



The trends of the antioxidant drug “U-74389G” on potassium levels during hypoxia reoxygenation injury in rats

C. TSOMPOS¹
C. PANOULIS²
K. TOUTOUZAS³
A. TRIANTAFYLLOU⁴
G. ZOGRAFOS⁵
A. PAPALOIS⁶

¹ Constantinos Tsompos: Consultant A
Department of Obstetrics & Gynecology
Mesologi County Hospital, Etoloakarnania, Greece

² Constantinos Panoulis: Assistant Professor
Department of Obstetrics & Gynecology
Aretaieion Hospital, Athens University, Attiki, Greece

³ Konstantinos Toutouzias: Assistant Professor
Department of Surgery, Ippokrateion General
Hospital, Athens University, Attiki, Greece

⁴ Aggeliki Triantafyllou: Associate Professor
Department of Biologic Chemistry, Athens University
Attiki, Greece

⁵ George Zografos: Professor, Department of Surgery
Ippokrateion General Hospital, Athens University
Attiki, Greece

⁶ Apostolos Papalois: Director, Experimental Research
Center ELPEN Pharmaceuticals, S.A. Inc., Co., Attiki
Greece

Correspondence:

Tsompos Constantinos
Department of Obstetrics & Gynecology
Mesologi County Hospital
Nafpaktou street
Mesologi 30200
Etoloakarnania
Greece
E-mail: Tsomposconstantinos@gmail.com

Key words: hypoxia, U-74389G, potassium reoxygenation

Received October 26, 2015.
Revised March 21, 2016.
Accepted March 23, 2016.

Abstract

Background: This experimental study examined the trends of the antioxidant drug “U-74389G”, on a rat model and particularly in a hypoxia – reoxygenation (HR) protocol. The trends of that molecule were studied biochemically using blood mean potassium levels.

Methods: 40 rats of mean weight 231.875 g were used in the study. Potassium (K⁺) levels were measured at 60 min of reoxygenation (groups A and C) and at 120 min of reoxygenation (groups B and D) with administration of the drug U-74389G in groups C and D.

Results: U-74389G administration non significantly decreased the K⁺ levels by 2.14%±5.06% (p= 0.6730). Reoxygenation time non-significantly increased the K⁺ levels by 8.66%±4.85% (p= 0.0934). However, U-74389G administration and reoxygenation time together non-significantly increased the K⁺ levels by 2.07%±3.03% (P= 0.4853).

Conclusions: U-74389G administration, reoxygenation time and their interaction have miscellaneous non significant short – term trends on potassium levels. Perhaps, a longer study time or a higher drug dose may reveal clearer and significant effects.

INTRODUCTION

Permanent or transient damage with serious implications on adjacent organs and systems may be due to tissue hypoxia reoxygenation (HR). The use of U-74389G in HR has been a challenge for many years. However, although the progress was significant, several practical questions have not been clarified yet. 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 HR 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 HR 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).

The aim of this experimental study was to evaluate the effect of U-74389G in a rat model of HR using mean blood potassium (K⁺) levels. The certain protocol and dose height were determined by following ex-

