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Original Article

The effect of the antioxidant drug "U-74389G" on testosterone levels during ischemia reperfusion injury in rats

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Abstract
Background: This experimental study examined the effect of the antioxidant drug "U-74389G", on a rat model and particularly in an adrenal ischemia - reperfusion protocol. The effects of that molecule were studied biochemically using blood mean testosterone (T) levels.
Materials and methods: 40 rats of mean weight 231.875 g were used in the study. Testosterone levels were measured at 60 min of reperfusion (groups A and C) and at 120 min of reperfusion (groups B and D), A and B without but C and D with U-74389G administration.
Results: U-74389G administration significantly increased the T levels by 52.17%±28.69% (p=0.0451). Reperfusion time significantly decreased the T levels by 85.62%±26.33% (P= 0.0019). However, U-74389G administration and reperfusion time together produced a non-significant combined effect in increasing the T levels by 11.18%±17.97% (p= 0.5245).
Conclusions: U-74389G administration interacted or not with reperfusion time increased short – term the testosterone levels.

1. Introduction

Permanent or transient damage with serious implications on adjacent organs and systems may be due to tissue ischemia - reperfusion (IR). The use of U-74389G in IR has been a challenge for many years. However, although the progress was significant, several practical questions have not clarified. They include: a) how potent U-74389G should be b) when should it be administered and c) at what optimal dose U-74389G should be administered. The promising effect of U-74389G in tissue protection has been noted in several IR studies. U-74389G or also 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 an antioxidant which prevents both arachidonic acid-induced and iron-dependent lipid peroxidation[1]. It protects against IR injury in animal organs such as heart, liver and kidney models. These membrane-associating antioxidants are particularly effective in preventing permeability changes in brain microvascular endothelial cells monolayers[2]. A meta-analysis of 26 published seric variables, coming from the same experimental setting, tried to provide a numeric evaluation of the U-74389G efficacy at the same endpoints (Table 1). Several publications addressed trials of other similar antioxidant molecules to which the studied molecule U-74389G belongs to.

The **aim** of this experimental study was to examine the effect of the antioxidant drug "U-74389G" on rat model and particularly in a generalized adrenal ischemia - reperfusion (IR) protocol. The effects of that molecule were studied by measuring blood mean testosterone (T) levels.

2. Materials and methods

2.1 Animal preparation

This basic experimental research was licensed by Veterinary Address of East Attiki Prefecture under 3693/12-11- 2010 & 14/10-1-2012 decisions. All consumables, equipment and substances, were a grant of Experimental Research Centre of ELPEN Pharmaceuticals Co. Inc. S.A. at Pikermi, Attiki. Accepted standards of human animal care were adopted for Albino female Wistar rats. 7 days pre-experimental normal housing included *ad libitum* diet in laboratory. Prenarcosis of animals proceeded of continuous intra-experimental general anesthesia[3-5], oxygen supply, electrocardiogram and acidometry. Post-experimental euthanasia did not permitted awakening and preservation of the animals. Rats were randomly delivered to four experimental groups by 10 animals in each one, using following protocols of IR: 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 dose of U-74389G was 10 mg/Kg body mass of animals. Ischemia was caused by laparotomic clamping inferior aorta over renal arteries with forceps for 45 min. The clamp removal restored the inferior aorta patency and reperfusion. U-74389G was administered at the time of reperfusion; through catheterized inferior vena cava. The T levels were determined at 60th min of reperfusion (for A and C groups) and at 120th min of reperfusion (for B and D groups). Fourty female Wistar albino rats were used (mean weight 231.875 g [Standard Deviation (SD): 36.59703 g], with minimum weight 165 g and maximum weight 320 g. Rats' weight could be potentially a confusing factor, e.g. more obese rats to have higher T levels. This assumption was also investigated.

2.2 Model of ischemia reperfusion injury

Control groups: 20 control rats of mean weight 252.5 g [SD: 39.31988 g] experienced ischemia for 45 min followed by reperfusion.
Group A: Reperfusion which lasted 60 min concerned 10 control rats of mean weight 243 g [SD: 45.77724 g] and mean T levels 0.091 ng/ml [SD: 0.0455705 ng/ml] (Table 2).

Group B: Reperfusion which lasted 120 min concerned 10 control rats of mean weight 262 g [SD: 31.10913 g] and mean T levels 0.034 ng/ml [SD: 0.0084327 ng/ml] (Table 2).

