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The Effect of the Antioxidant Drug "U-74389G" on Creatine Phosphokinase Levels during Ischemia Reperfusion Injury in Rats

ORIGINAL INVESTIGATION

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ABSTRACT

Objective: This experimental study examines the effect of the antioxidant drug "U-74389G" on rat model, particularly in an ischemia–reperfusion (IR) protocol. The effects of this molecule were biochemically studied using mean creatinine phosphokinase (CPK) levels in blood.

Materials and Methods: Forty rats with a mean weight of 231.875 g were used in the study. CPK levels were measured at 60 min (groups A and C) and at 120 min of reperfusion (groups B and D); A and B were groups without U-74389G administration, but C and D were groups with U-74389G administration.

Results: U-74389G administration significantly increased CPK levels by 35.34%+17.20% (p=0.0260). Reperfusion time non-significantly increased CPK levels by 13.17%+18.05% (p=0.4134). However, U-74389G administration and reperfusion time together non-significantly increased CPK levels by 18.52%+9.44% (p=0.0770).

Conclusion: U-74389G administration significantly increased CPK levels for a short term; however, these levels could not be restored, and the restoration capacity increases along with reperfusion time.

Keywords: Ischemia, U-74389G, creatinine phosphokinase, reperfusion

INTRODUCTION

Tissue ischemia–reperfusion (IR) remains one of the main causes of permanent or transient damage with serious implications on adjacent organs and, certainly, on patients' health. Although important progress has been made regarding the usage of U-74389G in managing this kind of damages, satisfactory answers have not yet been given to fundamental questions such as how powerful should an antioxidant be, when should it be administered, and what dosage should be administered. The particularly satisfactory effect of the antioxidant U-74389G in tissue protection has been noted in several experiments performed. However, only few relative reports were found concerning U-74389G trial in IR experiments, not completely covering this particular matter. Also, several publications addressed trials of other similar molecules of the aminosteroid (lazaroids) group to which the studied molecule belongs. 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 (1). It protects against IR injury in animal heart, liver, and kidney models. These membrane-associated antioxidants are particularly effective in preventing permeability changes in brain microvascular endothelial cell monolayers (2). The same authors of that study found the influence of U-74389G, as depicted in Table 1, on some seric variable levels in related IR injury experiments at the same endpoints with the present experiment after clamp removal in rats (3, 4).

Creatinine phosphokinase (CPK) is an enzyme expressed by various tissues and cell types. It catalyzes the conversion of creatinine and consumes adenosine triphosphate (ATP) to create phosphocreatinine (PCr) and adenosine diphosphate (ADP). Some tissues and cells rapidly consume ATP such as brain, photoreceptor cells of the retina, hair cells of the inner ear, spermatozoa, smooth muscles, particularly skeletal muscles. The aim of this experimental study was to examine the effect of the antioxidant drug "U-74389G" on rat model, particularly in an inferior suprarenal aorta IR protocol. The occluded regions concern renal, pelvic, and inferior limbs muscular tissues, which express CPK enzymes. The effects of this molecule were studied by measuring the mean CPK levels in the blood.

MATERIALS and METHODS

Animal preparation

This experimental study was designed at the Experimental Research Center of ELPEN Pharmaceuticals Co. Inc. S.A. at Pikermi, Attiki. It was licensed by Veterinary Address of East Attiki Prefecture (Ethics Committee) under

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Table 1. The U-74389G influence (+Std. Dev.) on the levels of some seric variables 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
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
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
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
Calcium4	0%+1.75%	1.0000	−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

