

The Effect of the Antioxidant Drug U-74389G on Magnesium Levels During Hypoxia–Reoxygenation Injury in Rats

Objective: The aim of this experimental study was to examine the effect of the antioxidant drug U-74389G in a rat model of hypoxia–reoxygenation using the quoted established protocol. Effects of treatments were evaluated via magnesium (Mg^{2+}) levels in blood. **Methods:** A controlled study was performed. Mg^{2+} levels were determined in 60 min (groups A and C) and 120 min (groups B and D) after starting the reoxygenation. Groups A and B received no drugs, whereas rats from groups C and D were administered with U-74389G. **Results:** 40 rats 16–18 weeks old of a mean weight of 231.9 g were employed in the study. It is demonstrated that U-74389G administration did not alter the Mg^{2+} levels (decrease in Mg^{2+} concentration was $0.28 \pm 2.75\%$; $p = 0.917$). Reoxygenation non-significantly increased the Mg^{2+} levels by $4.27 \pm 2.66\%$ ($p = 0.107$). Together, the U-74389G administration and reoxygenation non-significantly increased the Mg^{2+} levels by $0.36 \pm 1.64\%$ ($p = 0.823$). **Conclusion:** U-74389G administration, alone or in concert with reoxygenation did not significantly affect Mg^{2+} level in blood after experimental hypoxia in rats.

Key words: hypoxia, reoxygenation, U-74389G, magnesium.

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INTRODUCTION

Hypoxia–reoxygenation (HR) is recognized for specific events that occur following an injury and common signs and symptoms associated with tissue damage and repair [1]. A critical step in this context is the deepening of the understanding of how the body repairs damaged tissues using antioxidant substances. However, although the progress was significant, several practical questions have not been clarified. They include a) how potent the antioxidant should be, b) when should it be administered, and c) at which optimal

dose the drug should be administered. The promising effect of the antioxidant, the aminosteroid U-74389G in tissue protection has been noted in several studies. International bibliography demonstrated that U-74389G which belongs to the family of aminosteroids (lazaroids), is very popular in HR experiments (Table 1). U-74389G (or 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 factor [2]. Its main capacity is both arachidonic acid-induced and iron-dependent lipid peroxidation prevention. It has been proved beneficial in some animal tissues as heart, liver and kidney

Table 1. Influence of U-74389G ($M \pm SD$) on the levels of seric variables [4] depending on the reoxygenation (reo.) time

Variable	1 hour reo.	p-Value	1.5 h reo.	p-Value	2 h reo.	p-Value	Interaction of U-74389G and reo.	p-Value
RBC, $10^6/mm^3$	$+1.39\% \pm 0.71\%$	0.716	$+0.64\% \pm 0.32\%$	0.811	$-0.10\% \pm 0.05\%$	0.976	$+1.05\% \pm 0.53\%$	0.491
Hemoglobin, g/dl	$+5.2\% \pm 2.8\%$	0.093	$+3.9\% \pm 2.1\%$	0.060	$+2.7\% \pm 3.2\%$	0.354	$+2.5\% \pm 1.3\%$	0.042
Mean corpuscular hemoglobin, pg	$+1.77\% \pm 0.96\%$	0.066	$+2.40\% \pm 0.57\%$	<0.001	$+3.03\% \pm 0.71\%$	<0.001	$1.33\% \pm 0.36\%$	<0.001
Glucose, mmol/l	$-6.41\% \pm 3.50\%$	0.066	$-8.57\% \pm 2.06\%$	<0.001	$-10.74\% \pm 2.52\%$	<0.001	$-4.76\% \pm 1.28\%$	<0.001
Total protein, g/dl	$-5.48\% \pm 2.99\%$	0.066	$-7.34\% \pm 1.76\%$	<0.001	$-9.20\% \pm 2.16\%$	<0.001	$-4.08\% \pm 1.10\%$	<0.001
Alkaline phosphatase, IU/l	$+22.66\% \pm 12.37\%$	0.066	$+31.91\% \pm 7.69\%$	<0.001	$+41.16\% \pm 9.65\%$	<0.001	$+17.75\% \pm 4.79\%$	0.001
Sodium, mmol/l	$+1.22\% \pm 0.66\%$	0.071	$+0.17\% \pm 0.61\%$	0.771	$-0.87\% \pm 1.03\%$	0.400	$-0.32\% \pm 0.36\%$	0.369
Chloride, mmol/l	$-0.58\% \pm 0.77\%$	0.453	$-0.97\% \pm 0.53\%$	0.088	$-1.36\% \pm 0.76\%$	0.111	$-0.75\% \pm 0.38\%$	0.016
Calcium, g/dl	$0\% \pm 1.75\%$	1	$-0.14\% \pm 1.10\%$	0.878	$-0.28\% \pm 1.54\%$	0.849	$+0.14\% \pm 0.64\%$	0.825
Phosphorus, g/dl	$-2.23\% \pm 5.51\%$	0.797	$-1.61\% \pm 3.32\%$	0.579	$-1\% \pm 4.48\%$	0.813	$-1.09\% \pm 2\%$	0.577
Mean	$+2.42\% \pm 9.22\%$	0.453	$+3.23\% \pm 12.92\%$	0.447	$+4.05\% \pm 16.67\%$	0.450	$+1.81\% \pm 7.20\%$	0.325

