

The Effect of the Antioxidant Drug “U-74389G” On Oviductal Congestion during Ischemia Reperfusion Injury in Rats

Constantinos Tsompos^{1*}, Constantinos Panoulis², Konstantinos Toutouzas³, George Zografos³, Apostolos Papalois⁴

¹Department of Obstetrics & Gynecology, Mesologi County Hospital, Etoloakarnania, Greece

²Department of Obstetrics & Gynecology, Aretaieion Hospital, Athens University, Attiki, Greece

³Department of Surgery, Ippokrateion General Hospital, Athens University, Attiki, Greece

⁴Experimental Research Center ELPEN Pharmaceuticals, S.A. Inc., Co., Attiki Greece

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ABSTRACT :

The effect of the antioxidant drug “U-74389G” was examined, on rat model and particularly in an oviductal ischemia reperfusion (IR) protocol. The probable effects of that molecule were studied pathologically using oviductal congestion (OC) lesions. 40 rats of mean weight 231.875 g were used in the study. OC lesions were evaluated at 60 min of reperfusion (groups A and C) and at 120 min of reperfusion (groups B and D) in rats. U-74389G was administered only in groups C and D. U-74389G administration non-significantly altered the OC scores by 0 without lesions [-0.3582422 - 0.3582422] (p=0.9608). Reperfusion time non-significantly increased the OC scores by 0.2 without lesions [-0.1521699 - 0.5521699] (p= 0.2317). However, U-74389G administration and reperfusion time together significantly increased the OC scores by 0.0909091 without lesions [-0.1230462 - 0.3048644] (p=0.3951). U-74389G administration whether it interacted or not with reperfusion time failed to restore the OC lesions within short-term time context of 2 hours.

Keywords: ischemia; U-74389G; fallopian tubes; oviductal congestion; reperfusion

INTRODUCTION:

Tissue ischemia and reperfusion (IR) are among the main causes of permanent or transient damage with serious implications on adjacent organs and certainly on patients' health. The usage of antioxidant substances as a treatment is a new concept. Furthermore, satisfactory answers have not been given yet to fundamental questions, such as, how fast does U-74389G act, when should U-74389G be administered, and at what dosage. The particularly satisfactory action of the antioxidant U-74389G in tissue protection has been noted in several performed experiments. After a careful literature search was conducted, it was realized that this certain antioxidant has been tried in IR experiments. However, just few relative reports were found, not covering completely this particular matter. Also, many publications addressed trials of other similar molecules of aminosteroids (lazaroids) to which the studied molecule also belongs to. U-74389G or better 21-[4-(2,6-di-1-pyrrolidinyl-4-pyrimidinyl)-1-piperazinyl]-pregna-1,4,9(11)-triene-3,20-dione maleate salt is an antioxidant which prevents both arachidonic acid-induced and iron-dependent lipid peroxidation

[www.caymanchem.com]. It protects against IR injury in animal heart, liver and kidney models. These membrane-associating antioxidants are particularly effective in preventing permeability changes in brain microvascular endothelial cells monolayers [Fenglin et al., 1995]. A meta-analysis of 13 published seric variables, coming from the same experimental setting, tried to provide a numeric evaluation of U-74389G efficacy at the same endpoints (Table 1).

The **aim** of this experimental study was to examine the effect of U-74389G on rat model and particularly in an oviductal IR protocol. The probable effects of that molecule were studied by evaluating the oviductal congestion (OC) lesions.