Rep: reperfusion
+Std. Dev.: standard deviation

3693/12-11-2010 and 14/10-1-2012 decisions. Informed consent was not needed. All settings required for the study, including consumables, equipment, and substances used, were the courtesy of that S. A. Albino female Wistar rats were used in accordance with the accepted standards of humane animal care. They were housed in the laboratory for 7 days before the experiment, having easy access to water and food. The experiment was acute, i.e., the experiment was not followed by awakening and preserving rodents. They were randomly assigned to the following four experimental groups (10 animals in each group): 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); and ischemia for 45 min followed by immediate U-74389G IV administration and reperfusion for 120 min (group D). The dose of the molecule U-74389G was 10 mg/kg body weight of animals. The sampling was performed after vena cava venipuncture by filling a 2-mL dimpled control stroke syringe with pre-set volume for withdrawal and sterile insulin needle. Then, the sample was transferred to a 2-mL vacuum blood collection tube (disposable vacutainer) according to both National Committee for Clinical Laboratory Standards (NCCLS) and the International Council for Standardization. CPK level measurements were performed by Nihon Kohden Celltac, a MEK-6450 K automated biochemistry analyzer with preset rat-type and cyanide-free reagents.

The experiment started with animals subjected to pre-narcosis followed by general anesthesia, as described in detail in related references (3, 4). Their electrocardiogram, acidometry, and oxygen supply were continuously provided. Their inferior aorta's flow was excluded using forceps. After exclusion, the protocol of IR was applied exactly in the same manner as that described in experimental groups. The molecules were administered at the time of reperfusion through the inferior vena cava after catheterization had been achieved. CPK level measurements were performed at 60 min of reperfusion (groups A and C) and at 120 min of reperfusion (groups B and D).

Ischemia was caused by clamping the inferior aorta over renal arteries for 45 min after laparotomic access was achieved. Reperfusion was induced by removing the clamp and re-establishing the patency of the inferior aorta. Forty female Wistar albino rats were used with a mean weight of 231.875 g [Std. Dev.: 36.59703 g], having a minimum weight of ≥165 g and maximum weight of ≤320 g. Weight of rats could potentially be a confusing factor, e.g., fatter rats tend to have greater blood CPK levels. This suspicion was also investigated.

Control groups

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

Group A

Reperfusion that for 60 min concerned 10 control rats with a mean weight of 243 g [Std. Dev.: 45.77 g] and mean CPK levels of 2170.3 IU/L [Std. Dev.: 817.92 IU/L] (Table 2).

Group B

Reperfusion that lasted for 120 min concerned 10 control rats with a mean weight of 262 g [Std. Dev.: 31.10 g] and mean CPK levels of 3107.6 IU/L [Std. Dev.: 2360.91 IU/L] (Table 2).

Lazaroid (L) group

Twenty rats with a mean weight of 211.25 g [Std. Dev.: 17.53 g] suffered from ischemia for 45 min followed by reperfusion, in

Table 2. Weight and CPK mean levels and Std. Dev. of groups

Groups	Variable	Mean	Std. Dev.
A	Weight	243 g	45.77 g
	CPK	2170.3 IU/L	817.92 IU/L
B	Weight	262 g	31.10 g
	CPK	3107.6 IU/L	2360.91 IU/L
C	Weight	212.5 g	17.83 g
	CPK	3789 IU/L	889.96 IU/L
D	Weight	210 g	18.10 g
	CPK	3662 IU/L	1883.55 IU/L

Std. Dev.: standard deviation; CPK: creatinine phosphokinase; IU/L: international units per liter

Table 3. Statistical significance of difference in mean values for groups after statistical paired t-test application

DG	Variable	Difference	p value
A - B	Weight	–19 g	0.2423
	CPK	–937.3 IU/L	0.1566
A - C	Weight	30.5 g	0.0674
	CPK	–1618.7 IU/L	0.0020
A - D	Weight	33 g	0.0574
	CPK	–1491.7 IU/L	0.0377
B - C	Weight	49.5 g	0.0019
	CPK	–681.4 IU/L	0.3634
B - D	Weight	52 g	0.0004
	CPK	–554.4 IU/L	0.4214
C - D	Weight	2.5 g	0.7043
	CPK	127 IU/L	0.8444

CPK: creatinine phosphokinase; DG: difference for groups; IU/L: international units per liter

the beginning of which 10-mg U-74389G/kg body weight was IV administered.

