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The Ovarian Congestion after "U-74389G" Administration in Rats

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Abstract

Object: The possible recessing capacity of the antioxidant drug "U-74389G" was studied in a rat model. It included the evaluation of the mean Ovarian Congestion (OC) lesions after induced ovarian Ischemia-Reperfusion (IR) injury.

Methods: The 40 used rats of mean mass 231.875 g were classified at 2 evaluation endpoints: one of 60 min for groups A and C and one of 120 min for groups B and D after reperfusion. The U-74389G was administered only in groups C and D.

Results: U-74389G administration non-significantly declined the OC lesions scores by 0.6 mild [-1.208205–0.0082055] (p=0.0832). Reperfusion time non-significantly augmented the OC lesions scores by 0.4 without lesions [0.7678806–0.56788075] (p=0.2860). The interaction of U-74389G administration with reperfusion time non-significantly declined the OC lesions scores by 0.2727273 without lesions [-0.6477081–0.1022535] (p=0.1492).

Conclusion: The U-74389G administration presented a no significant short-term recess direction for OC scores without lesions alteration. A more long-term study time or an enhanced U-74389G dose may outcome more significant effects.

Keywords: Ischemia; U-74389G; Ovarian congestion; Reperfusion

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Introduction

The lazaroid chemical family is a C21-amino-steroid, devoid of activity on carbohydrate metabolism (glucoactive activities) and mineralocorticoid [1]. It has a powerful effect against the pathological lipid peroxidation of lipid membranes, with a steroid-like mechanism, but without the side effects typical of high-dose steroid (methylprednisolone) [2]. All the lazaroids act as "scavengers" of oxygen free radicals (ROS) such as superoxide anion, hydroxyl radical and lipid peroxides; well as inhibiting the lipoxygenase and the production and release of arachidonic acid. The U-74389G is one of the more popular antioxidant agent of this family.

Actually, U-74389G implicates over 253 published biomedical studies. The experimental kind of the 45 (17.78%) at least of these studies belong to the tissue Ischemia-Reperfusion (IR) style. The assumption whether U-74389G can reverse induced IR injuries of either tissues or adjacent organs or the patients' health was raised. Popular questions concern the drug reaction velocity, the time of its administration and its dosage height. This antioxidant agent may be more beneficial than its original action. Such specific matters are always hardly met in related reports. Certain numeric efficacy of U-74389G was provided by a meta-analysis of 30 published related studies **(Table 1)**. This biomedical work tested the effect of U-74389G on a rat ovarian model. The U-74389G effect was estimated on mean OC lesions after induced IR.

Table 1 The U-74389G influence (±SD) on the levels of some seric variables [3] concerning reperfusion (rep) time.

Variable	1 h rep	p-value	1.5 h rep	p-value	2 h rep	p-value	Interaction of U-74389G and rep	p-value
WBCC	0.3544	0.0914	0.4199	0.0045	0.5609	0.0185	0.2973	0.0004
RBCC	±1.39% ± 0.71%	0.7161	0.0096	0.8106	-0.10% ± 0.05%	0.9762	±1.05% ± 0.53%	0.4911
Hematocrit	0.0858	0.0852	0.0698	0.0435	0.0733	0.2608	0.0449	0.0196
Hemoglobin	0.08	0.0925	0.06	0.0604	0.059	0.3544	0.038	0.0423
MCH	0.0273	0.0663	0.0297	0.0001	0.0374	0.0003	1.33%±0.36%	0.0005
MCHC	0.0024	0.482	-0.0032	0.1124	-0.0028	0.1603	-0.0032	0.0655
RbcDW	-0.024	0.0667	-0.0269	0.0175	-0.0073	0.1383	-0.0115	0.679
Platelet count	-0.0839	0.0647	-0.0704	0.0303	-0.0005	0.2939	-0.0254	0.0857
Platelet-crit	0.1367	0.6373	0.1552	0.1064	0.2369	0.0833	0.1045	0.0712
PDW	0.0198	0.2368	0.0255	0.0314	0.0382	0.0807	0.0142	0.0396
Glucose	-0.0291	0.0663	-0.0651	0.0001	-0.0822	0.0003	-0.0348	0.0005
Creatinine	-0.0725	0.0663	-0.1596	0.0001	-0.1997	0.0003	-0.0853	0.0005
Uric acid	0.353	0.1614	0.2453	0.096	0.2211	0.3946	0.1042	0.3873
Total protein	-0.0249	0.0663	-0.0558	0	-0.0704	0	-0.0298	0
γGT	0.3793	0.2362	0.2171	0.6442	0.1439	0.7809	0.1023	0.8877
ALP	±22.66% ± 12.37%	0.0663	0.396	0.0001	0.5081	0.0003	0.2254	0.0005
ACP	-0.9159	0.0006	-1.1361	0	-1.2274	0	-0.6482	0
CPK	0.6807	0.0012	0.5254	0.026	0.4661	0.4951	0.2796	0.077
Sodium	±1.22% ± 0.66%	0.0707	0.0078	0.7714	-0.87% ± 1.03%	0.3995	-0.32% ± 0.36%	0.3693
Potassium	-0.053	0.0579	0.0292	0.673	0.1262	0.3801	0.051	0.4853
Chloride	-0.58% ± 0.77%	0.4533	-0.0044	0.0879	-0.006	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	0.0492	0.7033	0.0247	0.9171	0.0338	0.7161	0.0494	0.8228
Mean	0.2755	0.2618	0.2817	0.2454	0.301	0.3044	0.1657	0.2476

