# THE EFFECT OF THE ANTIOXIDANT DRUG U-74389G ON URIC ACID LEVELS DURING ISCHEMIA REPERFUSION INJURY IN RATS <br> Constantinos Tsompos ${ }^{1}$, Constantinos Panoulis ${ }^{2}$, Konstantinos Toutouzas ${ }^{3}$, Aggeliki Triantafyllou ${ }^{4}$, George Zografos ${ }^{5}$, Apostolos Papalois ${ }^{6}$ <br> ${ }^{1}$ Department of Obstetrics \& Gynecology, Mesologi County Hospital, Etoloakarnania, Greece <br> ${ }^{2}$ Department of Obstetrics \& Gynecology, Aretaieion Hospital, Athens University, Attiki, Greece <br> ${ }^{3}$ Department of Surgery, Ippokrateion General Hospital, Athens University, Attiki, Greece <br> ${ }^{4}$ Department of Biologic Chemistry, Athens University, Attiki, Greece <br> ${ }^{5}$ Department of Surgery, Ippokrateion General Hospital, Athens University, Attiki, Greece <br> ${ }^{6}$ Experimental Research Centre ELPEN Pharmaceuticals, S.A. Inc., Co., Attiki, Greece 

EFEKTI ANTIOKSIDACIONOG LEKA U-74389G
NA VREDNOSTI MOKRACNE KISELINE TOKOM
ISHEMIJSKO REPERFUZIONE POVREDE KOD PACOVA
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#### Abstract

This experimental study examined the effect of the antioxidant drug U-74389G in a rat model using a renal isch-aemia-reperfusion (IR) protocol. The effects of the molecule were studied biochemically by assessing mean serum uric acid levels (SUA). In total, 40 rats (mean weight $=231.875$ g) were used in the study. SUA levels were measured at 60 min of reperfusion for groups $A$ and $C$ and at 120 min of reperfusion for groups B and D. The drug U-74389G was administered only in groups C and D. U-74389G administration non-significantly increased the SUA levels by $15.43 \% \pm 9.10 \%(p=0.096)$ at the representative endpoint of 1.5 h . The reperfusion time non-significantly decreased the SUA levels by $13.61 \% \pm 9.18 \%(p=0.126)$. However, the interaction of U-74389G administration and reperfusion time non-significantly increased the SUA levels by $4.78 \% \pm 5.64 \%$ ( $p=0.387$ ). Whether it interacted with the reperfusion time, U-74389G administration non-significantly increased SUA levels. It seems that U-74389G cannot reverse injury to IR tubular epithelial cells within 2 hours.


Keywords: ischaemia, U-74389G, uric acid, reperfusion

## SAŽETAK

U ovoj eksperimentalnoj studiji ispitivani su efekti antioksidacionog leka LU-74389G tokom ishemije i reperfuzije bubrega na modelu pacova. Efekti ispitivanog molekula su izučavani biohemijski, merenjem srednje vrednosti nivoa mokraćne kiseline u serumu. U studiji je korišćeno 40 pacova (prosečna telesna masa= $231,875 \mathrm{~g}$ ). Nivoi mokraćne kiseline u serumu su mereni za grupe $A$ i $C$ u šezdesetom minutu, a za grupe B i D u stodvadesetom minutu reperfuzije. U-74389G je primenjivan samo u grupi C i D. Administracija U-74389G nije dovela do statistički značajnog povećanja niova mokraćne kiseline u krajnjoj tački u devedesetom minutu $15,43 \% \pm 9,10 \%$ ( $p=0,096$ ). Reperfuzija nije dovela do statistički značajnog smanjenja nivoa mokraćne kiseline u serumи $13,61 \% \pm 9,18 \%$ ( $p=0,126$ ). Bez obzira na reperfuziono vreme administracija U-74389G nije statistički značajno povisila nivo mokraćne kiseline u serumu. Izgleda da u toku dva sata U-74389G ne može popraviti povredu tubularnih epitelijalnih ćelija nastalu ishemijom i reperfuzijom.