MATERIALS AND METHODS

Animal preparation

This experimental study was licensed by the Veterinary Address of East Attiki Prefecture under 3693/12-11-2010 & 14/10-1-2012 decisions. It was done at the Experimental Research Centre of ELPEN Pharmaceuticals Co. Inc. S.A. at Pikermi, Attiki. Everything needed for the study

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*Corresponding author:

Constantinos Tsompos

Department of Obstetrics & Gynecology, Mesologi County Hospital, Etoloakarnania, Greece

Email: Constantinostsompos@yahoo.com

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including consumables, equipment and substances, were a courtesy of Elpen. 40 Albino female Wistar rats were used in accordance with accepted standards of humane animal care, which spent 7 days in laboratory before the experiment with easy access to water and food. The experiment was acute, that is, the animal usage was completed even by euthanasia after the following experimental set of times without awakening and preservation of the rodents. Rats were randomly assigned to four experimental groups with 10 animals in each one: Ischemia for 45 min followed by reperfusion for 60 min (group A). Ischemia for 45 min followed by reperfusion for 120 min (group B). Ischemia for 45 min followed by immediate U-74389G intravenous (IV) administration and reperfusion for 60 min (group C). Ischemia for 45 min followed by immediate U-74389G IV administration and reperfusion for 120 min (group D). The molecule U-74389G dose was 10 mg/Kg body weight of animals.

The experiment started by the submission of animals into preanesthesia followed by general anesthesia. Their electrocardiogram and acidometry were continuously monitored. Their inferior aorta's flow was excluded by forceps. After exclusion, the protocol of IR was applied, exactly as described above. The molecules were administered at the time of reperfusion, through catheterized inferior vena cava. The OC evaluations were performed at 60 min of reperfusion (groups A and C) and at 120 min of reperfusion (groups B and D).

The detailed anesthesiologic technique is described in related references [Tsompos *et al.*, 2015]. Continuous oxygen supply was administered during whole experiment performance. Ischemia was caused by clamping inferior aorta over renal arteries for 45 min after laparotomic access. Reperfusion was induced by removing the clamp and reestablishment of inferior aorta patency. Forty (40) female Wistar albino rats were used, mean weight 231.875 g [Std. Dev: 36.59703 g], with min weight ≥ 165 g and max weight ≤ 320 g. Rats' weight could be potentially a confusing factor, e.g. more obese rats to have greater OC lesions scores. This suspicion was investigated. Also, detailed pathological study and grading of OC findings was performed by scores, this is: 0 lesions were not found, 1 mild lesions were found, 2 moderate lesions were found and 3 serious lesions were found [Osmanağaoğlu *et al.*, 2012]. The previous grading was transformed as follows: (0-0.499) without lesions, (0.5-1.499) the mild lesions, (1.5-2.499) the moderate lesions and (2.5-3) the serious lesions damage, because the study concerns score ranges rather than point scores.

Model of ischemia reperfusion injury

Control groups

20 control rats of mean weight 252.5 g [Std. Dev: 39.31988 g] suffered by ischemia for 45 min followed by reperfusion.

Group A

Reperfusion which lasted 60 min concerned 10 control

rats of mean weight 243 g [Std. Dev: 45.77724 g] and mean mild OC score 0.5 [Std. Dev: 0.5270463] (Table 2).

Group B

Reperfusion which lasted 120 min concerned 10 control rats of mean weight 262 g [Std. Dev: 31.10913 g] and mean without lesions OC score 0.4 [Std. Dev: 0.6992059] (Table 2).

Lazaroid (L) group

20 rats of mean weight 211.25 g [Std. Dev: 17.53755 g] suffered by ischemia for 45 min followed by reperfusion in the beginning of which 10 mg U-74389G /kg body weight were IV administered.

Group C

Reperfusion which lasted 60 min concerned 10 L rats of mean weight 212.5 g [Std. Dev: 17.83411 g] and mean without lesions OC score 0.2 [Std. Dev: 0.421637] (Table 2).

Group D

Reperfusion which lasted 120 min concerned 10 L rats of mean weight 210 g [Std. Dev: 18.10463 g] and mean mild OC score 0.7 [Std. Dev: 0.4830459] (Table 2).

Statistical analysis

Rats of each group were compared by weight and OC scores with each other by statistical paired t-tests and Wilcoxon signed-rank test (Table 3). Any significant difference among OC levels, was investigated whether owed in potent significant weight correlations. The application of generalized linear models (glm) with dependant variable the OC scores and independent variables the U-74389G or no drug, the reperfusion time and both variables in combination was followed. Inserting the rats weight also as an independent variable at generalized linear models analysis, a non significant relation resulted in ($p= 0.4159$), so as to further investigation was not needed.