Materials and Methods

Rat's preparation

Formal vet licenses were ascribed under No 3693/12-11-2010 and 14/10-1-2012 decisions of the local Prefecture in which ELPEN Pharmaceuticals Co Inc. S.A. belongs. This Co also offered all the substances and consumables. Appropriate humanistic care was applied for the female albino Wistar rats. This care was delivered inter-experimentally. It began 7 days already pre-experimentally by housing in laboratory the rats on ad libitum diet. Prenarcosis and non-stop general anesthesia [3-7], electrocardiogram, acidometry, oxygen supply were intra-experimentally provided. Finally, post-experimental euthanasia was followed. The rats were classified into four random groups. Each one contained 10 animals. All groups were submitted into 45 min ischemia after laparotomic clamping of inferior aorta higher than renal arteries level. The following clamp removal was restoring the inferior aorta patency and the reperfusion. The reperfusion was lasting 60 min for group A, 120 min for group B, 60 min plus U-74389G intravenous (IV) administration for group C and 120 min plus U-74389G IV administration for group D. The drug dosage was assessed at 10 mg/kg body mass per animal. The U-74389G was administered through inferior vena cava catheter upon reperfusion initiation. The evaluation of OC lesions scores was performed at 60 min of reperfusion for A and C groups; and at 120 min of reperfusion for B and D groups. The mean mass (M) of the above forty (40) Wistar albino rats was 231.875 g [Standard Deviation (SD): 36.59703 g], from min weight 165 g until max and the classification of OC values was weight 320 g. Thus, the pathologic evaluation [8] as scores: 0 for no noted lesions, 1 for mild ones, 2 for moderate ones, and 3 for serious scores. The original classification was transformed like this: (0-0.49) without OC lesions, (0.5-1.499) mild lesions, (1.5-2.499) moderate lesions and (2.5-3) serious ones since scores received also decimal values. The 1st Pathology Department of Clinical–Laboratory Sector in Faculty of Medicine of Athens University contributed in OC lesions evaluation.

The Ovarian Ischemia-Reperfusion Model

Control rats branch

This rats branch included 20 control ones of M: 252.5 g [SD: 39.31988 g] submitted into 45 min ischemia. The sub-group A was reperfused by 60 min (10 controls rats of M: 243 g [SD: 45.77724 g] and mean mild OC lesions score 1.6 [SD: 1.074968]. The sub-group B was reperfused by 120 min (10 controls rats of M: 262 g [SD: 31.10913 g] and mean mild OC lesions score 1.9 [SD: 0.9944289] **(Table 2)**.