Ključne reči: ishemija, U-74389G, mokraćna kiselina, reperfuzija


## INTRODUCTION

Tissue ischaemia－reperfusion（IR）injury can induce permanent or transient damage with serious implications to adjacent organs and systems．The use of U－74389G in IR has been a challenge for many years．However，although progress has been significant，several practical questions have not been clarified．They include：
a）how potent U－74389G should be；
b）when it should be administered；and
c）the optimal dose at which U－74389G should be ad－ ministered．

The promising effect of U－74389G in tissue protec－ tion has been noted in several IR studies．U－74389G，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 that prevents both arachidonic acid－ induced and iron－dependent lipid peroxidation（1）．Laza－ roids，a novel series of glucocorticoid compounds such as 21－aminosteroids，promote free radical scavenging．U－ 74389 G is one of the 132 similar lazaroid compounds．It has a molecular weight of $726.90406 \mathrm{~g} / \mathrm{mol}$ and a selective action on vascular endothelium with vitamin E－like prop－ erties．It also exhibits neuroprotection and membrane－sta－ bilizing properties．It protected against IR injury in heart， liver and kidney models．These membrane－associated an－ tioxidants are particularly effective in preventing perme－ ability changes in monolayers of brain microvascular en－ dothelial cells（2）．A meta－analysis of 15 published studies， including red blood cell counts，haemoglobin and mean corpuscular haemoglobin levels，platelet count，platelet－ crit，platelet distribution width，glucose，total protein， alkaline phosphatase，creatine phosphokinase，sodium， chloride，calcium，phosphorus and magnesium levels，ex－ amined in the same experimental setting，provided a total numeric evaluation of the U－74389G anabolic efficacy of approximately $+2.14 \% \pm 7.18 \%(p$－value $=0.227)$ at the same endpoints（3，4）．Several publications addressed trials of other similar antioxidant molecules to which the studied molecule U－74389G belongs．

The aim of this experimental study was to evaluate the effect of $U-74389 \mathrm{G}$ in a rat model of renal IR using mean serum uric acid levels（SUA）．

## MATERIALS AND METHODS

## Animal preparation

This basic experimental study was licensed by Veterinary Address of East Attiki Prefecture under 3693／12－11－2010 \＆ 14／10－1－2012．All consumables，equipment and substances used were grants of the Experimental Research Centre of ELPEN Pharmaceuticals Co．Inc．S．A．at Pikermi，Attiki．Ac－ cepted standards of humane animal care were adopted for albino female Wistar rats．Seven days of normal pre－experi－
mental housing allowed for an ad libitum diet in the labora－ tory．In total， 40 female albino Wistar rats（16－18 weeks old） were used（mean weight $\pm$ standard deviation（SD）： $232 \pm 37$ g ），with a minimum weight of 165 g and a maximum weight of 320 g ．Rats＇weights could be a confounding factor；e．g．， more obese rats have higher SUA levels．This was also in－ vestigated．Post－experimental awakening of animals was not permitted，even if euthanasia was needed．Rats were ran－ domly sorted into four experimental groups with 10 animals in each group using the following IR protocols：ischemia for 45 min followed by reperfusion for 60 min （group A）； ischaemia for 45 min followed by reperfusion for 120 min （group B）；ischaemia for 45 min followed by immediate U － 74389G intravenous（IV）administration and reperfusion for 60 min （group C）；and ischaemia for 45 min followed by immediate U－74389G IV administration and reperfusion for 120 min （group D）．The dose of U－74389G was $10 \mathrm{mg} / \mathrm{kg}$ body mass．The protocol and doses were determined via the following experiments with favourable outcomes．Flessas I et al．found（5）that the role of U－74389G was protective in many emergency clinical situations of intestinal IR．Bimpis A et al．showed limited brain damage after（6）U－74389G administration．Tsaroucha AK et al．（7）showed attenuated liver damage after U－74389G administration．Andreadou I et al．showed that the small intestine（8）was protected after U－74389G administration．

The detailed prenarcotic and general anaesthesiologic techniques were described previously（3，4）．A continuous intra－experimental oxygen supply，electrocardiogram and acidometry were maintained．Ischaemia was caused by lapa－ rotomic clamping of the inferior aorta over the renal arteries with forceps for 45 min ．Reperfusion was induced by removing the clamp and re－establishing the patency of the inferior aorta． After the exclusion of a blood flow，the IR protocol was applied as described above for each experimental group．U－74389G was administered at the time of reperfusion through the cath－ eterized inferior vena cava．The SUA levels were determined at the 60th min of reperfusion（for the $A$ and $C$ groups）and at the 120th min of reperfusion（for the $B$ and $D$ groups）．

## Control groups

Twenty control rats（ $252 \pm 39 \mathrm{~g}$ ）underwent ischaemia for 45 min followed by reperfusion．

