Research Journal of Pharmacology and Toxicology 02[02] 2016
www.asdpub.com/index.php/rjpt

Original Article

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

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Keywords:
Ischemia,
U-74389G,
Creatine kinase - MB,
Reperfusion

Abstract

Background: This experimental study examined the effect of the antioxidant drug "U-74389G", on a rat model and particularly in a liver ischemia - reperfusion protocol. The effects of that molecule were studied biochemically using blood mean creatine kinase - MB (CK-MB) levels.
Methods: 40 rats of mean weight 231.875 g were used in the study. CK-MB levels were measured at 60 min of reperfusion (groups A and C) and at 120 min of reperfusion (groups B and D). The drug U-74389G was administered only in groups C and D.
Results: U-74389G administration kept significantly increased the CK-MB levels by 31.60% ± 13.10% (p = 0.0148). Reperfusion time kept non-significantly increased the CK-MB levels by 5.40% ± 14.09% (p= 0.6358). However, U-74389G administration and reperfusion time together kept non-significantly increased the CK-MB levels by 14.50% ± 8.16% (p=0.0745).
Conclusions: U-74389G administration reduced at non-significantly increased the CK-MB levels, getting on decline them from significant to non-significant level.

1. Introduction

The chemical group of lazaroid is a C_{21}-amino-steroid complex, devoid of effect on mineralocorticoid and carbohydrate metabolism (glucoactive activity)[1]. Contrary, this complex has a powerful effect against the pathologic lipoperoxidation occurred on lipid membranes. This effect is performed by a steroid-like mechanism but devoid of the side effects typical of high-dose steroids as methylprednisolone[2]. All the lazaroid members act as "scavengers" of oxygen free radicals (ROS) such as hydroxyl radical, lipid peroxides and superoxide anion. Along, it inhibits the lipoxygenase and the production and release of arachidonic acid. The U-74389G is the most famous antioxidant agent of that family.

The U-74389G implicates over 254 published biomedical studies at present. The methodology at least of the 46 (18.11%) of these experiments are classified at the tissue ischemia-reperfusion (IR) style. The assumption concerned whether the U-74389G can reverse induced IR injuries in tissues, surrounding organs or even the patients' health. Common affairs were the drug reaction rapidity, the timing of its administration and the dosage height. This antioxidant agent may be proved more beneficial than described. So specific matters are always hardly met in bibliography. A meta-analysis of 35 published related studies yielded a certain numeric efficacy of U-74389G (Table 1). This certain biomedical work tested the effect of U-74389G on a rat liver model. The U-74389G effect was calculated on mean creatine kinase - MB (CK-MB) levels.

2. Materials and methods

2.1 Animal preparation

Legal vet licenses were ascribed under the 3693/November 12, 2010 & 14/January 10, 2012 decisions by the local Prefecture in which the acknowledged Co. Inc SA belongs. All the consumed and non substances, settings and equipment were offered by that Co. at Pikermi - Attiki. The Albino female Wistar rats were managed by the precicted humanistic care. *Ad libitum* diet accompanied the 7 days pre-experimental normal housing in laboratory. Prenarcosis engaged the nonstop intra-experimental care including general anesthesia[3], oxygen supply, electrocardiogram and acidometry. Post-experimental euthanasia excluded awakening and preservation of the animals. The rats were successively delivered to four experimental groups; each one containing 10 animals. Thus, the following protocol of IR was performed: The ischemia stage of 45 min preceded and was common at all groups. However, it was followed by reperfusion for: 60 min for group A; 120 min for group B; 60 min with immediate U-74389G intravenous (IV) administration for group C and 120 min with immediate U-74389G IV administration for group D. The dose of U-74389G was assessed at 10 mg/Kg body mass of animals. Ischemia was induced by laparotomic clamping inferior aorta over the renal arteries with forceps for 45 min. The clamp removal restored the inferior aorta patency and reperfusion. The U-74389G was administered just at initiation of reperfusion; through inferior vena cava catheter. The CK-MB levels were assessed at 60th min of reperfusion for A,C groups and at 120th min of reperfusion for B,D groups. The fourty female Wistar albino rats were described with mean weight (W): 231.875 g [Standard Deviation (SD): 36.59703 g], with minimum W: 165 g and maximum W: 320 g. Rats' W could be perhaps a confusing factor, e.g. the more obese rats, the higher CK-MB levels. This assumption was also tested.