Group C

Reperfusion that lasted for 60 min concerned 10 L rats with a mean weight of 212.5 g [Std. Dev.: 17.83 g] and mean CPK levels of 3789 IU/L [Std. Dev.: 889.96 IU/L] (Table 2).

Group D

Reperfusion that lasted for 120 min concerned 10 L rats with a mean weight of 210 g [Std. Dev.: 18.10 g] and mean CPK levels of 3662 IU/L [Std. Dev.: 1883.55 IU/L] (Table 2).

Statistical analysis

Each from four rats weight groups was compared with each other from three remaining groups applying statistical paired t-test (Table 3). Any emerging significant difference among CPK levels owing to the abovementioned significant weight correlations was investigated. Also, CPK levels in each of the four rat groups were compared with the three remained groups applying statistical paired t-test (Table 3). Application of generalized linear models (GLM) with dependent variable the CPK levels and independent variables the U-74389G administration or not, the reperfusion time and their interaction was followed.

RESULTS

GLM resulted in the following: U-74389G administration significantly increased CPK levels by 1618.7 IU/L [815.6505–2421.749 IU/L] (p=0.0005) at 1 hour of reperfusion in the U-74389G group than in the sham-operated one. This finding was in accordance with the results of paired t-test (p=0.0020). U-74389G administration significantly increased CPK levels by 1086.55 IU/L [49.52376–2123.576 IU/L] (p=0.0405) at 1.5 hours of reperfusion in the U-74389G administered group than in the sham-operated one. This finding was in accordance with the results of the paired t-test (p=0.0115). U-74389G administration non-significantly increased CPK levels by 554.4 IU/L [–1452.138 IU/L–2560.938 IU/L] (p=0.5688) at 2 hours of reperfusion in the U-74389G group than in the sham-operated one. This finding was in accordance with the results of the paired t-test (p=0.4214). Also, reperfusion time non-significantly increased CPK levels by 405.15 IU/L [–683.447 IU/L–1493.747 IU/L] (p=0.4558) in accordance with the paired t-test (p=0.3711) at 1.5 hours of reperfusion in sham animals. However, U-74389G administration and reperfusion time together non-significantly increased CPK levels by 569.5727 IU/L [–64.75793 IU/L–1203.903 IU/L] (p=0.0770) at 1.5 hours of reperfusion in U-74389G administered group than in the sham-operated one. Reviewing the abovementioned results and those in Table 3, Table 4 summarizes data concerning alterations in the influence of U-74389G versus reperfusion time. Iterating the above reasoning concisely according to rates (%), the following results were obtained: U-74389G administration significantly increased CPK levels by 54.32%±13.75% at 1 hour of reperfusion in the U-74389G group than in the sham-operated one (p=0.0012). U-74389G administration significantly increased CPK levels by 35.34%±17.20% at 1.5 hours of reperfusion in U-74389G administered group than in the sham-operated one (p=0.0260). U-74389G administration non-significantly increased CPK levels by 16.37%±30.24% at 2 hours of reperfusion in the U-74389G group than in the sham operated one (p=0.4951). Reperfusion time non-significantly increased CPK levels by 13.17%±18.05% (p=0.4134) at 1.5 hours of reperfusion in sham animals. However, U-74389G administration and reperfusion time together non-significantly increased CPK levels by 18.52%±9.44% (p=0.0770) at 1.5 hours of reperfusion in U-74389G administered group than in the sham-operated one. Reviewing the above results and those in Table 4, Table 5 summarizes data concerning alterations in the influence of U-74389G versus reperfusion time. Also, by adding the weight of rats as an independent variable in GLM analysis, a non-significant relation results (p=0.1075); therefore, as to further investigation is not needed.

