

DOI: 10.21767/2471-9749.100039

The Co-evaluation of Endometrial Edema and Uterus Inflammation after the “U-74389G” Effect on Uterine Ischemia Reperfusion Injury

Tsompos C^{1*},
Panoulis C², Toutouzas K³,
Triantafyllou A⁴, Zografos G⁵
and Papalois A⁶

Abstract

Context: This study co-evaluated the 2 quoted histologic variables after the antioxidant lazaroid agent “U-74389G” (L) administration.

Objective: The calculation was based on the results of 2 preliminary studies, each one evaluating a respective histologic variable of endometrial edema (EE) or uterus inflammation (UI) in an induced ischemia reperfusion (IR) animal experiment.

Design: The 2 main experimental endpoints at which the EE and UI scores were evaluated was the reperfusion 60th min (for A & C groups) and the reperfusion 120th min (for B & D groups).

Setting: The groups A and B were processed without drugs, whereas the groups C and D after L administration.

Participants: Female *Wistar* Albino rats.

Main outcome measures: The first preliminary study showed that L non significantly reduced the EE scores by the grade “without lesions” 0.1636364 ± 0.1839975 (p-value=0.3641). The other preliminary study showed that L significantly reduced the UI scores by the grade “without lesions” 0.3636364 ± 0.13840105 (p-value=0.0099).

Result: 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.

Conclusion: L has a non-significant recessing potency for these histologic parameters at the “without lesions” grade 0.2636364 ± 0.14594051 (p-values=0.0698) since they were co-evaluated together.

Keywords: Ischemia; U-74389G; Endometrial edema; Uterus inflammation; Reperfusion

Received: December 05, 2017; **Accepted:** January 04, 2018; **Published:** January 11, 2018

Introduction

U-74389G is a new antioxidant agent implicating just only 255 published studies. The ischemia reperfusion (IR) type of experiments is noted in 4.31% of these studies. A tissue protective feature of U-74389G is obvious in such IR studies. The U-74389G chemically known as 21-[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-pregna-1,4,9(11)-triene-3,20-dione maleate salt is antioxidant complex, which inhibits the lipid peroxidation either

iron-dependent, or arachidonic acid-induced one. Animal kidney, liver, brain micro vascular endothelial cells monolayers and heart models are protected by U-74389G after IR injury. U-74389G also attenuates the leukocytes; down-regulates the proinflammatory gene; treats the endotoxin shock; produces cytokine; enhances the mononuclear immunity; protects the endothelium and presents antismoke property. 2 histologic variables in an uterine ischemia reperfusion (UIR) experiment was tested for this purpose. The one variable was that of endometrial edema (EE)

- 1 Department of Gynecology, General Hospital of Thessaloniki "St. Dimitrios", Thessaloniki, Greece
- 2 Department of Obstetrics & Gynecology, Aretaieion Hospital, Athens University, Athens, Attiki, Greece
- 3 Department of Surgery, Ippokrateion General Hospital, Athens University, Athens, Attiki, Greece
- 4 Department of Biologic Chemistry, Athens University, Athens, Attiki, Greece
- 5 Department of Surgery, Ippokrateion General Hospital, Athens University, Athens, Attiki, Greece
- 6 Experimental Research Centre ELPEN Pharmaceuticals, SA Inc Co, Pikermi, Attiki, Greece

*Corresponding author: Tsompos C

✉ Tsomposconstantinos@gmail.com

Department of Gynecology, General Hospital of Thessaloniki "St. Dimitrios", Thessaloniki, Greece.

Tel: 00302313322100

Citation: Tsompos C, Panoulis C, Toutouzas K, Triantafyllou A, Zografos G, et al. (2018) The Co-evaluation of Endometrial Edema and Uterus Inflammation after the “U-74389G” Effect on Uterine Ischemia Reperfusion Injury. J Contracept Stud Vol.3 No.1:6

which was recessed by the grade "without lesions" 0.1636364 ± 0.1839975 (p -value=0.3641) [1]. The other variable was that of uterus inflammation (UI) and was restored by the grade "without lesions" 0.3636364 ± 0.13840105 (p -value=0.0099) [2]. The present experimental work tried to co-evaluate these EE and UI variables together and to compare its outcome with each one separately, from the same rat induced UIR protocol.