Table 1: The U-74389G influence (\pm SD) on the levels of some seric variables⁷ 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
WBCC8	+22.99% \pm 53.60%	0.0914	+30.12% \pm 10.87%	0.0050	+37.25% \pm 93.02%	0.0212	+23.64% \pm 6.32%	0.0003
RBCC	+1.39% \pm 0.71%	0.7161	+0.64% \pm 0.32%	0.8106	-0.10% \pm 0.05%	0.9762	+1.05% \pm 0.53%	0.4911
Hematocrit ⁹	+5.58% \pm 3%	0.0852	+4.73% \pm 2.25%	0.0435	+3.89% \pm 3.44%	0.2608	+3.16% \pm 1.33%	0.0196
Hemoglobin	+5.2% \pm 2.8%	0.0925	+3.9% \pm 2.1%	0.0604	+2.7% \pm 3.2%	0.3544	+2.5% \pm 1.3%	0.0423
MCH	+1.77% \pm 0.96%	0.0663	+2.40% \pm 0.57%	0.0001	+3.03% \pm 0.71%	0.0003	1.33% \pm 0.36%	0.0005
Platelet count ²	-17.79% \pm 9.40%	0.0647	-12.83% \pm 5.79%	0.0303	-7.88% \pm 7.83%	0.2939	-6.12% \pm 3.58%	0.0857
Platelet-crit	+3.80% \pm 9.87%	0.6373	+9.23% \pm 6.29%	0.1064	+14.66% \pm 9.03%	0.0833	+6.72% \pm 3.73%	0.0712
PDW	+1.1% \pm 0.88%	0.2368	+1.79% \pm 0.76%	0.0314	+2.49% \pm 1.33%	0.0807	+0.96% \pm 0.46%	0.0396
Glucose	-6.41% \pm 3.50%	0.0663	-8.57% \pm 2.06%	0.0001	-10.74% \pm 2.52%	0.0003	-4.76% \pm 1.28%	0.0005
Total protein	-5.48% \pm 2.99%	0.0663	-7.34% \pm 1.76%	0.0000	-9.20% \pm 2.16%	0.0000	-4.08% \pm 1.10%	0.0000
ALP	+22.66% \pm 12.37%	0.0663	+31.91% \pm 7.69%	0.0001	+41.16% \pm 9.65%	0.0003	+17.75% \pm 4.79%	0.0005
ACP	-112.54% \pm 20.95%	0.0006	-128.45% \pm 14.84%	0.0000	-144.36% \pm 21.62%	0.0000	-74.45% \pm 9.63%	0.0000
CPK	+54.32% \pm 13.75%	0.0012	+35.34% \pm 17.20%	0.0260	+16.37% \pm 30.24%	0.4951	+18.52% \pm 9.44%	0.0770
Sodium	+1.22% \pm 0.66%	0.0707	+0.17% \pm 0.61%	0.7714	-0.87% \pm 1.03%	0.3995	-0.32% \pm 0.36%	0.3693
Chloride	-0.58% \pm 0.77%	0.4533	-0.97% \pm 0.53%	0.0879	-1.36% \pm 0.76%	0.1113	-0.75% \pm 0.38%	0.0159
Calcium	0% \pm 1.75%	1	-0.14% \pm 1.10%	0.8782	-0.28% \pm 1.54%	0.8492	+0.14% \pm 0.64%	0.8245
Phosphorus	-2.23% \pm 5.51%	0.7966	-1.61% \pm 3.32%	0.5789	-1% \pm 4.48%	0.8129	-1.09% \pm 2%	0.5771
Magnesium	+1.33% \pm 3.59%	0.7033	-0.28% \pm 2.75%	0.9171	-1.90% \pm 5.28%	0.7161	+0.36% \pm 4.58%	0.8228
Mean	-1.31% \pm 31.71%	0.2897	-2.22% \pm 34.31%	0.2415	-3.11% \pm 37.99%	0.3030	-0.85% \pm 20.13%	0.1909

periments with favorable outcomes. Actually, Flessas I et al found (3) the U-74389G protective in many emergency clinical situations of intestinal IR. Bimpis A et al limited brain damage itself after (4) U-74389G administration. Tsaroucha AK et al (5) attenuated liver damage after U-74389G administration. Andreadou I et al protected the small intestine (6) after U-74389G administration. Along, ovarian TNF α and malondialdehyde levels were evaluated at the same endpoints. The levels of TNF α were stayed rather undisturbed; whereas the malondialdehyde levels were kept significantly increased. Nevertheless, the recommendation was that these 2 body mass related biomarkers not to be considered because they were not representative of all tissues under the ischemia level. Contrary, potassium is a systemic variable concerning all these tissues and not only the ovaries. The consequence of the applied treatment was the investigation of the short-term trends of U-74389G on general metabolism and certainly whether it is anabolic or catabolic. Table 1 depicts that after the evaluation of 18 seric variables, the short-term trend of the drug is rather catabolic; without the potassium trend included. This table was built-up by the successive stepwise addition of published studies (7–9). Although the addition of every variable was incidental, it seems that the profil of the table and generally of the drug is improbable to change, as many other variables even if be added.