U-74389G (L) rats branch

This rats branch included 20 L ones of M: 211.25 g [SD: 17.53755 g] submitted into 45 min ischemia. The perfusion was companioned by 10 mg U-74389G/kg body weight IV administration. The sub-group C was reperfused by 60 min plus U-74389G (10 L rats of M: 212.5 g [SD: 17.83411 g] and mean mild OC lesions score 1 [SD:

0.8164966]. The sub-group D was reperfused by 120 min plus U-74389G (10 L rats of M: 210g [SD: 18.10463 g] and mean mild OC lesions score 1.3 [SD: 0.9486833] **(Table 2)**.

Statistical analysis

A t-test is any statistical hypothesis test in which the test statistic follows a normal distribution under the null hypothesis [9]. It can be used to determine if two sets of data are significantly different from each other. Since the test statistic of rats' weight follows a normal distribution under the null hypothesis, the standard t-test can be used to determine if the 4 sets of weight data are significantly different from each other **(Table 3)**. The Wilcoxon signed-rank test is a non-parametric statistical hypothesis test used when comparing two related samples to assess whether their population mean ranks differ [10]. It can be used as an alternative to the t-test for dependent samples when the population cannot be assumed to be normally distributed. Since ovarian congestion lesions scores are not normally distributed, the Wilcoxon signed-rank test can be used for comparison of the 4 related samples of this variable, in order to assess whether their population mean ranks differ **(Table 3)**. Any raised significant difference among OC lesions scores, was tested weather was due to any respective probable significant mass one **(Table 3)**. The general linear model is a statistical linear model [11]. It consists of a dependant variable as a matrix with series of multivariate measurements, of an independent variable as a matrix that might be a design matrix, of parameters that are usually to be estimated and of errors or noise. The errors are usually assumed to be uncorrelated across measurements, and follow a multivariate normal distribution. If the errors do not follow a multivariate normal distribution, generalized linear models may be used to relax assumptions about the dependant variable and errors or noise. The general linear model incorporates a number of different statistical models including ordinary linear regression for non-parametric variables. Hypothesis tests with the general linear model can be made in two ways: multivariate or as several independent univariate tests. In multivariate tests the columns of dependant variable are tested together. Since ovarian congestion lesions scores can be considered a dependant variable; the drug or no administration, the reperfusion time and their interaction can be considered as multivariate independent variables; the general linear models can be used to determine how the dependant variable is influenced by the independent ones **(Table 3)**.

Table 2 Weight and ovarian congestion (OC) lesions mean scores and Std. Dev. of groups.

Groups	Variable	Mean	Std. Dev
A	Weight	243 g	45.77724 g
	OC	Moderate lesions 1.6	1.074968
B	Weight	262 g	31.10913 g
	OC	Moderate lesions 1.9	0.994429
C	Weight	212.5 g	17.83411 g
	OC	Mild lesions 1	0.816497
D	Weight	210 g	18.10463 g
	OC	Mild lesions 1.3	0.948683

The assumption of rats' mass as a confusing factor; e.g. the more obese rats to have higher OC lesions scores; was rejected since the successive insertion of rats' mass as independent variable at glm, revealed a non-significant correlation with OC lesions scores (p=0.0953). The statistical analysis was performed by Stata 6.0 statistical software [Stata 6.0, StataCorp LP, Texas, USA].

Results

U-74389G administration non-significantly declined the OC scores by 0.6 mild [-1.208205 - 0.0082055] (p=0.0530); accordant with the result of Wilcoxon signed-rank test (p=0.1135). Reperfusion time non-significantly augmented the OC scores by 0.3 without lesions [-0.3316902, -0.9316902] (p=0.3424), approximately accordant with the Wilcoxon signed-rank test one by 0.5 mild [-1.204071, -0.2040713] (p=0.2297). However, the interaction of U-74389G administration with reperfusion time significantly declined the OC scores by 0.2727273 without lesions [-0.6477081, 0.1022535] (p=0.1492). The above and **Table 3** constitute the **Tables 4 and 5** which show the decreasing influence of U-74389G versus reperfusion time.