ischemia–reperfusion (IR) models, as well in protecting brain microvascular endothelial cells monolayers against permeability changes [3].

The aim of this experimental study was to examine the effect of the antioxidant drug U-74389G in a rat model of HR using blood magnesium (Mg^{2+}) levels as possible biomarker of effect of the drug.

METHODS

Study design

A controlled study was performed.

Eligibility criteria

Forty female Wistar albino rats (mean weight 231.9 ± 36.9 g) were used.

Testing environment

This experimental study was performed at the Experimental Research Center of ELPEN Pharmaceuticals Co. Inc. S.A. at Pikermi, Attiki (Greece). All settings needed for the study including consumables, equipment and substances used, were a courtesy of that S.A.

Duration of study

The experiment lasted 15 days.

Description of medical intervention

The experiment started with preanesthesia followed by general anesthesia of each animal. The detailed anesthesiologic technique was described in related references [4, 5]. Continuous oxygen supply was administered during whole experiment performance. The electrocardiogram and acidometry were continuously monitored. Hypoxia was caused by clamping inferior aorta over renal arteries with forceps for 45 min after laparotomic access was achieved. Reoxygenation was induced by removing the clamp and reestablishment of inferior aorta patency. After exclusion of a blood flow, the protocol of HR was applied as described above for each of experimental group. U-74389G was administered at the time of reoxygenation through inferior vena cava after the catheterization had been achieved.

Study outcomes

Primary outcome of the study

Control groups included 20 control rats (252.5 ± 39.3 g) submitted to hypoxia for 45 min followed by reoxygenation.

- Group A: reoxygenation lasted for 60 min ($n = 10$, 243 ± 45.77 g, Mg^{2+} levels 2.98 ± 0.19 mg/dl; Table 2).

- Group B: reoxygenation lasted for 120 min ($n = 10$, 262 ± 31.1 g, Mg^{2+} levels 3.18 ± 0.26 mg/dl; see Table 2).
Secondary outcomes of the study
- Lazaroid (L) group included 20 experimental rats (211.25 ± 17.5 g) submitted to hypoxia for 45 min followed by reoxygenation at the beginning of which 10 mg U-74389G /kg body weight were intravenously administered.
- Group C: reoxygenation lasted for 60 min ($n = 10$, 212.5 ± 17.83 g, Mg^{2+} levels 3.02 ± 0.24 mg/dl; see Table 2).
- Group D: reoxygenation lasted for 120 min ($n = 10$, 210 ± 18.1 g, Mg^{2+} levels 3.12 ± 0.41 mg/dl; see Table 2).

Subgroup analysis

Rats were randomly assigned to four experimental groups (10 animals in each group) 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 intravenous administration of U-74389G and reoxygenation for 60 min (group C); hypoxia for 45 min followed by immediate intravenous administration of U-74389G and reoxygenation for 120 min (group D). The dose of U-74389G was 10 mg/Kg body mass of animals.

Outcome recording techniques

The Mg^{2+} levels were determined at 60th min of reoxygenation (groups A and C) and at 120th min of reoxygenation (groups B and D).

Ethics review

The study was licensed by Veterinary Address of East Attiki Prefecture under 3693/12-11-2010 & 14/10-1-2012 decisions. Animals were used and maintained in accordance with accepted standards of humane animal care.