MATERIALS AND METHODS

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 used, were a grant of Experimental Research Centre of ELPEN Pharmaceuticals Co. Inc. S.A. at Pikermi, Attiki. Accepted standards of humane animal care were adopted for Albino female Wistar rats. 7 days pre-experimental normal housing included *ad libitum* diet in laboratory. Post-experimental awakening and preservation of animals was not permitted even if euthanasia was required. Rats were randomly delivered to four experimental groups by 10 animals in each one, using following protocols of HR: Hypoxia for 45 min followed by reoxygenation for 60 min (group A). Hypoxia for 45 min followed by reoxygenation for 120 min (group B). Hypoxia for 45 min followed by immediate U-74389G intravenous (IV) administration and reoxygenation for 60 min (group C). Hypoxia for 45 min followed by immediate U-74389G IV administration and reoxygenation for 120 min (group D). The molecule U-74389G dosage was 10 mg/Kg body weight of animals.

Prenarcosis preceded of continuous intra-experimental general anesthesia, oxygen supply, electrocardiogram and

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

Groups	Variable	Mean	Std. Dev
A	Weight	243 g	45.77724 g
	Potassium	6.85 mmol/l	0.8449194 mmol/l
B	Weight	262 g	31.10913 g
	Potassium	6.82 mmol/l	0.9507891 mmol/l
C	Weight	212.5 g	17.83411 g
	Potassium	6.19 mmol/l	0.3784471 mmol/l
D	Weight	210 g	18.10463 g
	Potassium	7.23 mmol/l	1.040353 mmol/l

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

DG	Variable	Difference	p-value
A-B	Weight	-19 g	0.3555
	Potassium	0.03 mmol/l	0.9483
A-C	Weight	30.5 g	0.0674
	Potassium	0.66 mmol/l	0.0789
A-D	Weight	33 g	0.0574
	Potassium	-0.38 mmol/l	0.3987
B-C	Weight	49.5 g	0.0062
	Potassium	0.63 mmol/l	0.0361
B-D	Weight	52 g	0.0009
	Potassium	-0.41 mmol/l	0.3905
C-D	Weight	2.5 g	0.7043
	Potassium	-1.04 mmol/l	0.0164

acidometry. Hypoxia was caused by laparotomic clamping inferior aorta over renal arteries with forceps for 45 min. Reoxygenation was induced by removing the clamp and reestablishment the inferior aorta patency. After exclusion of blood flow, the protocol of HR was applied, as described above for each experimental group. U-74389G was administered at the time of reoxygenation through inferior vena cava catheter. The K⁺ levels were determined at 60th min of reoxygenation (for A and C groups) and at 120th min of reoxygenation (for B and D groups). Forty 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 K levels. This assumption was also investigated.

Control groups

20 control rats (mean mass 252.5 g [SD: 39.31988 g]) experienced hypoxia for 45 min followed by reoxygenation.

Group A

Reoxygenation lasted for 60 min (n=10 controls rats) mean mass 243 g [SD: 45.77724 g], mean K⁺ levels 6.85 mmol/l [SD: 0.8449194 mmol/l] (Table 2).

Group B

Reoxygenation lasted for 120 min (n=10 controls rats) mean mass 262 g [SD: 31.10913 g], mean K⁺ levels 6.82 mmol/l [SD: 0.9507891 mmol/l] (Table 2).

Lazaroid (L) group

20 L rats (mean mass 211.25 g [SD: 17.53755 g]) experienced hypoxia for 45 min followed by reoxygenation in the beginning of which 10 mg U-74389G /kg body weight were IV administered.

Group C

Reoxygenation lasted for 60 min (n=10 L rats) mean mass 212.5 g [SD: 17.83411 g], mean K⁺ levels 6.19 mmol/l [SD: 0.3784471 mmol/l] (Table 2).

Group D

Reoxygenation lasted for 120 min (n=10 L rats) mean mass 210 g [SD: 18.10463 g], mean K⁺ levels 7.23 mmol/l [SD: 1.040353 mmol/l] (Table 2).

