The effect of oxytocin and Kisspeptin-10 in ovary and uterus of ischemia-reperfusion injured rats". *Taiwanese Journal of Obstetrics and Gynecology* 56.4 (2017): 456-462.

17. Tsompos C., *et al*. "The Antioxidant Drug "U-74389g" Effect on Alanine Aminotransferase Levels". *Journal of Analytical and Pharmaceutical Research* 4.2 (2017): 00095.

Volume 3 Issue 1 November 2019