Group A
Reperfusion lasted for 60 min （ $\mathrm{n}=10$ control rats， $243 \pm 46$ g ），and SUA levels were $1.03 \pm 0.176 \mathrm{mg} / \mathrm{dl}$（Table 1）．

## Group B

Reperfusion lasted for $120 \mathrm{~min}(\mathrm{n}=10$ control rats， $262 \pm 31 \mathrm{~g}$ ），and SUA levels were $0.95 \pm 0.295 \mathrm{mg} / \mathrm{dl}$（Table 1）．

Groups receiving the lazaroid（L）drug U－74389G
The 20 rats $(211+17 \mathrm{~g})$ receiving $L$ experienced isch－ aemia for 45 min followed by reperfusion after 10 mg of U－74389G $/ \mathrm{kg}$ body weight was IV administered．



## Group C

Reperfusion lasted for $60 \mathrm{~min}(\mathrm{n}=10 \mathrm{~L}$ rats, $212 \pm 17 \mathrm{~g}$ ), and SUA levels were $1.27 \pm 0.457 \mathrm{mg} / \mathrm{dl}$ (Table 1).

## Group D

Reperfusion lasted for $120 \mathrm{~min}(\mathrm{n}=10 \mathrm{~L}$ rats, $210 \pm 18 \mathrm{~g}$ ), and SUA levels were $1.05 \pm 0.201 \mathrm{mg} / \mathrm{dl}$ (Table 1).

## STATISTICAL ANALYSIS

Every weight and SUA level group was compared with each other using paired t-tests. Significant differences among SUA levels were investigated. A generalized linear model (GLM) was applied with SUA levels as the dependent variable. The 3 independent variables were: the presence/absence of U-74389G, the reperfusion time, and both variables in combination. Using rat weight as an independent variable in the GLM analysis, a non-significant relationship was detected ( $p=0.4431$ ), so further investigation was not needed.

## RESULTS

The application of GLM resulted in the following findings: U-74389G administration non-significantly increased SUA levels by $0.17 \mathrm{mg} / \mathrm{dl}[-0.026 \mathrm{mg} / \mathrm{dl}-0.366 \mathrm{mg} /$ $\mathrm{dl}](\mathrm{p}=0.088)$. This finding was in accordance with a paired t-test ( $\mathrm{p}=0.103$ ). The reperfusion time non-significantly decreased SUA levels by $0.15 \mathrm{mg} / \mathrm{dl}[-0.348 \mathrm{mg} / \mathrm{dl}-0.048$ $\mathrm{mg} / \mathrm{dl}](\mathrm{p}=0.134)$, also in accordance with the results of a paired t -test ( $\mathrm{p}=0.118$ ). However, U-74389G administration and reperfusion time non-significantly increased SUA levels by $0.052 \mathrm{mg} / \mathrm{dl}[-0.069 \mathrm{mg} / \mathrm{dl}-0.174 \mathrm{mg} / \mathrm{dl}]$ ( $\mathrm{p}=$ 0.387 ). Tables 2 and 3 summarize the changes in the influence of U-74389G in connection with reperfusion time.

## DISCUSSION

SUA is considered to be a reliable index of renal function. Its production is influenced by ischaemia and by a certain mode. Chiquete E et al. showed (9) that SUA is a potent antioxidant, and its serum concentration increases rapidly after acute ischaemic stroke (AIS). They associated the magnitude of cerebral infarction with the mean SUA concentration and favourable outcomes ( $p=0.004$ ) in patients with AIS upon hospital arrival. Logallo N et al. positively correlated (10) the adjusted SUA level with clinical improvement ( $\mathrm{p}=0.02$ ) and favourable stroke outcome ( $\mathrm{p}=0.04$ ) in patients with tissue plasminogen activator thrombolysis upon admission. Seifert J et al. found (11) SUA levels on day 7 and ribose ingestion after 14 days. Hellsten-Westing Y et al. found (12) that SUA is taken up by the strenuous muscle, which is the main source of plasma hypoxanthine in the blood. SUA is also taken up by the

Table 1: Weight and mean serum uric acid levels and SD of the groups.

