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The Co-Evaluation of Endosalpingeal Edema and Karyorrhexis after the "U-74389G" Effect on Fallopian Ischemia Reperfusion Injury

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ABSTRACT

Aim: *This study co-evaluated the 2 quoted histologic variables after the antioxidant lazaroid agent "U-74389G" (L) administration. The calculation was based on the results of 2 preliminary studies, each one evaluating a respective histologic variable of endosalpingeal edema (E) or endosalpingeal karyorrhexis (K) in an induced ischemia reperfusion (IR) animal experiment.*

Materials and methods: *The 2 main experimental endpoints at which the E and K 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 L administration.*

Results: *The first preliminary study showed that L non significantly deflated the E scores by the grade "without lesions" 0.0226438 [-0.0493716 - 0.0040841] (p-value=0.0945). The second preliminary study showed that L did not influence the K scores by the grade "without lesions" 0.00 (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: *L hardly non significantly inflated both scores for these histologic parameters at the grade of "without lesions" 0.0113219 [-0.0246858 - 0.002042] (p-value=0.0945) since they were co-evaluated together.*

Keywords: *ischemia, U-74389G, endosalpingeal edema, endo salpingeal karyorrhexis, reperfusion;*

INTRODUCTION

U-74389G is a new antioxidant agent implicating just only 256 published studies. The ischemia reperfusion (IR) type of experiments is noted in 17.96% 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 microvascular

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 antishock property. 2 histologic variables in a fallopian ischemia reperfusion (FIR) experiment was tested for this purpose. The one variable was that of endosalpingeal edema (E) which was recessed by the grade "without lesions" 0.0226438±0.01155293 (p-value=0.0945)[1]. The other variable was that of endosalpingeal karyorrhexis (K) but was not influenced by the grade "without lesions" 0.00 (p-value=1.0000)[2]. he present experimental work tried to co-evaluate these E and K 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 L intravenous (IV) administration and 60 min reperfusion in group C; and immediate L 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. L was administered at the time of reperfusion; through an inferior vena cava catheter. The E and K 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 only E scores (p-value= 0.0432) whereas no with K ones (p-values=1.0000). Thus, the predicted values were used for E scores.

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 E and K (E&K) score as the mean of E score and K one (Table 1). Group B 120 min reperfusion concerned 10 placebo rats of combined E&K (cE&K) score as the mean of E and K 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 cE&K score as the mean of E score and K one (Table 1). Group D 120 min reperfusion concerned 10 L rats of cE&K score as the mean of E score and K one (Table 1).

Table1. *Endosalpingeal edema (E), endosalpingeal karyorrhexis* (K) *and their mean and SD scores*

	Mean E score ±SD	**Mean K score ±SD**	**Mean E&K score ±SD**
Group A	without lesions 0.0715645±0.0887336	without lesions 0	without lesions 0.0715645±0.0887336
Group B	without lesions 0.1083937±0.0603013	without lesions 0	without lesions 0.1083937±0.0603013
Group C	without lesions 0.0124439±0.0345692	without lesions 0	without lesions 0.0124439±0.0345692
Group D	without lesions 0.007598±0.0350936	without lesions 0	without lesions 0.007598±0.0350936

Statistical Analysis

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

RESULTS

L administration hardly none significantly deflated the cE & K scores by the "without alterations" grade 0.0142955 [-0.0349313 - 0.00634025] (p=0.1989) by both Wilcoxon signed-rank test and glm methods respectively. Reperfusion time non significantly inflated the cE&K scores by "without alterations" grade 0.0094496 [-0.01155015 - 0.0304493] (p=0.1216) by the similar methodology.

Finally, L administration and reperfusion time together also hardly non significantly deflated the cE&K scores by the "without alterations" grade 0.0113219 [-0.0246858 - 0.002042] (p-value=0.0945). A concise form of the above findings is depicted at table 4.

Table2. *The values difference for groups (DG) after Wilcoxon signed-rank test for mean E&K scores.*

DG	Difference	p-value
A-B	0.0184146	0.2014
A-C	-0.0295602	0.0926
A-D	-0.0319832	0.0745
B-C	-0.0479748	0.0093
B-D	-0.0503978	0.0069
C-D	-0.002423	0.9183

Table3. *The alteration influence of L in connection with reperfusion time*

Alteration	95% c. in.	Reperfusion time	wilkoxon	glm
without alterations -0.0295602	-0.0617267 - 0.0026062	1h	0.0926	
without alterations -0.0072689	-0.043012 0.0284742	1h		0.6743
without alterations -0.0142955	-0.0368147 0.0082236	1.5h		0.2065
without alterations -0.0142955	-0.0330479 - 0.0044569	1.5h	0.1913	
without alterations -0.0503978	-0.0710814 - -0.0297142	2h	0.0069	
without alterations -0.0193837	-0.0497716 0.0110041	2h		0.1969
without alterations -0.0133263	-.0359095 .0092568	reperfusion		0.2396
without alterations 0.0322255	0.0128092 0.0516418	reperfusion	0.0036	
without alterations -0.0113219	-0.0246858 0.002042	interaction		0.0945

Table4. *Concise form of the table 3*

Increase	95% c. in.	Reperfusion time	p-value
without alterations -0.01841455	-0.05236935 0.0155402	1h	0.3834
without alterations -0.0142955	-0.0349313 0.00634025	1.5h	0.1989
without alterations -0.03489075	-0.0604265 -0.00935505	2h	0.1019
without alterations 0.0094496	-0.01155015 0.0304493	reperfusion	0.1216
without alterations -0.0113219	-0.0246858 0.002042	interaction	0.0945

Table5. *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	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

DISCUSSION

Adamyan LV et al considered[3] 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 protected against salpingitis, other pelvic infections and against tubal pregnancies after combined oral contraceptives administration[4]. 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 reported[5] the case of a pregnant presenting with acute lateropelvic pain. Normal adnexal torsion is rare during pregnancy. Çılgın H et al indicated[6] 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 reported[7] 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 evaluated[8] 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 reviewed[9] 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 concluded[10] a decrease in elastic fibers with ultra structural abnormalities and an over expression 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 described[13] 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 observed[12] 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 found that adnexal torsion and detorsion significantly increased[13] 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 found[14] 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 associated[15] 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 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 identified[17] cell necrosis by high mobility group box 1 protein and apoptosis by Caspase 3a staining of tissue samples taken at 3 endpoints post burn.

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 found[18] the mean number of preantral and small antral follicles lower and only AMH levels significantly decreased following the 3-hour IR ($P < .05$) in detorsion group than those of the sham group ($P < .01$). After torsion, anti-Müllerian hormone (AMH), estradiol, and inhibin B levels were decreased significantly than preoperative and postoperative periods ($P = 0.032$). A numeric evaluation[19] 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 hardly non significantly deflated the cE&K scores by the "without alterations" grade (p-values= 0.0945) creating a suspicion for beneficial usage in situations such as tubal pregnancies, fertility, elastic and desmin ultra structure, 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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