The Effect of the Antioxidant Drug “U-74389G” on Creatinine Levels during Ischemia Reperfusion Injury in Rats

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Key Words

Ischemia • U-74389G • Creatinine • Reperfusion

Abstract

Objective: The aim of this experimental study was to examine the effect of the antioxidant drug “U-74389G” on a rat model using an ischemia reperfusion protocol. The effect of U-74389G was studied biochemically by measuring mean blood creatinine levels. **Materials and Methods:** Forty rats were used in the study. Creatinine levels were measured at 60 min of reperfusion (groups A and C) or at 120 min of reperfusion (groups B and D), where groups A and B were controls and groups C and D received U-74389G administration. **Results:** U-74389G administration significantly decreased the predicted creatinine levels by $21.02 \pm 5.06\%$ ($p = 0.0001$). Reperfusion time non-significantly increased the predicted creatinine levels by $4.20 \pm 6.12\%$ ($p = 0.4103$). However, U-74389G administration and reperfusion time together produced a significant combined effect in decreasing the predicted creatinine levels by $11.69 \pm 3.16\%$ ($p = 0.0005$). **Conclusion:** Independent of reperfusion time, U-74389G administration significantly decreased the creatinine levels in an ischemic rat model. This study demonstrates that short-term U-74389G administration improves renal function by increasing creatinine excretion. Copyright © 2015 S. Karger AG, Basel

Introduction

Ischemia and reperfusion (IR) remains one of the main causes of permanent or transient tissue damage with serious implications for adjacent organs and certainly on patients' health. Although important progress has been made regarding the usage of U-74389G in managing this kind of damage, many fundamental questions remain unanswered, such as, U-74389G's mechanism of action, the optimal time-point for administration, and the optimal dosage. The effective action of U-74389G as antioxidant agent has been noted in several studies. However, few reports regarding U-74389G administration in IR experiments have been performed, and these important questions are yet to be addressed. Also, many studies have examined related antioxidant molecules within the same chemical class. 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. It protects against IR injury in animal heart, liver, and kidney models [1]. These membrane-associating antioxidants are particularly effective in preventing permeability changes in brain microvascular endothelial cell monolayers [2]. A meta-analysis of 7 published seric variables, using the same experimental setting, attempts to provide a numeric

Table 1. Meta-analysis of the U-74389G influence ($\pm$ SD) on the levels of some seric variables concerning reperfusion time coming from the same experimental setting [10]

Variable	1h rep	p	1.5h rep	p	2h rep	p	Interaction of U-74389G and rep	p
RBC	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
Total protein [12]	-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
Alkaline phosphatase	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
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 [11]	0 $\pm$ 1.75%	1.0000	-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
Mean	2.42% $\pm$ 9.22%	0.4527	3.23% $\pm$ 12.92%	0.4467	4.05% $\pm$ 16.67%	0.4499	1.81% $\pm$ 7.20%	0.3254

SD = Standard Deviation; rep = reperfusion; RBC = red blood cells.

evaluation of U-74389G efficacy at equivalent endpoints (Table 1). The aim of this experimental study was to examine the effect of U-74389G on a rat model using a renal IR protocol. The effect of U-74389G was studied by measuring mean blood creatinine (Cr) levels.

Materials and Methods

Animal Preparation

This experimental study was conducted 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 under 3693/12-11-2010 and 14/10-1-2012 decisions. Accepted standards of humane animal care were adopted for albino female Wistar rats. Normal housing in the laboratory 7 days before the experiment included continuous access to water and food. The experiment was acute and concluded with animal sacrifice. They were randomly divided into 4 experimental groups containing 10 animals each. In the control groups, ischemia for 45 min was followed by reperfusion for 60 min (group A) or for 120 min (group B). In the U-74389G-treated groups, ischemia for 45 min was followed by immediate U-74389G intravenous administration and reperfusion for 60 min (group C) or reperfusion for 120 min (group D).

The U-74389G dosage was 10 mg/kg body weight of animals. The dose volume and the IR duration were determined by following experiments with favorable outcomes. Chrysikos et al. [3] considered oxidative stress as a crucial factor in the pathophysiology of acute pancreatitis. They administered U-74389G 10 mg/kg intravenously after pancreatic IR (30 min/120 min) through the inferior vena cava in 2 groups of pigs. Histopathologic evaluation revealed that only a statistically significant edema seemed to be more pronounced in the placebo group ($p = 0.020$). Bimpis A et al. [4] considered the administration of U-74389G in a spontaneous intracerebral hemorrhage porcine model as a neuroprotective agent. Bimpis A et al. [5] implicated that intracerebral hemorrhage accounted for 10–15% of all strokes. They demonstrated the activation of AChE enzymes following U-74389G administration.