Statistical analysis

Every weight and K⁺ level group was compared with each other by statistical standard *t*-tests (Table 3). Any significant difference among K⁺ levels, was investigated whether owed in probable significant weight correlations.

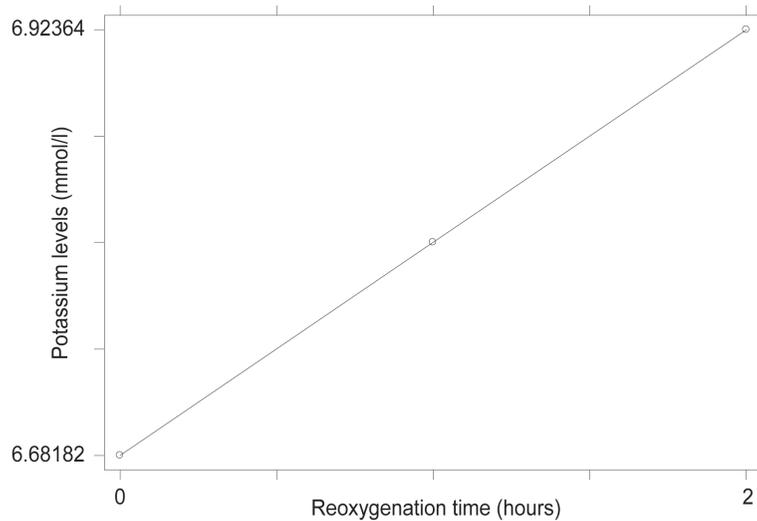


Figure 1: The increasing trend of U-74389G on potassium levels regarding reoxygenation time (p -value=0.4853).

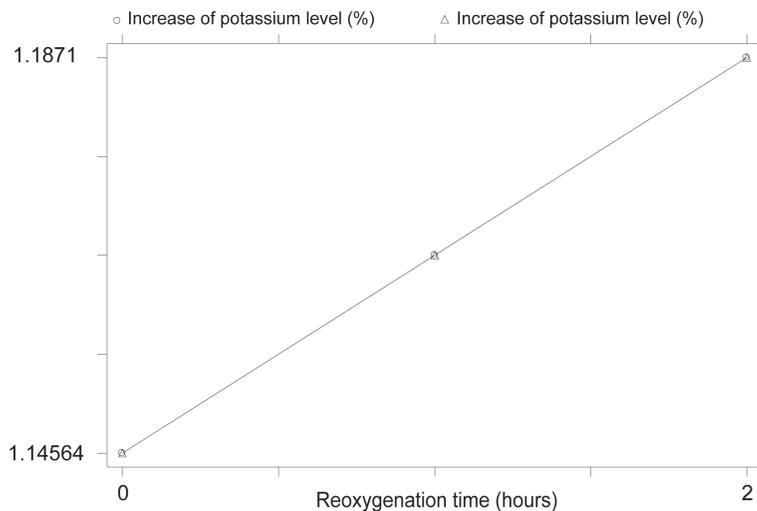


Figure 2: The (%) increasing trend of U-74389G on potassium levels regarding reoxygenation time (p -value=0.4853).

The generalized linear models (glm) with dependant variable the K^+ levels were applied. The 3 independent variables were the U-74389G or no drug, the reoxygenation time and both variables in combination. Inserting the rats' weight also as an independent variable at glm analysis, a non significant relation resulted in with K^+ levels ($p=0.4029$), so as to further investigation was not needed.

RESULTS

The application of glm analysis resulted in: U-74389G administration non significantly decreased the K^+ levels by 0.125 mmol/l [-0.7032406 mmol/l – 0.4532407 mmol/l] ($P=0.6641$). This finding was in accordance with the results of standard t-test ($p=0.6820$). Reoxygenation

time non-significantly increased the K^+ levels by 0.505 mmol/l [-0.0504671 mmol/l – 1.060467 mmol/l] ($P=0.0735$), also in accordance with standard t-test ($p=0.1134$). However, U-74389G administration and reoxygenation time together non-significantly increased the K^+ levels by 0.1209091 mmol/l [-0.2263983 mmol/l – 0.4682165 mmol/l] ($P=0.4853$). Reviewing the above and table 3, the figures 1 and 2 sum up concerning the increasing trend of U-74389G in connection with reoxygenation time.