Statistical processing of data

Rats of each group were compared by weight and level of Mg^{2+} in blood with each one by statistical paired t-test (Table 3). The application of generalized linear models (glm) with dependant variables (Mg^{2+} levels) and independent variables U-74389G or no drug, the reoxygenation time and both variables in combination was performed. The data for the dependant variables (Mg^{2+} levels) are depicted at Table 2. The dummy variables for U-74389G or no drug were stand for 1 or 0 respectively. The dummy variables for reoxygenation time were stand as 1 and 2 for 60 min and 120 min respectively. The dummy variables for their combination were found by their numerical

Table 2. Weight and magnesium (Mg^{2+}) mean levels and Standard Deviation (SD) of groups

Groups	Variable	Mean	SD
A	Weight	243	45.78
A	Mg^{2+}	2.9	0.20
B	Weight	262	31.11
B	Mg^{2+}	3.18	0.27
C	Weight	212.5	17.83
C	Mg^{2+}	3.02	0.25
D	Weight	210	18.10
D	Mg^{2+}	3.12	0.41

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	0.242
A-B	Mg ²⁺ , mg/dl	-0.2	0.101
A-C	Weight	30.5	0.067
A-C	Mg ²⁺ , mg/dl	-0.04	0.711
A-D	Weight	33	0.057
A-D	Mg ²⁺ , mg/dl	-0.14	0.427
B-C	Weight	49.5	0.002
B-C	Mg ²⁺ , mg/dl	0.16	0.175
B-D	Weight	52	<0.001
B-D	Mg ²⁺ , mg/dl	0.06	0.728
C-D	Weight	2,5	0.704
C-D	Mg ²⁺ , mg/dl	-0.10	0.509

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products. The statistical software of STATA 6.0 (Stata Corp., USA) was used.

RESULTS

Study object

The study was carried out on forty (40) 16–18 week-old female albino rats of Wistar strain having minimum weight 165 g and maximum weight 320 g (mean weight 231.9±36.9 g). The animals were divided into 4 groups.

Key findings

U-74389G administration possessed a trend to decrease the Mg²⁺ levels by 0.01 mg/dl (-0.20–0.18 mg/dl; *p* =0.916). This finding was in accordance with the results of a paired t-test (*p* =0.919). Reoxygenation time non-significantly increased the Mg²⁺ levels by 0.15 mg/dl (-0.03–0.33 mg/dl; *p* =0.106), also in accordance with the paired t-test results (*p* =0.109). However, U-74389G administration and reoxygenation time in combination non-significantly increased Mg²⁺ levels by 0.01 mg/dl (-0.10–0.13 mg/dl; *p* =0.823). Reviewing the above and Table 3, the Table 4 sums up the effect of U-74389G regarding reoxygenation time. Considering the weight of animals as an independent variable in generalized linear models analysis, a non-significant association with Mg²⁺ levels was revealed (*p* =0.495), suggesting that further investigations were not needed.

DISCUSSION

Unpleasantly, studies describing whether hypoxia may affect the Mg²⁺ levels have not been found in published literature. However, there were a lot of studies reporting how the fluctuations of Mg²⁺ levels affect functions of various organs. Nevertheless, isolated magnesium administration is impossible. However, it is meant that another drug or ion administered with Mg²⁺ might affect the Mg²⁺ levels. Siegler J.E. et al. found [6] non-significant difference in worse outcome (neurologic deterioration, ND), death, discharge disposition or poor short-term functional measures) in ischemic stroke patients, whose serum Mg²⁺ levels were decreased from a baseline during the first 24 hours of admission compared to patients with unchanged or increasing Mg²⁺ levels. Lee K.C. et al. considered [7] as safe the combination of St. Thomas cardioplegic arrest with histidine-tryptophan-ketoglutarate solution with an average short-term ischemia survival time ~225 minutes, nearly similar to other approaches, during low-pressure perfusion donor heart preservation. van den Bergh W.M. et al. tried [8] to prevent or reverse delayed cerebral ischemia (DCI) after aneurysmal subarachnoid haemorrhage (SAH) by administering magnesium as a neuroprotective agent at a continuous intravenous dosage of 64 mmol/L per day, which maintained serum Mg²⁺ levels within the range of 1.0–2.0 mmol/L for 14 days. Whitelaw A. et al. treated [9] hypoxic-ischemic labor-delivery-encephalopathy infants with magnesium sulphate. Ichiba H. et al. found [10] significantly

Table 4. The absolute and (%) alteration influence of U-74389G in connection with reoxygenation time. *p*-Values