Materials and Methods

Animal management

The Vet No 3693/12-November-2010 & 14/10-Januaru-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 U-74389G intravenous (IV) administration and 60 min reperfusion in group C; and immediate U-74389G 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. U-74389G was administered at the time of reperfusion; through an inferior vena cava catheter. The EE and UI 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 raised between animals' mass with neither EE scores (p -value=0.7779) nor with UI ones (p -value=0.0576).

Ethics

All animals received humane care according to the criteria outlined in the "Guide for the Care and Use of Laboratory Animals (1996)" prepared by the National Academy of Sciences.

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 EE and predicted UI (EE & UI) score as the mean of EE score and UI one (Table 1).

Group B

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

L group

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

Group C

60 min reperfusion concerned 10 L rats of cEE & UI score as the mean of EE score and predicted UI one (Table 1).

Group D

120 min reperfusion concerned 10 L rats of cEE & UI score as the mean of EE score and predicted UI one (Table 1).

Statistics

Successive comparisons among the 4 cEE & UI groups were performed applying Wilcoxon signed-rank test (Tables 2 and 3). Then, the generalized linear models (GLM) were applied with dependent variable the cEE & UI scores Independent variables were used the L administration or no, the reperfusion time and their interaction.

Results

L administration non-significantly recessed the cEE & UI scores by mild alterations $0.55 [-1.011516 -0.0884839]$ ($p=0.0130$ and 0.0208) by Wilcoxon signed-rank test and GLM methods respectively. Reperfusion time hardly non-significantly deteriorated the Cee & UI scores by between without alterations $0.2 [-0.2912299-0.6912299]$ ($p=0.3934$ and 0.4150) respectively. However, L administration and reperfusion time together also non-significantly recessed the cEE & UI scores by without alterations $0.2636364 [-0.5496798-0.022407]$ ($p=0.0698$). A concise form of the above findings is depicted at (Table 4).

Discussion

Thaete et al. [3] used Pep-1 (inhibits low-molecular-weight hyaluronan (LMW-HA) due to binding to toll-like receptor 4 [TLR4]). TLR4 has a regulatory role for two anti-inflammatory cytokines: the interferon-B1 decreased in wild-type mice and the interleukin-10 increased in TLR4-deficient mice ($P<0.001$), in response to UIR. Pep-1 completely inhibited the UIR induced fetal growth restriction (FGR) ($P<0.001$), ascribing possible roles for the endogenous TLR4 ligand LMW-HA in UIR induced FGR. FGR is up to both TLR4 and endogenous ligand(s), including the breakdown products of HA. In addition, TLR4 plays a role in staving pregnancy loss after UIR. Reiter et al. [4] described placenta, in particular, often as a site of excessive free radical production due to suboptimal adhesion to the uterine wall, which leads to either persistent or intermittent hypoxia and reoxygenation. Both of these processes cause massive free radical production and organ dysfunction. These may induce pre-eclampsia and other disorders associating the pregnancy. Melatonin has prevented the above in non-human mammals. The optimal maternal circadian rhythmicity

Table 1 Endometrial edema (EE), Uterus inflammation (UI) and their mean and SD scores.

	Mean EE score ± SD	Mean UI score ± SD	Mean EE&UI score ± SD
Group A	Mild lesions 1.2 ± 0.7888106	Moderate lesions 1.5 ± 0.7071068	Mild lesions 1.35 ± 0.6687468
Group B	Mild lesions 1.3 ± 1.159502	Moderate lesions 1.8 ± 0.6324555	Moderate lesions 1.55 ± 0.831665
Group C	Mild lesions 0.7 ± 0.6749486	Mild lesions 0.9 ± 0.7378648	Mild lesions 0.8 ± 0.6324555
Group D	Mild lesions 1 ± 1.054093	Mild lesions 1 ± 0.6666667	Mild lesions 1 ± 0.781736

Table 2 The values difference for groups (DG) after Wilcoxon signed-rank test for mean EE&UI scores.

DG	Difference	p-Value
A-B	-0.2	0.6430
A-C	+0.55	0.1104
A-D	+0.35	0.1955
B-C	+0.75	0.0201
B-D	+0.55	0.0470
C-D	-0.2	0.3941

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

Alteration	95% c. in.	Reperfusion time	p-values	
			Wilcoxon	glm
Mild alterations -0.55	-0.1338636 1.233864	1 h	0.1104	0.0750
Mild alterations -0.55	-1.011516 -0.0884839	1.5 h	0.0130	0.0208
Mild alterations -0.55	0.0049396 1.09506	2 h	0.0470	0.1449
Without alterations 0.2	-0.2912299 0.6912299	Reperfusion	0.3934	0.4150
Without alterations -0.2636364	-0.5496798 0.022407	Interaction		0.0698

Table 4 Concise form of the table 3.