Discussion

An association between ischemia and ovarian congestion is extracted congestion among pathological results. Akdemir A. et al. found [12] apparent congestion among pathological results in induced ovarian IR injury of rats. Sapmaz-Metin et al. assessed [13] pathological changes in post IR ovaries. Aslan et al. examined [14] post IR (peri) ovarian congestion. Aran et al. evaluated [15] high degrees of vascular congestion in twisted ovaries of Sprague-Dawley rats. Coskun et al. indicated [16] a gradually increasing congestion associated with respective increasing ischemic time for IR ovaries in rats. Kart et al. observed [17] severe congestion in ovarian IR of rabbits. Cigremis et al. observed [18] severe congestion in twisted ovaries of rabbits Smorgick et al. imaged [19] pathological series of congestion by ultrasound in twisted IR ovaries and necrosis in normal menstrual women. Kazez et al. assessed congestion [20] in both IR ovaries of female Wistar albino rats. Uguralp et al. showed [21] different degrees of congestion in contralateral ovaries after unilateral IR ones in albino Wistar rats. Taskin et al. showed [22] prominent congestion in all sections 36

Table 3 Statistical Standard t test application for mass and Wilcoxon signed-rank test for lesions scores.

DG	Variable	Difference	p-value
A-B	Weight	-19 g	0.2423
	OC	Without lesions -0.3	0.6009
A-C	Weight	30.5 g	0.0674
	OC	Mild 0.6	0.3259
A-D	Weight	33 g	0.0574
	OC	Without lesions 0.3	0.4655
B-C	Weight	49.5 g	0.0019
	OC	Mild 0.9	0.0313
B-D	Weight	52 g	0.0004
	OC	Mild 0.6	0.1882
C-D	Weight	2.5 g	0.7043
	OC	Without lesions -0.3	0.5294

Table 4 The decreasing influence of U-74389G versus reperfusion time.

Decrease	95% c. in.	Reperfusion time	Wilcoxon	p-values glm
Mild 0.6	-1.496831 - 0.296831	1 h	0.3259	0.1769
Mild 0.6	-1.208205 - 0.0082055	1.5 h	0.1135	0.053
Mild 0.6	-1.513089 - 0.3130891	2 h	0.1882	0.1843
Without lesions -0.3	-0.3316902 - 0.9316902	Reperfusion time	-	0.3424
Mild -0.5	-1.204071 - 0.2040713	Reperfusion time	0.2297	-
Without lesions 0.2727273	-0.6477081 - 0.1022535	Interaction	-	0.1492

Table 5 Concise presence of the decreasing influence of U-74389G versus reperfusion time.

Decrease	95% c. in.	Reperfusion time	p-values
mild -0.6	-1.496831 - 0.296831	1 h	0.2514
mild -0.6	-1.208205 - 0.0082055	1.5 h	0.0832
mild -0.6	-1.513089 - 0.3130891	2 h	0.1862
without lesions ±0.4	-0.7678806 - 0.56788075	Reperfusion time	0.286
without lesions -0.2727273	-0.6477081 - 0.1022535	Interaction	0.1492

h after adnexal IR in cycling female rats. Cavender and Murdoch paralleled [23] congestion chronologically along with ischemia during ovulation in ewe.

Richman hardly elucidated [24] that a disease progression is not equivalent to drug failure, which is not equivalent to drug resistance. Clinical disease progression is only indirectly linked to ovarian congestion. Drug resistance does appear to contribute to drug failure. High dosages may be active against ovarian congestion even in the presence of failed or resistant ovarian vessels. At the present time, vessel resistance and biological phenotype are not useful in the management of individual cases. Hwang et al. assessed [25] factors associated with regulatory approval or reasons for failure of investigational therapeutics in phase 3 or pivotal trials. Many investigational drugs fail in late-stage clinical development. They identified that 54% of therapeutics failed in clinical development, whereas 46% of them were approved. Most products failed due to inadequate efficacy (57%), while (17%) failed because of safety concerns and (22%) failed due to commercial reasons. In analyses adjusted for therapeutic area, agent type, firm size, orphan designation, fast-track status, trial year, and novelty of biological pathway, orphan-designated drugs were significantly more likely than nonorphan drugs to be approved (aOR: 2.3). Cancer drugs (aOR: 0.5) were significantly less likely to be approved.

Conclusion

U-74389G administration showed a non-significant short-term recessing trend for OC scores without lesions alteration. Since safety concerns were not raised yet for U-74389G, the future experiments ought to investigate whether a longer study time than 2 hours for the same dosage, or whether higher U-74389G dosage for the same study time may reveal more adequate decongestive efficacy.

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