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

Increase	95% CI	Reperfusion time	p values t-test	GLM
1618.7 IU/L	815.65 IU/L–2421.74 IU/L	1 h	0.0020	0.0005
1086.55 IU/L	49.52 IU/L–2123.57 IU/L	1.5 h	0.0115	0.0405
554.4 IU/L	–1452.13 IU/L–2560.93 IU/L	2 h	0.4214	0.5688
405.15 IU/L	–683.44 IU/L–1493.74 IU/L	reperfusion time	0.3711	0.4558
569.5727 IU/L	–64.75 IU/L–1203.90 IU/L	interaction		0.0770

GLM: generalized linear models; IU/L: international units per liter; t-test: homonymous statistical test; CI: confidence interval

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

Increase	+Std. Dev.	Reperfusion time	p values
54.32%	+13.75%	1 h	0.0012
35.34%	+17.20%	1.5 h	0.0260
16.37%	+30.24%	2 h	0.4951
13.17%	+18.05%	reperfusion time	0.4134
18.52%	+9.44%	interaction	0.0770

+Std. Dev.: +standard deviation

DISCUSSION

The folowing situations show the relation between CPK levels and ischemic kidneys. Radovanović et al. (5) claim that excessive narcotic consumption or abuse for a long period can lead to various consequences such as atraumatic rhabdomyolysis, acute renal failure, and electrolytic disorders. Rhabdomyolysis is characterized by the injury of the skeletal muscle with subsequent release of intracellular contents such as myoglobin, potassium, and CPK. In the case of heroin addicts, rhabdomyolysis is a consequence of the development of a compartment syndrome (CoS) because of immobilization of patients in the state of unconsciousness, prolonged compression of extremities, direct heroin toxicity, or ischemia of the extremities caused by intraluminal occlusion of blood vessels after intra-arterial injection of heroin. Laboratory analyses usually registers high hyperkalemia level, increased urea, creatinine, CPK, CK-MB, and myoglobin levels. Murata et al. (6) created a crush syndrome (CrS) model followed by rhabdomyolysis with markers such as potassium and CPK after bilateral hind limb IR in rats. Tachtsi et al. (7) described the compressive myopathy syndrome (SCM) as a lesion of skeletal muscles resulting in the subsequent release of intracellular contents (myoglobin, CPK, and potassium) into the circulatory system. The most common potentially lethal complication is acute renal failure. The occurrence of acute rhabdomyolysis should be considered in any patient who can remain stationary for long periods, is in a coma, is intoxicated in any form, or with a prolonged compression of an extremity. Byard et al. (8) observed marked pallor and edema of the left sternomastoid muscle and myoglobin casts in renal tubules corresponding to an antemortem increased CPK level in an unexpected cardiac arrest case. Alconcher et al. (9) reported rhabdomyolysis associated with severe renal insufficiency and CPK levels raised up to 40.824 UI/L in 24% of cocaine and other excessive drugs users. Fernandez et al. (10) classified the most common causes of rhabdomyolysis into cocaine, exercise, and immobilization, excluding patients with an initial serum CPK level of >1000 U/L if they had evidence of myocardial ischemia, cerebrovascular insufficiency, or rhabdomyolysis after hospitalization. Souza-Moraes et al. (11) investigated kidney alterations estimating blood CPK levels after infrarenal IR of hind limb skeletal muscles in rats. Ishikawa I reported acute renal failure induced by rhabdomyolysis after strenuous exercise associated with normal or only slightly elevated CPK and serum myoglobin concentrations, thereby suggesting damaged type 2 muscle fibers (12). Smoszna et al. (13) suggested immediate hemodialysis in the course of rhabdomyolysis in cases with high CPK activity in blood (>10,000 U/L). The causes of rhabdomyolysis were as follows: ischemia of lower limbs after vascular operations (36.36%), exhausting exercise with rapid body cooling (27.27%), multiorgan failure after a traffic accident, acute myositis (9.09%), status epilepticus (9.09%), rapid clinical course of viral infection (9.09%). Knobel et al. (14) presented a case of severe, acute renal failure during severe attacks of diarrhea caused by *Vibrio cholerae,* including a marked increase in CPK levels. Maccario et al. (15) diagnosed rhabdomyolytic, anuric, acute renal failure as a complication of cardiopulmonary bypass on 2nd post-operational day, with serum myoglobin values of 16000>88 µg/L and a CPK serum value of 8790 UI/L (with Mb fraction of <8%). Veenstra et al. (16) found the incidence of acute renal failure (ARF) to be 51%, higher in patients with CPK levels of >15,000 U/L and in patients with severe rhabdomyolysis with CPK levels of >5000 U/L, present in 0.074% of all admitted patients. Ischemia was the most frequent cause, and drugs, alcohol, and/or coma were the second most common cause of severe rhabdomyolysis. Burdmann et al. (17) reported ARF frequently after bites from the Viperidae snake family. However, IV injection of rats with 0.4-mg/kg venom of *Bothrops jararaca* did not affect serum CPK levels. Ralph (18) easily set the diagnosis of myoglobinuria caused by rhabdomyolysis as well as by elevated CPK levels, resulting from muscle trauma, ischemia, metabolic causes, drug-induced injury, or intrinsic muscle disorders.