Alteration, mg/dl	95% CI	Reoxygenation time, hours	t-Test	glm
+0.04 (+1.33%)	-0.17 – -0.25 (±3.59%)	1	0.711	0.696
-0.01 (-0.28%)	-0.20 – -0.18 (±2.75%)	1.5	0.919	0.916
-0.06 (-1.90%)	-0.39 – -0.27 (±5.28%)	2	0.728	0.704
+0.15 (+4.27%)	-0.03 – -0.33 (±2.66%)	Reoxygenation time alone (1.5 p)	0.109	0.106
+0.01 (+0.36%)	-0.10 – -0.13 (±4.58%)	Interaction of drug and reoxygenation time	–	0.823

more frequent the survival of infants 14 days old with severe birth asphyxia (5-min Apgar score ≤ 7) in treated group with postnatal MgSO_4 infusion than in control group. Khan I.A. et al treated [11] the acquired form of long $Q-T$ syndrome due to causes as stroke, myocardial ischemia and organophosphorus compounds by intravenous Mg^{2+} administration. Hoenicke E.M. et al found [12] that Krebs–Henseleit solution was equivalent to St. Thomas' solution but inferior to University of Wisconsin solution in rabbit hearts protection trials. Yano Y. et al. blocked [13] calcium damage by terminal Mg^{2+} cardioplegia recovering 79% of control aortic flow in rat IR hearts. Krause S. et al. significantly depressed [14] the canine Mg^{2+} -dependent ATPase activity by both global ischemia and pH 7.1 acidosis.

Heim C. et al. impaired [15] learning ability, injecting either 0.3 μg or 0.06 μg intraatrially ferric chloride (FeCl_3), one week after a 60-minute oligemic IR median brain episode in adult rats. Lazaroid U-74389G, a potent inhibitor of iron-induced lipid peroxidation, totally prevents the learning impairments in both median adult and aged animals, suggesting that iron-induced lipid peroxidation may be responsible for the late learning deficiencies. However, when U-74389G was administered alone one week after the oligemic episode, it also impaired the animals' learning ability. Moore R.M. et al. subjected [16] horses into IR condition with a reduced to 20% of baseline (BL) colonic arterial blood flow for 3 hours. Then the colon was reperfused during another 3 hours. 21-Aminosteroid U-74389G administered as 10 mg/kg intravenously 30 minutes prior to colonic reperfusion significantly increased mean pulmonary artery and mean right atrial pressure and decreased colonic arterial resistance at least for 3 hours during IR. These data demonstrate the potential of the 21-aminosteroid U-74389G to affect reperfusion, however, the concentration of Mg^{2+} in blood serum seem to be a pure biomarker of the effect of the drug in HR.

It is repeated that it is unknown how hypoxia affects the serum Mg^{2+} levels. Nevertheless, Mg^{2+} intravenous infusion has beneficial protective effects for nervous and cardiac muscle tissues and for respiratory acidosis [9, 10]. U-74389G although reinforces reperfusion does not protects nervous tissue if the criterion is the leaning ability and of course has unknown effects on serum Mg^{2+} levels. However this study although tried to correlate directly the effect of U-74389G on the serum Mg^{2+} levels, the outcome was non significant and confusing.

CONCLUSION

Administration of U-74389G, reoxygenation time and their interaction provide only a statistically non-significant trend for a short-term effects in Mg^{2+} levels. Levels of Mg^{2+} cannot be considered as biomarkers of the effect of U-74389G at least at studied dose. Study suggests that the longer study time or increased U-74389G dose may reveal any significant effects post-HR.

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CONFLICT OF INTEREST

The authors have indicated they have no any conflict of interest relevant to this article to disclose.

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CONTACT INFORMATION

Tsompos Constantinos, consultant A of the Department of Obstetrics & Gynecology, Mesologi County Hospital.
Address: Nafpaktou Str., Mesologi 30200, Etoloakarnania Greece, **Tel.:** 00302631360237,
E-mail: Constantinosompos@yahoo.com

Panoulis Constantinos, Assistant Professor of the Department of Obstetrics & Gynecology, Aretaieion Hospital.
Address: 76 Vas. Sofias Ave, Athens 11528, Attiki Greece, **Tel.:** 00302107286283, **E-mail:** Kwn.panoulis@gmail.com

Toutouzas Konstantinos, Assistant Professor of the Department of Surgery, Ippokrateion General Hospital.
Address: 114 Vas. Sofias Ave, Athens 11527, Attiki Greece, **Tel.:** 00302132088000, **E-mail:** Tousur@hotmail.com

Zografos George, Professor of the Department of Surgery, Ippokrateion General Hospital.
Address: 114 Vas. Sofias Ave, Athens 11527, Attiki Greece, **Tel.:** 00302132088000, **E-mail:** Gzografo@med.uoa.gr

Papalois Apostolos, Director of the Experimental Research Center ELPEN Pharmaceuticals, S.A. Inc., Co.
Address: 95 Marathonos Ave, Pikermi 19009, Attiki Greece, **Tel.:** 00302106039326-9, **E-mail:** Apapalois@elpen.gr