2.2 Control groups

The known preceded ischemia of 45 min for the 20 control rats (mW: 252.5 g [SD: 39.31988 g]) was followed by reperfusion.
Group A: Reperfusion lasting 60 min (10 controls rats) concerned mW: 243 g [SD: 45.77724 g] and mean CK-MB levels: 299.4 UI/L [SD: 176.6592 UI/L] (Table 2).

Group B: Reperfusion lasting 120 min (10 controls rats) concerned mW: 262 g [SD: 31.10913 g] and mean CK-MB levels: 315 UI/L [SD: 189.6535 UI/L] (Table 2).

Lazaroid (L) group

The known preceded ischemia of 45 min for the 20 L rats (mW: 211.25 g [SD: 17.53755 g] was followed by reperfusion in the beginning of which 10 mg U-74389G /kg body W were IV administered.

Group C: Reperfusion lasting 60 min (10 L rats) concerned mW: 212.5 g [SD: 17.83411 g] and mean CK-MB levels: 451.3 UI/L [SD: 54.75207 UI/L] (Table 2).

Group D: Reperfusion lasting 120 min (10 L rats) concerned mW: 210 g [SD: 18.10463 g] and mean CK-MB levels: 395.9 UI/L [SD: 141.9667 UI/L] (Table 2).

2.3 Statistical analysis

Every weight and CK-MB level group was compared with each other by statistical standard t-tests (Table 3). Any significant difference among CK-MB levels, was investigated whether owed in any potent significant weight one. The application of generalized linear models (glm) with dependant variable the CK-MB 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=0.0723), so as to further investigation was not needed.

3. Results

The glm resulted in: U-74389G administration kept significantly increased the CK-MB levels by 116.4 UI/L [210.9841 UI/L - 21.81587 UI/L] (p= 0.0172). This finding was in accordance with standard t-test (p=0.0124). Reperfusion time kept non-significantly increased the CK-MB levels by 19.9 UI/L [-121.707 UI/L - 81.90697 UI/L] (P= 0.6945), also in accordance with the results of standard t-test (p=0.5772). However, U-74389G administration and reperfusion time together kept non-significantly increased the CK-MB levels by 53.41818 UI/L [-5.545847 UI/L - 112.3822 UI/L] (p= 0.0745). Reviewing the above and table 3, the tables 4 and 5 sum up concerning the alteration influence of U-74389G versus 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	+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
MPV	+9.29%±5.07%	0.0663	+12.77%±3.07%	0.0001	+16.25%±3.81%	0.0003	+7.09%±1.91%	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
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
Urea	-8.51%±4.64%	0.0663	-11.35%±2.73%	0.0001	-14.19%±3.32%	0.0003	-6.31%±1.70%	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
Albumins	-2.70%±1.47%	0.0663	-3.63%±0.87%	0.0001	-4.57%±1.07%	0.0003	-2.02%±0.54%	0.0005
ALT[3]	+11.25%±6.19%	0.0686	-8.83%±11.25%	0.4168	-28.92%+24.55%	0.2206	-4.98%+6.77%	0.4527
AST	+33.94%±15.10%	0.0328	+24.97%±14.34%	0.0593	+16%±22.72%	0.4077	+16.23%±8.58%	0.0583
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
CK-MB	+40.46%± 16.70%	0.0147	+31.60%+13.10%	0.0148	+22.75%± 22.59%	0.2865	+14.50%±8.16 %	0.0745
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	-10.12%±4.8%	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
Amylase	-6.28%±3.43%	0.0663	-8.40%±2.02%	0.0001	-10.53%+2.47%	0.0003	-4.67%±1.26%	0.0005
Progesterone	-46.14%±25.19%	0.0663	-58.04%±13.99%	0.0001	-69.94%±16.41%	0.0003	-32.29%±8.73%	0.0005
Testosterone	+40.35%±28.70%	0.1261	+52.17%±28.69%	0.0451	+64%±44.32%	0.1380	+11.18%±17.97%	0.5245
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