Furthermore, the following situations show the relation between CPK levels and ischemic muscles. Lintz et al. (19) did not notice any differences in ischemic pre- and post-conditioning CPK levels in skeletal muscle injury caused by IR in rats. Sobhani et al. (20) found dramatically increased CPK levels after common femoral artery IR in soleus muscle biopsies of Wistar albino rats. Lin et al. (21) biochemically investigated the IR-injured cremaster muscle by CPK isoenzyme levels in male Sprague Dawley rats. Pan et al. (22) found a significantly improvement in the serum CPK-MM isoenzyme level on the 8th day after lower limb gastrocnemius muscle IR injury in male rats. Arieli et al. (23) sampled blood for CPK serum level counts because microsurgical free-tissue transfer or replantation of amputated digits involves an obligatory ischemic period leading to regional tissue edema, rhabdomyolysis, systemic acidosis, hypercalcemia, and multiple organ dysfunction syndrome, thereby reflecting IR injury. Wang et al. (24) found serum CPK levels in the University of Wisconsin solution to be significantly lesser than those in the control after 2-h IR in rat limbs. Ozyurt et al. (25) found elevated plasma CPK activities in IR skeletal muscles. Silva et al. (26) presented significantly increased CPK levels among groups by means of infrarenal aorta IR in Wistar male rats. Avni et al. (27) evidenced an aggravation of IR injury upon reoxygenation during blood reperfusion by the significantly ($p<0.05$) higher CPK activity at 7 days after IR injury in the gastrocnemius muscles of rats. Irie et al. (28) estimated the elevated CPK levels after 24-h reperfusion in rat gastrocnemius and tibialis anterior muscles using the hind limb amputation model. Ihnken et al. (29) counted systemic a CPK level of 88,000 U/L and myoglobin level of 27,000 ng/mL after the successful surgical revascularization of ischemic limbs. Carter et al. (30) found significant increases in CPK levels, which were well correlated with cranial tibialis and quadricep muscle histopa-

thology scores after suprarenal aorta IR in mice. Hirose et al. (31) showed elevated serum CPK levels during muscle injury. Lindsay et al. (32) noticed complete CPK recycling occurrence in skeletal muscles after an acute prolonged arterial occlusion. Narita found the peak of CPK release to be clearly dissociated for 30 min upon reoxygenation after the isolation of rats hind limb following their aorta and vena cava clamp (33).

Enzymes can be compartmentalized in different cellular compartments, for example, in the cytosol, endoplasmic reticulum, and the Golgi apparatus (34). Cellular compartments in cell biology comprise all of the closed parts within the cytosol of eukaryotic cells, usually surrounded by a single or double lipid layer membrane. Release of intracellular enzymes to the extracellular space is a marker of cell damage in various diseases, e.g., liver, heart, and muscle diseases. In the normal state, the plasma membrane is impermeable to enzymes and enzyme release; therefore, it indicates a severe change of the membrane integrity. Cellular changes lead to enzyme release. These changes may be caused either by energy depletion, e.g., in ischemia or shock. Inhibition of the energy metabolism results in ATP depletion leading to Na^+, K^+ and Cl^- fluxes down their gradients across the membrane and cell swelling. Subsequently, Ca^{++} leak into the cell activates phospholipases and the formation of eicosanoids, affecting the cytoskeleton, and perhaps, activates the formation of oxidants. The result of these reactions and probably other unknown reactions as well is membrane damage. This is morphologically evident at first by the formation of blebs that appears in the reversible phase, and later on by rupture of the membrane, a sign of irreversible damage. Excessive nitric oxide produced during reperfusion reacts with superoxide to produce potent reactive species. These nitric oxide radicals and reactive oxygen species (ROS) also attack cell membrane lipids, proteins, and glycosaminoglycans, causing further damage. An antioxidant like U-74389G may interfere in irreversible phase or accelerate the formation of new cellular compartments.