The lazaroid U-74389G seems to limit the damage at the brain itself. Tsaroucha et al. [6] administered U-74389G at 10 mg/kg after liver IR (30 min/120 min) in pigs. Histopathological evaluation, tissue malondialdehyde levels, and TNF α values revealed statistically significant amelioration in portal infiltration of the liver tissue in the treated group when compared to the control group ($p = 0.01$). Andreadou et al. [7] administered U-74389G at 10 mg/kg after intestinal IR (60 min/60 min) in rats. The number of polymorphonuclear leukocytes in the terminal ileum intestinal mucosa and the small intestine tissue malondialdehyde levels were lower in the U-74389G group than in controls. U-74389G protected the rat small intestine from oxidative damage by inhibiting lipid peroxidation. Although 2 studies were found associating U-74389G with renal function, direct association of U-74389G with serum creatinine levels does not exist. Hori et al. [8] evaluated 0.9 mg/kg intravenous cisplatin-induced (7–10 days) nephrotoxicity in Fisher 344 rats and found no significant difference in serum BUN level and little difference in renal histopathological findings between the 10 mg/kg U-74389G administered group and controls. Salahudeen et al. [9] reduced hydrogen peroxide-induced lipid degradation and peroxidation, protected the cells against hydrogen peroxide-induced cytolysis inhibiting excess F₂-isoprostane production, and ameliorated renal dysfunction by U-74389G administration in experimental models of acute renal injury in renal proximal tubular (LLC-PK1) cell layers.

At first, the animals were induced into preanesthesia followed by general anesthesia. The detailed anesthesiologic technique is described in related references [10–12]. Oxygen supply, electrocardiogram, and acidometry were continuously provided during the entire experimental performance. Although an ideal method would include histopathological renal specimen evaluations to allow for improved kidney structure and function results, it was unfortunately not done with this protocol. Some study limitations are that larger samples could be used, the urine creatinine levels could be measured, and histopathological renal specimens could be evaluated for greater reliability.

The IR protocol was followed. Ischemia was caused by clamping forceps to the inferior aorta over renal arteries for 45 min after laparotomic access had been achieved. Reperfusion was induced by removing the clamp and allowing reestablishment of inferior aorta patency. The U-74389G molecules were administered at

Table 2. Weight, mean creatinine levels, and standard deviation of groups

Group	Variable	Mean	Std. Dev
A	Weight	243 g	45.77724 g
A	Creatinine	0.37 mg/dl	0.0823273 mg/dl
B	Weight	262 g	31.10913 g
B	Creatinine	0.49 mg/dl	0.137032 mg/dl
C	Weight	242.8 g	29.33636 g
C	Creatinine	0.28 mg/dl	0.1135292 mg/dl
D	Weight	243 g	32.84644 g
D	Creatinine	0.29 mg/dl	0.1286684 mg/dl

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

DG	Variable	Difference	p
A–B	Weight	-19 g	0.2423
A–B	Creatinine	-0.12 mg/dl	0.0239
A–C	Weight	30.5 g	0.0674
A–C	Creatinine	0.09 mg/dl	0.0187
A–D	Weight	33 g	0.0574
A–D	Creatinine	0.08 mg/dl	0.1039
B–C	Weight	49.5 g	0.0019
B–C	Creatinine	0.21 mg/dl	0.0040
B–D	Weight	52 g	0.0004
B–D	Creatinine	0.2 mg/dl	0.0048
C–D	Weight	2.5 g	0.7043
C–D	Creatinine	-0.01 mg/dl	0.7804

DG = Difference of Groups.

reperfusion, through the inferior vena cava after catheterization had been achieved. The Cr level measurements were performed at 60 min of reperfusion (for groups A and C) or at 120 min of reperfusion (for groups B and D). Forty female Wistar albino rats of mean weight 231.875 g were used, with min weight ≥ 165 g and max weight < 320 g. Since weight could be a potentially confounding factor (i.e. fatter rats tend to have greater Cr levels), we investigated this possibility.

Model of Ischemia Reperfusion Injury

Control group: 20 rats of mean weight 252.5 g induced with ischemia for 45 min followed by reperfusion.

Group A: Reperfusion which lasted 60 min consisted of 10 control rats of mean weight 243 g and mean Cr levels 0.37 mg/dl (Table 2).

Group B: Reperfusion which lasted 120 min consisted of 10 control rats of mean weight 262 g and mean Cr levels 0.49 mg/dl (Table 2).

Lazaroid (L) group: 20 rats of mean weight 211.25 g induced with ischemia for 45 min followed by reperfusion that were first intravenously administered with U-74389G at 10 mg/kg body weight.

Group C: Reperfusion which lasted 60 min consisted of 10 L rats of mean weight 212.5 g and mean Cr levels 0.28 mg/dl (Table 2).

