The effect of the antioxidant *drug U-74389G* on urea levels during ischemia reperfusion injury in rats

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ABSTRACT

This experimental study examined the effect of the antioxidant *drug U-74389G*, on a rat model and particularly in a renal ischemia-reperfusion protocol. The effects of that molecule were studied biochemically using blood mean urea levels. Forty rats of mean weight 231.875 g were used in the study. Urea levels were measured at 60 min of reperfusion (groups A and C) and at 120 min of reperfusion (groups B and D). The drug U-74389G was administered only in groups C and D. U-74389G administration significantly decreased the predicted urea levels by 11.35%±2.73% (P=0.0001). Reperfusion time non-significantly increased the predicted urea levels by 2.26%±3.29% (P=0.4103). However, U-74389G administration and reperfusion time together significantly decreased the predicted urea levels by 6.31%±1.70% (P=0.0005). U-74389G administration whether it interacted or not with reperfusion time had significant decreasing effect on the urea serum levels, reflecting a respective renal function augmentation.

Introduction

Permanent or transient damage with serious implications on adjacent organs and systems may be due to tissue ischemia-reperfusion (IR). The use of U-74389G in IR has been a challenge for many years. However, although the progress was significant, several practical questions have not clarified. They include: i) how potent U-74389G should be; ii) when should it be administered; and iii) at what optimal dose of U-74389G should be administered. The promising effect of U-74389G in tissue protection has been noted in several IR 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 IR 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] A meta-analysis of 20 published seric variables, coming from the same experimental setting, tried to provide a numeric evaluation of the U-74389G efficacy at the same endpoints (Table 1). 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 examine the effect of the antioxidant *drug U-74389G* on rat model and particularly in a renal IR protocol. The effects of that molecule were studied by measuring blood mean serum urea levels.

Materials and Methods

Animal preparation

This basic experimental research was licensed by

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Veterinary Address of East Attiki Prefecture under 3693/12-11-2010 and 14/10-1-2012 decisions. All consumables, equipment and substances, 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. Prenarcosis of animals preceded by continuous intra-experimental general anesthesia,[3-7] oxygen supply, electrocardiogram and acidometry. Post-experimental awakening and preservation of animals were not permitted, even if euthanasia was needed. Rats were randomly delivered to four experimental groups by 10 animals in each one, using following protocols of IR: 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); ischemia for 45 min followed by immediate U-74389G IV administration and reperfusion for 120 min (group D). The dose of U-74389G was 10 mg/kg body mass of animals. Ischemia was caused by laparotomic clamping inferior aorta over renal arteries with forceps for 45 min. Reperfusion was induced by removing the clamp and re-establishing inferior aorta patency. U-74389G was administered at the time of reperfusion; through catheterized inferior vena cava. The urea levels were determined at 60th min of reperfusion (for A and C groups) and at 120th min of reperfusion (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 have higher urea levels. This assumption was also investigated.

Table 1. The U-74389G influence (±standard deviation) on the levels of some seric variables3 concerning reperfusion 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
WBCC[4]	+22.99±12.45	0.0914	+30.85±11.14	0.0045	+38.70±17.39	0.0185	+23.45±6.28	0.0004
RBCC	+1.39±0.71	0.7161	+0.64±0.32	0.8106	–0.100.05	0.9762	+1.05±0.53	0.4911
Hematocrit[5]	+5.58±3	0.0852	+4.73±2.25	0.0435	+3.89±3.44	0.2608	+3.16±1.33	0.0196
Hemoglobin	+5.2±2.8	0.0925	+3.9±2.1	0.0604	+2.7±3.2	0.3544	+2.5±1.3	0.0423
MCH	+1.77±0.96	0.0663	+2.40±0.57	0.0001	+3.03+0.71	0.0003	1.33+0.36	0.0005
MCHC[7]	–0.5±0.74	0.4820	–0.95±0.63	0.1124	–1.4±1.12	0.1603	–0.69±0.37	0.0655
RbcDW[6]	–6.13±3.73	0.0667	–4.96±2.27	0.0175	–3.80±3.07	0.1383	–2.54±1.39	0.679
Platelet count	–17.79±9.40	0.0647	–12.83±5.79	0.0303	–7.88±7.83	0.2939	–6.12±3.58	0.0857
Plateletcrit	+3.80±9.87	0.6373	+9.23±6.29	0.1064	+14.66±9.03	0.0833	+6.72±3.73	0.0712
PDW	+1.1±0.88	0.2368	+1.79±0.76	0.0314	+2.49±1.33	0.0807	+0.96±0.46	0.0396
Glucose	–6.41±3.50	0.0663	–8.57±2.06	0.0001	–10.74±2.52	0.0003	–4.76±1.28	0.0005
Total protein	–5.48±2.99	0.0663	–7.34±1.76	0.0000	–9.20±2.16	0.0000	–4.08±1.10	0.0000
ALP	+22.66±12.37	0.0663	+31.91±7.69	0.0001	+41.16±9.65	0.0003	+17.75±4.79	0.0005
ACP	–112.54±20.95	0.0006	–128.45+14.84	0.0000	–144.36±21.62	0.0000	–74.45±9.63	0.0000
CPK	+54.32±13.75	0.0012	+35.34±17.20	0.0260	+16.37±30.24	0.4951	+18.52±9.44	0.0770
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
Calcium	0±1.75	1	–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
Magnesium	+1.33±3.59	0.7033	–0.28±2.75	0.9171	–1.90±5.28	0.7161	+0.36±4.58	0.8228
Mean	–1.51±30.02	0.2881	–2.25±32.50	0.2238	–2.99±36.02	0.2875	–0.94±19.03	0.1785

rep, reperfusion; WBCC, white blood cells count; RBCC, red blood cells count; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; RbcDW, red blood cell distribution width; PDW, platelet distribution width; ALP, alkaline phosphatase; ACP, acid phosphatase; CPK, creatine phosphokinase.