| Groups | Variable | Mean | SD |
| :--- | :--- | :--- | :--- |
| A | Weight | 243 g | 46 g |
| A | Uric acid | $1.03 \mathrm{mg} / \mathrm{dl}$ | $0.176 \mathrm{mg} / \mathrm{dl}$ |
| B | Weight | 262 g | 31 g |
| B | Uric acid | $0.95 \mathrm{mg} / \mathrm{dl}$ | $0.295 \mathrm{mg} / \mathrm{dl}$ |
| C | Weight | 212.5 g | 18 g |
| C | Uric acid | $1.27 \mathrm{mg} / \mathrm{dl}$ | $0.457 \mathrm{mg} / \mathrm{dl}$ |
| D | Weight | 210 g | 18 g |
| D | Uric acid | $1.05 \mathrm{mg} / \mathrm{dl}$ | $0.201 \mathrm{mg} / \mathrm{dl}$ |

Standard deviation: SD

Table 2: The increasing influence of U-74389G associated with reperfusion time.

| Alteration | $95 \%$ c. in. | Reperfusion <br> time | t-test | GLM |
| :--- | :--- | :--- | :--- | :--- |
| $+0.24 \mathrm{mg} / \mathrm{dl}$ | $-0.085 \mathrm{mg} / \mathrm{dl}$ <br> $-0.565 \mathrm{mg} / \mathrm{dl}$ | 1 h | 0.184 | 0.138 |
| $+0.17 \mathrm{mg} / \mathrm{dl}$ | $-0.026 \mathrm{mg} / \mathrm{dl}$ <br> $-0.366 \mathrm{mg} / \mathrm{dl}$ | 1.5 h | 0.103 | 0.088 |
| $+0.1 \mathrm{mg} / \mathrm{dl}$ | $-0.137 \mathrm{mg} / \mathrm{dl}$ <br> $-0.337 \mathrm{mg} / \mathrm{dl}$ | 2 h | 0.401 | 0.388 |
| $-0.15 \mathrm{mg} / \mathrm{dl}$ | $-0.348 \mathrm{mg} / \mathrm{dl}$ <br> $-0.048 \mathrm{mg} / \mathrm{dl}$ | reperfusion <br> time | 0.118 | 0.134 |
| $+0.052 \mathrm{mg} / \mathrm{dl}$ | $-0.069 \mathrm{mg} / \mathrm{dl}$ <br> $-0.174 \mathrm{mg} / \mathrm{dl}$ | interaction |  | 0.387 |

confidence interval: c. in;

Table 3: The (\%) alteration influence of U-74389G associated with reperfusion time.

| Alteration | $\pm$ SD | Reperfusion time | p-values |
| :--- | :--- | :--- | :--- |
| $+20.86 \%$ | $\pm 14.44 \%$ | 1 h | 0.161 |
| $+15.43 \%$ | $\pm 9.10 \%$ | 1.5 h | 0.096 |
| $+10 \%$ | $\pm 12.11 \%$ | 2 h | 0.394 |
| $-13.61 \%$ | $\pm 9.18 \%$ | reperfusion time | 0.126 |
| $+4.78 \%$ | $\pm 5.64 \%$ | interaction | 0.387 |

liver, where most of it is converted to uric acid. Lazzarino $G$ et al. found (13) significantly increased levels of SUA after cerebral IR in rats.

Although SUA predicts AIS and leads to gout, how renal SUA excretion is influenced by U-74389G is unknown. Moreover, only reperfusion time resulted in a non-significant decline of SUA levels, reflecting a non-significant increase in renal SUA excretion. All the other endpoints implicated by U-74389G administration exhibited discouraging but non-significant results. U-74389G increased SUA levels, reflecting a decrease in renal SUA excretion. This may stand for a generalized lack of amelioration of renal function by U-74389G administration. U-74389G
cannot reverse the injury to IR tubular epithelial cells．If the injury is severe，death by apoptosis and necrosis（acute tubular necrosis）occur，with the functional impairment of water and electrolyte homeostasis and reduced excre－ tion of metabolic waste products，including SUA．A longer study duration or a higher U－74389G dosage may reverse apoptosis and necrosis of tubular epithelial cells．The body mass，as mentioned above，had no impact on protocol；the most pronounced mass difference between the B and D groups（ p －value $=0.0004$ ）reflected a non－significant differ－ ence at their respective SUA levels（ p －value＝0．4013）．

## CONCLUSION

Whether it interacted with reperfusion time，U－74389G administration non－significantly increased SUA levels．U－ 74389G cannot reverse injury to IR tubular epithelial cells within 2 hours．Perhaps either a longer study time or a higher U－74389G dosage may reverse tubular apoptosis and prevent acute tubular necrosis．

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