RESULTS

The application of glm with dependant variable the OC scores and independent variables the U-74389G administration or no, the reperfusion time and their interaction, resulted in: U-74389G administration non-significantly altered the OC scores by 0 [-0.3582422 - 0.3582422] ($p=1.0000$). This finding was in accordance with the results of Wilcoxon signed-rank test ($p=0.9217$). Reperfusion time non-significantly increased the OC scores by 0.2 [-0.1521699 - 0.5521699] ($p= 0.2575$), also in accordance with the Wilcoxon signed-rank test ($p= 0.2059$). However, U-74389G administration and reperfusion time together significantly increased the OC scores by 0.0909091 [-0.1230462 - 0.3048644] ($p=0.3951$). Reviewing the above and table 3, the tables 4 and 5 sum up concerning the altering influence of U-74389G in connection with reperfusion time.

Variable	1h rep	p-value	1.5h rep	p-value	2h rep	p-value	interaction of U-74389G and rep	p-value
RBC	+1.39%±0.71%	0.7161	+0.64%±0.32%	0.8106	-0.10%±0.05%	0.9762	+1.05%±0.53%	0.4911
Hemoglobin	+5.2%±2.8%	0.0925	+3.9%±2.1%	0.0604	+2.7%±3.2%	0.3544	+2.5%±1.3%	0.0423
Mean corp he	+1.77%±0.96%	0.0663	+2.40%±0.57%	0.0001	+3.03%±0.71%	0.0003	1.33%±0.36%	0.0005
Platelet-crit	+3.80%±9.87%	0.6373	+9.23%±6.29%	0.1064	+14.66%±9.03%	0.0833	+6.72%±3.73%	0.0712
Glucose	-6.41%±3.50%	0.0663	-8.57%±2.06%	0.0001	-10.74%±2.52%	0.0003	-4.76%±1.28%	0.0005
Total protein	-5.48%±2.99%	0.0663	-7.34%±1.76%	0.0000	-9.20%±2.16%	0.0000	-4.08%±1.10%	0.0000
Alkaline phosphatase	+22.66%±12.37%	0.0663	+31.91%±7.69%	0.0001	+41.16%±9.65%	0.0003	+17.75%±4.79%	0.0005
Creatine phosphokinase	+54.32%±13.75%	0.0012	+35.34%±17.20%	0.0260	+16.37%±30.24%	0.4951	+18.52%±9.44%	0.0770
Sodium	+1.22%±0.66%	0.0707	+0.17%±0.61%	0.7714	-0.87%±1.03%	0.3995	-0.32%±0.36%	0.3693
Chloride	-0.58%±0.77%	0.4533	-0.97%±0.53%	0.0879	-1.36%±0.76%	0.1113	-0.75%±0.38%	0.0159
Calcium	0%±1.75%	1	-0.14%±1.10%	0.8782	-0.28%±1.54%	0.8492	+0.14%±0.64%	0.8245
Phosphorus	-2.23%±5.51%	0.7966	-1.61%±3.32%	0.5789	-1%±4.48%	0.8129	-1.09%±2%	0.5771
Magnesium	+1.33%±3.59%	0.7033	-0.28%±2.75%	0.9171	-1.90%±5.28%	0.7161	+0.36%±4.58%	0.8228
Mean	+5.92%±16.16%	0.3643	+4.97%±13.48%	0.3259	+4.03%±13.50%	0.3691	+2.87%±7.33%	0.2532

Table 1: The U-74389G influence (±SD) on the levels of some seric variables concerning reperfusion (rep) time [Tsompos et al., 2015].