Table 2: Weight and CK-MB levels and Std. Dev. of groups

Groups	Variable	Mean	Std. Dev
A	Weight	243 g	45.77724 g
	CK-MB	299.4 UI/L	176.6592 UI/L
B	Weight	262 g	31.10913 g
	CK-MB	315 UI/L	189.6535 UI/L
C	Weight	212,5 g	17.83411 g
	CK-MB	451.3 UI/L	54.75207 UI/L
D	Weight	210 g	18.10463 g
	CK-MB	141.9667	395.9 UI/L UI/L

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	0.2423
	CK-MB	-15.6UI/L	0.7558
A -C	Weight	30.5 g	0.0674
	CK-MB	-151.9 UI/L	0.0113
A -D	Weight	33 g	0.0503
	CK-MB	-96.5 UI/L	0.2064
B -C	Weight	49.5 g	0.0019
	CK-MB	-136.3 UI/L	0.0473
B -P	Weight	52 g	0.0004
	CK-MB	-80.9 UI/L	0.2786
C-D	Weight	2.5 g	0.7043
CK-MB		55.4 UI/L	0.3009

Table 4: The restore influence of U-74389G versus reperfusion time

			p-values	
Increase	95% c. in.	Reperfusion time	t-test	glm
151.9 UI/L	29.02519 UI/L - 274.7748 UI/L	1h	0.0182	0.0113
116.4 UI/L	21.81587 UI/L - 210.9841 UI/L	1.5h	0.0124	0.0172
80.9 UI/L	-76.49116 UI/L - 238.2912 UI/L	2h	0.2945	0.2786
-19.9 UI/L	-121.707 UI/L - 81.90697 UI/L	reperfusion temps	0.6945	0.5772
-5.545847	53.41818 UI/L UI/L - 112.3822 UI/L	interaction	- 0.0745	

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

Increase	+SD	Reperfusion time	p-values
40.46 %	± 16.70 %	1h	0.0147
31.60 %	±13.10 %	1.5h	0.0148
22.75 %	± 22.59 %	2h	0.2865
-5.40%	± 14.09%	reperfusion temps	0.6358
14.50%	± 8.16 %	interaction	0.0745

4. Discussion

The CK-MB levels can be influenced by ischemic cases. Gonçalves ES *et al*[4] noted a significant increase of the CK-MB plasma levels after 30 minutes IR in pilot rats treated by ornithin α-ketoglutarate. Jebeli M *et al*[5] found significantly elevated the CK-MB serum levels in ischemia or myocardial infarction among patients presenting either deterioration of the left ventricular function [(FEVG) < 35%] or acute phase of myocardial infarction (MI), suffered from ventricular tachycardia (VT). Sala MF *et al*[6] estimated more frequently by 3.95-fold the peak of CK-MB levels > 300 IU/L in acute phase of MI patients suffering from VT. Serruys PW *et al*[7] noted no case of raised CK-MB levels 10 times over the upper normal limit in patients after the success of their first percutaneous coronary intervention (PCCI) randomly receiving treatment with either fluvastatine 80 mg/kg or placebo one, during 3 to 4 years after hospital exit. Savchuk VI *et al*[8] calculated increased the total activity of CK-MB by 57.3±11.7 mE/ml ($p < 0.001$) in coronary blood after myocardial ischemia induction via short run in dogs.

Nediani C *et al* attenuated all the modifications, particularly, the neutrophiles infiltration by aminosteroid administration (4 mg/kg) in coronary IR of pigs. The 21-lazaroid aminosteroids[9] trap free radicals and work as membrane stabilizers. The energy load reduction was reversed in the treated group. Myocardial concentration of malondialdehyde was raised in all animals after the reperfusion, but this effect was significantly marked with aminosteroids treatment. Moreover, an increase in infarctions of ascorbic acid contents and a potency to reduce the serum peroxidation rate exposed in animals treated than untreated ones, indicate an improvement of the antioxidant protection induced by administration of aminosteroids. In addition, the serum rates of CK-MB isoenzyme suggest the possibility of the aminosteroids to attenuate the modifications of the membrane permeability induced by IR damage. The aminosteroid treatment is effective in the reduction of morphological and biochemical deteriorations which occur in an IR myocardium.

4. Conclusion

U-74389G administration reduced at non-significantly increased the CK-MB levels, getting on decline them from significant to non-significant level.

Acknowledgment

This study was funded by Scholarship by the Experimental Research Centre ELPEN Pharmaceuticals (E.R.C.E), Athens, Greece. The research facilities for this project were provided by the aforementioned institution.

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