Lazaroid (L) group: 20 rats of mean weight 211.25 g [SD: 17.53755 g] experienced 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 [SD: 17.83411 g] and mean T levels 0.137 ng/ml [SD: 0.0851208 ng/ml] (Table 2).

Group D: Reperfusion which lasted 120 min concerned 10 L rats of mean weight 210 g [SD: 18.10463 g] and mean T levels 0.066 ng/ml [SD: 0.0648417 ng/ml] (Table 2).

3. Results

Every weight and T level group was compared with each other by statistical standard t-tests (Table 3). Any significant difference among T levels, was investigated whether owed in any potent significant weight one. The application of generalized linear models (glm) with dependant variable the T levels was followed. The 3 independent variables were the U-74389G or no drug administration, the reperfusion time and both variables in combination. Inserting the rats' weight also as an independent variable at glm analysis, a non significant relation resulted in (p= 0.1741), so as to further investigation was not needed.

The glm resulted in: U-74389G administration significantly increased the T levels by 0.039 ng/ml [-0.0030339 ng/ml - 0.0810339 ng/ml] (p= 0.0680). This finding was in accordance with the results of standard t-test (p=0.0223). Reperfusion time significantly decreased the T levels by 0.064 ng/ml [-0.1025895 ng/ml - -0.0254105 ng/ml] (p= 0.0018), in accordance also with standard t-test (P= 0.0021). However, U-74389G administration and reperfusion time together produced a non-significant combined effect in increasing the T levels by 0.0083636 ng/ml [-0.0179916 ng/ml - 0.0347188 ng/ml] (p= 0.5245). Reviewing the above and table 3, the tables 4 and 5 sum up concerning the increasing influence of U-74389G along with reperfusion time.

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

Variable	1h rep	p-value	1.5h rep	p-value	2h rep	p-value	interaction of U-74389G and rep	p-value
WBCC	+22.99%±12.45%	0.0914	+30.85%±11.14%	0.0045	+38.70%±17.39%	0.0185	+23.45%±6.28%	0.0004
RBCC	+1.39%±0.71%	0.7161	+0.64%±0.32%	0.8106	-0.10%±0.05%	0.9762	+1.05%±0.53%	0.4911
Hematocrit	+5.58%±3%	0.0852	+4.73%±2.25%	0.0435	+3.89%±3.44%	0.2608	+3.16%±1.33%	0.0196
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
MCH	+1.77%±0.96%	0.0663	+2.40%±0.57%	0.0001	+3.03%+0.71%	0.0003	1.33%+0.36%	0.0005
MCV[5]	+2.12%±1.16%	0.0663	+2.88%±0.69%	0.0001	+3.64%±0.85%	0.0003	+1.6%±0.43%	0.0005
MCHC	-0.5%±0.74%	0.4820	-0.95%±0.63%	0.1124	-1.4%±1.12%	0.1603	-0.69%±0.37%	0.0655
RbcDW	-6.13%±3.73%	0.0667	-4.96%±2.27%	0.0175	-3.80%±3.07%	0.1383	-2.54%±1.39%	0.679
Platelet count	-17.79%±9.40%	0.0647	-12.83%±5.79%	0.0303	-7.88%±7.83%	0.2939	-6.12%±3.58%	0.0857
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
PDW	+1.1%±0.88%	0.2368	+1.79%±0.76%	0.0314	+2.49%±1.33%	0.0807	+0.96%±0.46%	0.0396
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
Creatinine	-15.96%±8.71%	0.0663	-21.02%±5.06%	0.0001	-26.09%±6.12%	0.0003	-11.69%±3.16%	0.0005
Uric acid	+20.86%±14.44%	0.1614	+15.43%±9.10%	0.0960	+10%±12.11%	0.3946	+4.78%±5.64%	0.3873
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
γGT	+19.35%±18.58%	0.2362	+6.82%±14.89%	0.6442	-5.71%±20.10%	0.7809	+1.23%±9%	0.8877
ALP	+22.66%±12.37%	0.0663	+31.91%±7.69%	0.0001	+41.16%±9.65%	0.0003	+17.75%±4.79%	0.0005
ACP	-112.54%±20.95%	0.0006	-128.45%+14.84%	0.0000	-144.36%±21.62%	0.0000	-74.45%±9.63%	0.0000
CPK	+54.32%±13.75%	0.0012	+35.34%±17.20%	0.0260	+16.37%±30.24%	0.4951	+18.52%±9.44%	0.0770
LDH	+13.56%±7.40%	0.0663	+18.78%±4.52%	0.0001	+24.01%±5.63%	0.0003	+10.43%±2.82%	0.0005
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
Potassium[4]	-10.12%±4.82%	0.0579	-2.14%±5.06%	0.6730	+5.83%±6.79%	0.3801	+2.07%±3.03%	0.4853
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	-0.01%±27.22%	0.2468	-0.93%±29.14%	0.2265	-1.85%±32.36%	0.2810	-0.40%±16.94%	0.2286