Groups	Variable	Mean	Std. Dev
A	Weight	243 g	45.77724 g
A	OC	mild 0.5	0.5270463
B	Weight	262 g	31.10913 g
B	OC	without lesions 0.4	0.6992059
C	Weight	212.5 g	17.83411 g
C	OC	without lesions 0.2	0.421637
D	Weight	210 g	18.10463 g
D	OC	mild 0.7	0.4830459

Table 2: Weight and oviductal congestion (OC) score mean levels and Std. Dev. of groups

DG	Variable	Difference	p-value
A-B	Weight	-19 g	0.2423
A-B	OC	without lesions 0.1	0.7055
A-C	Weight	30.5 g	0.0674
A-C	OC	without lesions 0.3	0.2568
A-D	Weight	33 g	0.0574
A-D	OC	without lesions -0.2	0.4142
B-C	Weight	49.5 g	0.0019
B-C	OC	without lesions 0.2	0.5225
B-D	Weight	52 g	0.0004
B-D	OC	without lesions -0.3	0.2864
C-D	Weight	2.5 g	0.7043
C-D	OC	mild -0.5	0.0588

Table 3: Statistical significance of mean values difference for groups (DG) after statistical paired t test application for weight and Wilcoxon signed-rank test for scores.

Alteration	95% c. in	p-values		
		Reperfusion time	Wilcoxon	glm
without lesions 0.3	-0.7484155 - 0.1484155	1h	0.2568	0.1769
without lesions 0	-0.3582422 - 0.3582422	1.5h	0.9217	1.0000
without lesions -0.3	-0.2646058 - 0.8646058	2h	0.2864	0.2790
without lesions -0.2	-0.1521699 - 0.5521699	reperfusion time	0.2059	0.2575
without lesions -0.0909091	-0.1230462 - 0.3048644	interaction	-	0.3951

Table 4: The alteration influence of U-74389G in connection with reperfusion time.

Alteration	95% c. in	Reperfusion time	p-values
without lesions 0.3	-0.7484155 - 0.1484155	1h	0.2168
without lesions 0	-0.3582422 - 0.3582422	1.5h	0.9608
without lesions -0.3	-0.2646058 - 0.8646058	2h	0.2827
without lesions -0.2	-0.1521699 - 0.5521699	reperfusion time	0.2317
without lesions -0.0909091	-0.1230462 - 0.3048644	interaction	0.3951

Table 5: Concise presentation of the alteration influence of U-74389G in connection with reperfusion time.

DISCUSSION

The following situations show the association between ischemia and congestion in oviducts. Ajayi OL et al observed severe congestion, hyperemia, edema, dilatation and devitalization in the affected portion of an oviductal 360 degrees volvulus clockwise around the dorsal ligament during routine postmortem examination in an 11 months old chicken (*Gallus gallus domesticus*) [Ajayi et al., 2008]. Gordts S et al elucidated the process of human ovum retrieval by fimbriae. The fimbriae on the ovulatory side appeared congested, tumescent and showed pulsatile movements synchronous with the heartbeat. Vascular congestion causing erection and pulsatile movements of the fimbriae play a role in the retrieval of the ovum [Gordts et al., 1998]. Tuffrey M et al suggested that severe mucus congestion accompanied by tubal edema and

loss of ciliated epithelia play a major role in the aetiology of chlamydial-induced tubal damage [Tuffrey *et al.*, 1990]. Kleinstein J *et al* supposed that the oviduct damage caused only by the mechanical influence of the secretion congestion, is the reason for the unfavourable pregnancy rate after salpingoneostomy of a chronic atrophied hydrosalpinx [Kleinstein *et al.*, 1982].

CONCLUSION

U-74389G administration whether it interacted or not with reperfusion time failed to restore the OC lesions within short-term time context of 2 hours. Perhaps, a longer study time or a higher drug dose, may reveal significant and beneficial results.

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Authors' contribution: All authors contributed equally. Constantinos Tsompos analyzed and interpreted the data, Constantinos Panoulis pick up the data, Konstantinos Toutouzas drafted the article, George Zografos revised the article and Apostolos Papalois designed the concept.

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