Increase	95% c. in.	Reperfusion time	p-value
Mild alterations -0.55	-0.1338636-1.233864	1 h	0.0927
Mild alterations -0.55	-1.011516-0.0884839	1.5 h	0.0169
Mild alterations -0.55	0.0049396-1.09506	2 h	0.0959
Without alterations 0.2	-0.291229-0.6912299	Reperfusion	0.4042
Without alterations -0.2636364	-0.5496798-0.022407	Interaction	0.0698

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.

35 Variables	1 h rep	p-value	1.5 h rep	p-value	2 h 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

via the melatonin rhythm, oscillates the developing one of the fetus. However, disturbed maternal circadian rhythms, known as chronodisruption, and disturbed melatonin cycles have ominous consequences for the maturing fetal oscillators, which may lead to neonatal psychological and behavioral problems. Melatonin, of any origin, promotes fetal maturation and placenta/uterine homeostasis. The peripheral reproductive organs circadian clock genes have important roles in reproductive and organismal (fetal and maternal) physiology. Indole amine may be beneficial for the treatment of pre-eclampsia, intrauterine growth restriction (IUGR), placental and fetal IR. This benefit is due to the possible antioxidant actions of melatonin along with its virtual absence of toxicity. The nocturnal propensity for parturition may relate with the interaction of nocturnal increase in melatonin with oxytocin. Sahin 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 et al. [6] improved fertility after bone marrow derived stem cells (BMDSC) transplant in Asherman's Syndrome mice, demonstrating a potential novel prevention and treatment for murine Asherman's Syndrome after uterine injury. Trifonova et al. [7] studied a cluster of 63 differentially expressed genes (DEG) up-regulated in preeclampsia patients including not only the known candidate genes identified in many other genome-wide studies (e.g., BHLHB2, LEP, SIGLEC6, BCL6, RDH13), but also new ones (SYDE1, ANKRD37, ITGB2, CYBA, etc.), considered as new biological markers of preeclampsia with increasing interest. So, the development of preeclampsia may be related with a immune processes, a stress response, the intracellular signaling cascades, the regulation of cell-cell interactions, etc. Iran-Nejad et al. [8] found the uterus weight augmented by estradiol (P<0.05) after renal IR injury in female rats. Drobyshevsky et al. [9] showed a significant 3.72-fold hypoperfusion of the maternal

placenta in reperfusion phase in the saline than the antioxidant group dynamic contrast enhanced (DCE) MRI, relative to pre-occlusion values correspondingly. 31% systematic hypoperfusion of placenta by steepest slope DCE MRI is significant on fetal antenatal ischemia in a rabbit model. Vafapour et al. [10] found uterus weight decreased significantly in female rats treated with GABA. Atalay et al. [11] found remifentanyl to protect the UIR and thus safe in uterus transplantation in exposed rats. Talebi et al. [12] found the uterus weight increased significantly after estradiol administration ($P < 0.05$) in ovariectomized rats. Tang et al. [13] indicated that the soy isoflavone (SI) phytoestrogen, similar chemically with endogenous estrogen-estradiol; protects myocardial IR injury in ovariectomized rats increasing PI3K/Akt/eNOS signal pathway and decreasing the oxidative stress. Ingles et al. [14] defined the preconditioning as "the preparation for a subsequent action." The unfolded protein response (UPR) is a cellular stress response controlled at the level of the endoplasmic reticulum. 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 et al. [15] revealed a moderate inflammation of the endometrium and serosa at 90 min 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 et al. [16] found antioxidant effects on the uterus and specially a cellular damage of uterus reduce in oxytocin and kisspeptin administered IR group than only kisspeptin one. A numeric evaluation [17] of the L 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).

Conclusion

L has a non significant recessing potency for EE and UI together (p -values=0.0698) creating a suspicion for beneficial usage in situations such as fetal growth restriction, pregnancy loss, pre-eclampsia, IUGR, placental and fetal IR, fertility, Asherman's syndrome, uterus transplantation, preterm labor, endometritis.

Acknowledgement

Acknowledged in preliminary studies.