DISCUSSION

Bibliography lacks references concerning whether hypoxia can influence the potassium levels. Potassium levels influence (10, 11) multiple physiological processes, includ-

ing resting cellular-membrane potential and the propagation of action potentials in neuronal, muscular, and cardiac tissue (12). Also K influences the hormone secretion and action, the vascular tone, controls the systemic blood pressure and the gastrointestinal motility. It joins in acid–base homeostasis, in glucose and insulin metabolism, in mineralocorticoid action, in renal concentrating ability and in fluid and electrolyte balance. Isolated potassium administration is impossible. The reason is that K⁺ has a single electron in its outer electron shell, which readily gives it up to create an atom with a positive charge – a cation and oxidizes rapidly in air. After oxidation, it reacts vigorously with water combining with anions to form salts. Potassium occurs only in ionic salts usually associated with another drug or a factor. This last chemical conjugate probably influences the potassium occurrence. So, the administration of potassium is by means of a salt. Chiu PY *et al* associated (13) the inhibition of the mitochondrial permeability transition through the opening of mitochondrial K⁺(ATP) channels, affording protection against myocardial ischemia reperfusion (IR) injury in rat myocardium. Kuhrt H *et al* found (14) that the expression of inwardly rectifying K⁺ (Kir) currents and transient A-type K⁺ currents alterations are characteristic features of retinal glial (Müller) cells after transient retinal IR. Nosaman BD *et al* associated (15) the vasodilator free radical peroxynitrite (ONOO⁻) with a cGMP⁻dependent mechanism in the hindlimb vascular bed of the cat. Pollesello P *et al* have shown (16) that the protective vasodilatory and antiischemic effects are mediated via the opening of ATP-sensitive K⁺ channels in vascular smooth-muscle cells and also in mitochondrial ATP-sensitive K⁺ (mito-KATP) channels in heart. Chicco AJ *et al* (17) demonstrated that resistance to myocardial IR injury is dependent on sarcolemmal K⁺(ATP) activity during IR in rats. García González MJ *et al* associated (18) the vasodilatory effect with ATP-dependent K⁺ channel K⁺(ATP) opening properties in cardiogenic shock. Bittner HB *et al* combined (19) the post-transplant increased mortality rate by 40% with the low-K⁺ dextran flush solution for the procurement of donor lungs. Reinés A *et al* found (20) that endogenous inhibitor endobain E, was able to inhibit both enzyme activity and ligand binding on synaptosomal membrane Na⁺K⁺-ATPase activity, binding to cerebral IR cortex membranes in rats. Müllenheim J *et al* found that ischemic late preconditioning (ILPC) blocks (21) K⁺(ATP) channels and cardioprotection against prolonged ischemia in isolated cells of rabbits coronary IR. Reshef A *et al* (22) conferred protection against ATP-depleting crisis, opening the ATP-sensitive K⁺(ATP) channels in a model of primary rat IR neuronal cultures. Schmidt TA *et al* related the high concentration of Na⁺K⁺ATPase with large pressure work (23) in animal IR myocardium.

Also, K⁺ levels are perhaps influenced by U-74389G administration. Stanimirovic DB *et al* abolished (24) the

hydrogen peroxide-evoked decrease in Na,K-ATPase activity in presence of the steroid antioxidants U-74389G (5–20 μM) in rats cerebromicrovascular endothelial cells (RCEC). The oxidant-induced inhibition of Na,K-ATPase activity implicate the mechanism responsible for the delayed free radical-induced increase in RCEC membrane ‘permeability’.

CONCLUSION

U-74389G administration, reoxygenation time and their interaction have miscellaneous non significant short – term trends on K⁺ levels. Perhaps, a longer study time or a higher U-74389G dosage may reveal clearer and significant effects; including also an equal number of male rats for probable gender bias exclusion.

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

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