Unpleasantly, examples of U-74389G will be borrowed from brain and heart tissues because renal and muscular ones lack. Horáková et al. (35) used thiobarbituric acid reactive substances (TBARS) as a marker of lipid peroxidation in induced oxidative stress and protein modification of brain homogenate. The preventive effect concerning lipid peroxidation was decreased by U-74389G (160 IC50 in µmol/L). The antioxidant non-glucocorticoid steroid U-74389G had preventive effects concerning CPK activity as a selective marker of the oxidative modification of proteins. Perna et al. (36) implicated lipid peroxidation for important tissue damages in IR syndromes. The 21-aminosteroids (lazaroids) exhibit beneficial effects in the post-traumatic lesions of the central nervous system. The typical morphological aspects of lipoperoxidative injury were shown in heterotopic hearts transplanted without treatment in rats, which were as follows: swollen mitochondria with disrupted cristae, damaged endothelial cells with the nucleolus bulging into the lumen, and a discontinued endothelial lining with diffuse edema among fibers. Lazaroid treatment for 30 min attenuated most of these damages in their hearts. Biochemically, the hearts transplanted in the presence of U-74389G treatment had significantly higher CPK levels ($p<0.01$) than the hearts transplanted without treatment. Furthermore, serum CK activity was lower in treated than in untreated recipient animals ($p<0.05$). Taken together, all these results indicate that U-74389G treatment is effective in protecting cardiac muscle from structural and functional IR injuries. These situations show the restoration effect of U-74389G on the nervous and heart tissues.

The action mechanism of U-74389G can be linked by its damaging oxygen-free radical ($O^=$) concentration decline. Olas et al. (37) inhibited ADP-induced platelet aggregation by GS–Pt complex, which as a major metabolite from the loss of both protein -SH and the thiol groups of GSH was found to induce the very active platelet LPO measured as $O^=$ generation in the cytosol of blood platelets in pigs. Fan et al. (38) protected myocardial cell membrane, reduced attacks of angina pectoris, and improved myocardial ischemia, thereby reducing plasma LPO due to the accelerated clearance of $O^=$ by the increased superoxide dismutase (SOD) and sulfhydryl group, which inhibit platelet aggregation by TXA_2/PGI_2 ratio regulation. Késmárky et al. (39) associated coronary stenosis, endothelial injury, and IR caused by balloon inflation and deflation with markedly increased plasma fibrinogen concentration and corrected blood viscosities with increased superoxide production of leukocytes that can effectively scavenge superoxide radicals with increased spontaneous platelet aggregation, elevated /wiki/Reactive_Oxygen_Species ROS in blood and OH^- levels (all $p<0.05$) during the hospital phase in patients undergoing percutaneous transluminal coronary angioplasty (PTCA). Wang et al. (40) and Chen et al. (41) claimed that a potent $O^=$ scavenger improved the histopathological outcomes attenuating the spinal cord tissue IR injury in New Zealand White rabbits. Peire et al. (42) determined that $O^=$ scavengers and thromboxane synthase inhibitors reduce thrombus growth. Taylor et al. (43) found that $O^=$ reaction products (MDA) significantly increased by 22.22%, plasma thiol reduced by 3.82%, higher levels of collagen were significantly induced by 67.44%, and spontaneous whole blood platelet aggregation increased by 24.32% (all $p<0.05$) in renal transplant patients compared with controls. Renal transplant patients are subject to oxidative cell damage and may be at increased risk of vascular thrombosis. Chiu et al. (44) showed the benefit for neurons after an effective scavenging of $O^=$ by SOD in rat brain astrocytes. Poelstra et al. (45) proposed the following sequence of events: $O^=$ produced by activated neutrophils reduce glomerular ADPase activity, leading to the facilitation of thrombus formation in an $O^=$ dependent manner in rat experimental glomerulonephritis models. Nakamura et al. (46) prevented diabetic neuropathy by decreasing $O^=$ mediation. Although Bednar et al. administered tPA, autologous clot embolization resulted in a trend toward significantly rising approximately 2.5-fold neutrophil count and their acute activation (aggregation, $O^=$;, $p<0.001$) in New Zealand white rabbit model (47). Emrecan et al. (48) improved blood flow and histologic scores according to the presence of tubular necrosis and atrophy, regenerative atypia, and hydropic degeneration by the inhibition of $O^=$ production, which decreases neutrophil activation and aggregation in rabbit renal model. Chan et al. (49) associated platelet activation and dysfunction with $O^=$ generation and matrix metalloproteinase (MMP)-9 activation. Platelet aggregation was significantly reduced with a rightward shift of concentration–response curve along with decreased plasma MMP-9 activity in newborn piglets. Praticó et al. (50) associated collagen-induced whole blood aggregation with human platelet aggregation, activation, OH^- level increase by 6.51-fold, TxB_2 formation by 22.5-fold, and protein kinase C (PKC)