References

- 1 Tsompos C, Panoulis C, Toutouzas K, Zografos G, Papalois A (2014) The effect of the antioxidant drug "U-74389G" on endometrial edema during ischemia reperfusion injury in rats. *Acta Biol Szeged* 58: 69-72.
- 2 Tsompos C, Panoulis C, Toutouzas K, Zografos G, Papalois A (2015) The Effect of the Antioxidant Drug "U-74389G" on Uterus Inflammation during Ischemia Reperfusion Injury in Rats. *J Pharm Sci Emerg Drugs* 3: 1.
- 3 Thaete LG, Qu XW, Jilling T, Crawford SE, Fitchev P, et al. (2013) Impact of toll-like receptor 4 deficiency on the response to uterine ischemia/reperfusion in mice. *Reproduction* 145: 517-526.
- 4 Reiter RJ, Tan DX, Korkmaz A, Rosales-Corral SA (2014) Melatonin and stable circadian rhythms optimize maternal, placental and fetal physiology. *Hum Reprod* 20: 293-307.
- 5 Sahin S, Ozakpinar OB, Ak K, Eroglu M, Acikel M, et al. (2014) The protective effects of tacrolimus on rat uteri exposed to ischemia-reperfusion injury: a biochemical and histopathologic evaluation. *Fertil Steril* 101: 1176-1182.
- 6 Alawadhi F, Du H, Cakmak H, Taylor HS (2014) Bone Marrow-Derived Stem Cell (BMDSC) transplantation improves fertility in a murine model of Asherman's syndrome. *PLoS One* 12: e9e6662.
- 7 Trifonova EA, Gabdulina TV, Ershov NI, Serebrova VN, Vorozhishcheva AY, et al. (2014) Analysis of the placental tissue transcriptome of normal and preeclampsia complicated pregnancies. *Acta Naturae* 6: 71-83.
- 8 Iran-Nejad A, Nematbakhsh M, Eshraghi-Jazi F, Talebi A (2015) Preventive role of estradiol on kidney injury induced by renal ischemia-reperfusion in male and female rats. *Int J Prev Med* 20: 22.
- 9 Drobyshevsky A, Prasad PV (2015) Placental perfusion in uterine ischemia model as evaluated by dynamic contrast enhanced MRI. *J Magn Reson Imaging* 42: 666-72.
- 10 Vafapour M, Nematbakhsh M, Monajemi R, Mazaheri S, Talebi A, et al. (2015) Effect of Γ -aminobutyric acid on kidney injury induced by renal ischemia-reperfusion in male and female rats: Gender-related difference. *Adv Biomed Res* 27: 158.
- 11 Atalay YO, Aktas S, Sahin S, Kucukodaci Z, Ozakpinar OB (2015) Remifentanyl protects uterus against ischemia-reperfusion injury in rats. *Acta Cir Bras* 30: 756-761.
- 12 Talebi N, Nematbakhsh M, Monajemi R, Mazaheri S, Talebi A, et al. (2016) The Protective Effect of γ -aminobutyric Acid on Kidney Injury Induced by Renal Ischemia-reperfusion in Ovariectomized Estradiol-treated Rats. *Int J Prev Med*: 7: 6.
- 13 Tang Y, Li S, Zhang P, Zhu J, Meng G, et al. (2016) Soy Isoflavone Protects Myocardial Ischemia/Reperfusion Injury through Increasing Endothelial Nitric Oxide Synthase and Decreasing Oxidative Stress in Ovariectomized Rats. *Oxid Med Cell Longev* 2016: 14.
- 14 Ingles J, Kyathanahalli CN, Jeyasuria P, Condon JC (2017) Thinking Outside the Box. *J Cardiovasc Pharmacol Ther* 22: 337-346.
- 15 Tricard J, Ponsonnard S, Tholance Y, Mesturoux L, Lachatré D, et al (2017) Uterus tolerance to extended cold ischemic storage after auto-transplantation in ewes. *Eur J Obstet Gynecol Reprod Biol* 214: 162-167.

- 16 Aslan M, Erkanli Senturk G, Akkaya H, Sahin S, Yilmaz B (2017) The effect of oxytocin and Kisspeptin-10 in ovary and uterus of ischemia-reperfusion injured rats. *Taiwan J Obstet Gynecol* 56: 456-462.
- 17 Tsompos C, Panoulis C, Toutouzas K, Triantafyllou A, Zografos G, et al. (2017) The Antioxidant Drug “U-74389g” Effect on Alanine Aminotransferase Levels. *J Anal Pharm Res* 4: 00095.