Group D: Reperfusion which lasted 120 min consisted of 10 L rats of mean weight 210 g and mean Cr levels 0.29 mg/dl (Table 2).

Statistical Analysis

All rats were divided into 4 groups based on weight and each group was individually compared with the other 3 groups by applying a statistical standard *t*-test (Table 3) since weight is a parametric variable. Any emerging significant difference among Cr levels was investigated to determine if there were any weight-related correlations. Similarly, all rats were divided into 4 groups based on Cr levels and each group was individually compared with the other 3 groups by applying a statistical standard *t*-test since Cr level is also a parametric variable (Table 3). Although ANOVA analysis can determine significant variance for mean weight and Cr levels among all groups, we chose to obtain a *p*-value for every individualized pair of variables, as a more conservative approach. Also, generalized linear models (GLM) were applied. They included the Cr levels as the dependant variable and independent variables including the U-74389G administration or control, the reperfusion time, and their interaction. Using the rats weight as an independent variable with the GLM showed a very significant relation with Cr levels ($p = 0.0000$), which merits further investigation. The predicted Cr values adjusted for rats weight were calculated. The above procedure was iterated for predicted values. The differences between predicted mean Cr values were calculated by paired *t* tests. Using the GLM we reevaluated these relationships using the predicted Cr levels as the dependent variable. The STATA 6.0 software was used.

Results

U-74389G administration significantly decreased the Cr levels by 0.145 mg/dl (-0.2232323–0.0667677 mg/dl) ($p = 0.0006$). This finding was in accordance with the results of the standard *t*-test ($p = 0.0003$). Reperfusion time non-significantly increased the Cr levels by 0.065 mg/dl (-0.0240624–0.1540625 mg/dl) ($p = 0.1478$) and was also in accordance with the standard *t*-test ($p = 0.0441$). However, U-74389G administration and reperfusion time together produced a significant combined effect in decreasing the Cr levels by 0.0772727 mg/dl (-0.1263251–0.0282203 mg/dl) ($p = 0.0029$). Considering the above results and data in table 3, table 4 sums up the influence of U-74389G along with reperfusion time. The predicted Cr values adjusted for rat weight were calculated and are depicted in table 5. The differences between predicted mean Cr values as calculated by standard *t* tests are depicted in

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

Decrease	95% CI	Reperfusion time	t-test	GLM p
0.09 mg/dl	-0.1831698–0.0031698 mg/dl	1h	0.0187	0.0575
0.145 mg/dl	-0.2232323–0.0667677 mg/dl	1.5h	0.0003	0.0006
0.2 mg/dl	-0.3248827–0.0751173 mg/dl	2h	0.0048	0.0035
-0.065 mg/dl	-0.0240624–0.1540625 mg/dl	reperfusion time	0.0441	0.1478
0.0772727 mg/dl	-0.1263251–0.0282203 mg/dl	interaction	–	0.0029

GLM = Generalized linear models.

Table 5. Mean predicted creatinine levels and standard deviation of groups

Group	Mean	Std. Dev
A	0.3778797 mg/dl	0.0838588 mg/dl
B	0.4126856 mg/dl	0.0569884 mg/dl
C	0.3220072 mg/dl	0.0326701 mg/dl
D	0.3174274 mg/dl	0.0331657 mg/dl

Table 6. Statistical significance of mean predicted creatinine values difference for groups after statistical standard *t*-test application

DG	Difference	p
A–B	-0.0348059 mg/dl	0.2423
A–C	0.0558726 mg/dl	0.0674
A–D	0.0604523 mg/dl	0.0574
B–C	0.0906785 mg/dl	0.0019
B–D	0.0952582 mg/dl	0.0004
C–D	0.0045797 mg/dl	0.7043

DG = Difference of Groups.

table 6. The iterated GLM showed that U-74389G administration significantly decreased the predicted Cr levels by 0.0755654 mg/dl (-0.1112671–0.0398636 mg/dl) ($p = 0.0001$). This finding was in accordance with the results of the standard *t*-test ($p = 0.0002$). Reperfusion time non-significantly increased the predicted Cr levels by 0.0151131 mg/dl (-0.0280818–0.0583079 mg/dl) ($p = 0.4831$) and is also in accordance with the standard *t*-test ($p = 0.3375$). However, U-74389G administration and reperfusion time together produced a significant

combined effect in decreasing the predicted Cr levels by 0.0420501 mg/dl (-0.0643377–0.0197626 mg/dl) ($p = 0.0005$). Considering the above results and the data in table 6, tables 7 and 8 sum up the decreasing influence of U-74389G along with reperfusion time.