translocation from the cytosol to the cell membrane, promoting atherosclerosis and coronary artery disease.

Clinically, CPK is assayed in blood tests as a marker of myocardial infarction (heart attack), rhabdomyolysis (severe muscle breakdown), muscular dystrophy, autoimmune myositides, and acute renal failure. It is evident that clinical implications of CPK can be extended to other tissues besides those of kidneys and muscles. Besides the influence of U-74389G on a single or double lipid layer membrane of cellular compartments, the main aim of this study ought to be remembered, i.e., CPK is a protein. Concentrations of Serum lipids, such as cholesterol, Low Density Lipoprotein (LDL), triglycerides, lipoprotein(a), which are among the most common risk factors for coronary artery disease development, and High Density Lipoprotein (HDL) with an opposite protective effect, nevertheless, are also more interesting as the means of drug direct antioxidant mechanism lipid peroxidation, and these could be studied exactly as CPK levels. In the present experiment, U-74389G administration significantly increased CPK levels at least by 16.37% at all short-term endpoints in the U-74389G group than in the sham-operated one ($p<0.4951$). This experimental setting failed to prove a potent short-term restoration capability of the U-74389G on CPK levels as mentioned in the last reference. This may have either statistical or physiological causes. A greater sample, a longer study period, greater dosage, or drug pretreatment may reveal significant restoration results. However, a declining effect over time is also revealed per endpoint by the present experiment: 54.32% → 35.34% → 16.37%, and 2-h reperfusion with U-74389G restores CPK levels in non-significant levels and encourages a better experimental design in the future. A prospective experimental setting should include muscle tissue assays for lipid peroxidation and pretreatment with U-74389G for more reliable and spectacular results. A potent beneficial effect on a range of diseases such as vascular thrombosis, myocardial cell membrane, attacks of angina pectoris, myocardial ischemia, atherosclerosis, coronary artery disease, PTCA, spinal cord tissue and brain injuries, diabetic neuropathy, skin flap and renal transplants, glomerulonephritis, bronchopulmonary dysplasia, inflammations even during mental stress is expected.

CONCLUSION

U-74389G administration significantly increased CPK levels for a short term; however, it was unable to restore these levels, but the restoration capacity increases with reperfusion time; 2-h reperfusion with U-74389G restores CPK levels in non-significant levels. Because a potent beneficial effect on a range of diseases is expected, further human clinical or molecular studies with the abovementioned settings are required to make this effect clearer.

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