Table 2: Weight and testosterone mean levels and Std. Dev. of groups

Groups	Variable	Mean	Std. Dev
A	Weight	243 g	45.77724 g
	Testosterone	0.091 ng/ml	0.0455705 ng/ml
B	Weight	262 g	31.10913 g
	Testosterone	0.034 ng/ml	0.0084327 ng/ml
C	Weight	212.5 g	17.83411 g
	Testosterone	0.137 ng/ml	0.0851208 ng/ml
D	Weight	210 g	18.10463 g
	Testosterone	0.066 ng/ml	0.0648417 ng/ml

Table 3: Statistical significance of mean values difference for groups (DG) after statistical standard t test application

DG	Variable	Difference	p-value
A-B	Weight	-19 g	0.3555
	Testosterone	0.057 ng/ml	0.0031
A-C	Weight	30.5 g	0.0674
	Testosterone	-0.046 ng/ml	0.1030
A-D	Weight	33 g	0.0574
	Testosterone	0.025 ng/ml	0.2674
B-C	Weight	49.5 g	0.0062
	Testosterone	-0.103 ng/ml	0.0043
B-D	Weight	52 g	0.0009
	Testosterone	-0.032 ng/ml	0.1369
C-D	Weight	2.5 g	0.7043
	Testosterone	0.071 ng/ml	0.0662

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

Increase	95% c. in	Reperfusion time	p-values	
			t-test	glm
0.046 ng/ml	-0.018146 ng/ml -0.110146 ng/ml	1h	0.1030	0.1493
0.039 ng/ml	-0.0030339 ng/ml - 0.0810339ng/ml	1.5h	0.0223	0.0680
0.032 ng/ml	-0.0114416 ng/ml - 0.0754416ng/ml	- 2h	0.1369	0.1391
0.064 ng/ml	-0.1025895 ng/ml -0.0254105 ng/ml	reperfusion time	0.0021	0.0018
0.0083636 ng/ml	-0.0179916 ng/ml - 0.0347188ng/ml	interaction	-	0.5245

Table 5: The (%) increasing influence of U-74389G in connection with reperfusion time

Increase	±SD	Reperfusion time	p-values
40.35%	±28.70%	1h	0.1261
52.17%	±28.69%	1.5h	0.0451
64%	±44.32%	2h	0.1380
-85.62%	±26.33%	reperfusion time	0.0019
11.18%	±17.97%	interaction	0.5245

4. Discussion

T is considered a reliable index substance of adrenals metabolism; being of great clinical significance. Examples are described herein concerning whether adrenal ischemia can influence the T levels. Cakir E et *al* found [6] total T levels significantly higher in PCOS women also having higher risk for cardiovascular disease (CVD) and myocardial ischemia than control subjects. Guven S *et al* found [7] significantly higher concentrations of serum total T (P = 0.031), elevated serum ischemia-modified albumin (IMA) concentrations - a clinical marker of ongoing myocardial ischemia - well correlated with total T levels (P = 0.022) in women with PCOS than control group. Shaw LJ *et al* [8] found more frequent and heavy angiographic coronary artery disease (CAD) (P = 0.04) and 9.8% less cumulative 5-yr cardiovascular (CV) event-free survival (P = 0.006) in women with clinical features of PCOS as defined by top T quartile (> 30.9 ng/dl) than normal control women. PCOS remained a significant predictor (P < 0.01) in prognostic models for suspected ischemia and CV disease. Kovalenko AN *et al* [9] determined peripheral blood T concentrations with consequences on metabolic background conducting to cerebral atherosclerosis and ischemic stroke development in clinically normal elderly than younger subjects.

5. Conclusion

U-74389G administration interacted or not with reperfusion time increased short – term the testosterone levels. The intervention of this molecule into the biosynthetic pathway of T worths further investigation.

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