Discussion

Serum Cr levels measurement is the most commonly used indicator of renal function [13]. A rise in blood Cr level is observed only with marked damage to functioning nephrons. Therefore, this test is suitable for detecting late-stage kidney disease. A better estimation of kidney function is given by calculating the estimated glomerular filtration rate (eGFR). Most clinical laboratories now align their Cr measurements against a new standardized isotope dilution mass spectrometry (IDMS) method to measure serum Cr. Renal function is influenced by ischemia and particularly by certain mode, as the next references shows. Xu et al. [14] found significantly increased serum Cr concentrations at 24 hours of reperfusion in male Sprague-Dawley rats IR kidneys. Wang et al. [15] found significantly increased serum Cr levels 3 days after induced IR renal failure which restored to normal levels within 4 weeks in male Lewis rats. Rabadi et al. [16] found a short-term serum Cr level deterioration after IR. Domínguez et al. [17] observed no difference in serum Cr levels after the 1st day in male Sprague-Dawley rats that served as concomitant kidney transplant donors and recipients versus control ones. Jang et al. [18] noted no difference in serum Cr levels among groups administered with different doses of either mouse anti-thymocyte globulin, rabbit immunoglobulin, or saline in different renal IR models. Nohara et al. [19] noted a significantly smaller short-term increase in serum Cr levels, IR injury minimization, and renal function preservation after anatomic

Table 7. The decreasing influence of U-74389G in connection with reperfusion time

Decrease	95% CI	Reperfusion time	t-test	GLM p
0.0558726 mg/dl	-0.1156645–0.0039193 mg/dl	1h	0.0674	0.0653
0.0755654 mg/dl	-0.1112671–0.0398636 mg/dl	1.5h	0.0002	0.0001
0.0952582 mg/dl	-0.1390645–0.0514519 mg/dl	2h	0.0004	0.0002
-0.0151131 mg/dl	-0.0280818–0.0583079 mg/dl	reperfusion time	0.3375	0.4831
0.0420501 mg/dl	-0.0643377–0.0197626 mg/dl	interaction	–	0.0005

GLM = Generalized linear models.

Table 8. The percentage decreasing influence of U-74389G in connection with reperfusion time

Decrease	± SD	Reperfusion time	p
-15.96%	± 8.71%	1h	0.0663
-21.02%	± 5.06%	1.5h	0.0001
-26.09%	± 6.12%	2h	0.0003
+4.20%	± 6.12%	reperfusion time	0.4103
-11.69%	± 3.16%	interaction	0.0005

nephrectomy compared with standard partial nephrectomy. O'Valle et al. [20] predicted short-term delay in total recovery of renal function and serum Cr levels from cold ischemia acute tubular necrosis in kidney allograft biopsies. Oliveira et al. [21] decreased tubular necrosis by 22% by administering intravenous fingolimod hydrochloride (1 mg/kg) immediately before an IR induction injury model compared to control mice. Lemos et al. [22] significantly correlated serum Cr levels with the protective role of both heme oxygenase-1 and vascular endothelial growth factor mRNA gene expressions at the first week of kidney posttransplantation. van der Hoeven et al. [23] showed a relationship between the increased serum Cr levels enhanced by hemodynamic instability with time duration after brain death in Wistar rats. Gueler et al. [24] suggested that hydroxy-3-methylglutaryl coenzyme A reductase inhibition reduced the Cr level by 40% ($p < 0.005$) and ameliorated the decreased eGFR by 350% ($p < 0.001$) 24 h after acute renal IR in male uninephrectomized Sprague-Dawley rats. Giovannini et al. [25] associated mortality to renal damage as indicated by serum Cr levels in ischemic male Wistar rats. Torras et al. [26] exerted functional and morphological protection against post-ischemic acute renal failure, as shown by Cr levels on the initial IR injury in warm ischemic uninephrecto-

mized male Sprague-Dawley rats. Caramelo et al. [27] improved renal function (6.673-fold GFR than controls, $p < 0.01$) only in the short-term post IR period in uninephrectomized rabbits.

Although new renal function markers with greater reliability have come into use, the serum Cr level measurement remains fundamental. eGFR can be accurately calculated using serum Cr concentration and some or all of the following variables: sex, age, weight, and race, as suggested by the American Diabetes Association without a 24-hour urine collection [28]. Many laboratories automatically calculate eGFR when a Cr test is requested. IDMS appears to give lower values than previous methods when the serum Cr values are relatively low, for example 0.7 mg/dl. The IDMS method would result in a comparative overestimation of the corresponding calculated GFR in some patients with normal renal function [29]. A serum Cr level decline stands consequently for a related improvement at eGFR, IDMS, and renal function. Also, Cr level is obviously influenced by U-74389G. The present study demonstrates the short-term protective role of U-74389G on renal function.

Conclusion

U-74389G administration, independent of reperfusion time, significantly decreases the Cr levels. This analysis indicates that short-term administration of U-74389G ameliorates renal dysfunction by increasing Cr excretion.

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