Control groups

Twenty control rats (mean mass 252.5 g [SD: 39.31988 g]) experienced ischemia for 45 min followed by reperfusion.

Group A

Reperfusion lasted for 60 min (n=10 controls rats) mean mass 243 g [SD: 45.77724 g], mean urea levels 55 mg/dL [SD: 8.137704 mg/dL].

Group B

Reperfusion lasted for 120 min (n=10 controls rats) mean mass 262 g [SD: 31.10913 g], mean urea levels 71.3 mg/dL [SD: 11.84202 mg/dL].

Lazaroid (L) group

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

Group C

Reperfusion lasted for 60 min (n=10 L rats) mean mass 212.5 g [SD: 17.83411 g], mean urea levels 52.2 mg/dL [SD: 9.715966 mg/dL].

Group D

Reperfusion lasted for 120 min (n=10 L rats) mean mass 210 g [SD: 18.10463 g], mean urea levels 50.9 mg/dL [SD: 9.904544 mg/dL].

Statistical analysis

Every weight and urea level group was compared with each other by statistical standard t-tests. Any significant difference among urea levels, was investigated due to weight fluctuation. The application of generalized linear models (glm) with dependent variable with urea levels as the dependant variable was followed. The 3 independent variables were the U-74389G or no drug, the reperfusion time and both variables in combination. Inserting the rats weight also as an independent variable at glm analysis, a significant relation resulted in (P=0.0030), so as to further investigation was needed. The predicted urea values for weight were calculated and are depicted in Table 2. The differences between predicted urea values groups calculated by standard t test are depicted in Table 3. The application of glm was followed with dependent variable used the predicted urea levels. The 3 independent variables were the same as in first glm.

Results

The first glm resulted in: U-74389G administration significantly decreased the urea levels by 11.6 mg/dL [–18.88982 mg/dL - –4.310184 mg/dL] (P=0.0026). This finding was in accordance with the results of standard t-test (P=0.0137). Reperfusion time non-significantly increased the urea levels by 7.5 mg/dl [–0.3477317 mg/dL - 15.34773 mg/dL] (P=0.0605), also in accordance with standard t-test (P=0.0762). However, U-74389G administration and reperfusion

Table 2. Mean predicted urea levels and standard deviation of groups.

Groups	Mean	Standard deviation
A	59.1111 mg/dL	7.246579 mg/dL
B	62.11881 mg/dL	4.924603 mg/dL
C	54.28292 mg/dL	2.823156 mg/dL
D	53.88717 mg/dL	2.86598 mg/dL

Table 3. Statistical significance of mean predicted urea levels difference for groups after statistical standard t test application.

Difference for groups	Difference	P-value
A-B	–3.007716 mg/dL	0.2423
A-C	4.828179 mg/dL	0.0674
A-D	5.223931 mg/dL	0.0574
B-C	7.835894 mg/dL	0.0019
B-D	8.231647 mg/dL	0.0004
C-D	0.3957523 mg/dL	0.7043

time together significantly decreased the urea levels by 6.563636 mg/dL [–11.03074 mg/dL - –2.096533 mg/dL] (P=0.0051).

The second glm resulted in: U-74389G administration significantly decreased the predicted urea levels by 6.529913 mg/dL [–9.615044 mg/dL - –3.444781 mg/dL] (P=0.0001). This finding was in accordance with the results of standard t-test (P=0.0002). Reperfusion time non-significantly increased the predicted urea levels by 1.305982 mg/dL [–2.426665 mg/dL - 5.038628 mg/dL] (P=0.4831), also in accordance with standard t-test (P=0.3375). However, U-74389G administration and reperfusion time together significantly decreased the predicted urea levels by 3.633726 mg/dL [–5.559681 mg/dL - –1.707771 mg/dL] (P=0.0005). Reviewing the above and Table 3, the Tables 4 and 5 sumup concern the decreasing influence of U-74389G in connection with reperfusion time.

Discussion

Serum urea is considered one of the two renal function indexes. Renal function is influenced by ischemia particularly by certain mode, as the next references show. Xu *et al.*[8] found urea serum concentrations significantly elevated after 45 min of kidneys IR in male Sprague-Dawley rats. Cakmaz *et al.*[9] found the extent of increased plasma D-amino acid oxidase levels and the one of decreased citrulline levels dependent on superior mesenteric artery IR in Wistar albino rats. Koetting *et al.* significantly enhanced[10] the renal function by 2 h hypothermic machine reperfusion with oxygenation HR-$O^{=}$ more than threefold increase in renal clearances of urea levels, after cold stored kidneys preservation; in female German Landrace pigs. Oliveira *et al.* investigated[11] the mechanisms involved in the protective activity of IV FTY720 (1 mg/kg) immediately before renal IR injury model about short-term (24 h) function outcome. Medina *et al.* found no difference in the degree of renal failure analyziing[12] serum urea levels at short-term kidneys IR between IGF-I50 µg/100 g reperfusion treatment than untreated controls male rats. Ansermet *et al.*[13] found plasma urea levels not depressed after the higher doses of toxic agents measured within 48 h after either mercuric chloride, or gentamicin, or tobramycin administration or renal arteries IR in adult male Wistar rats. Lin *et al.*[14] noted no significant difference in the urea kinetic study treating erythropoietin-hyporesponsive anemia by IV ascorbic acid in chronic hemodialysis patients with type II diabetes.

Also, urea is a factor influenced by U-74389G. Suitable U-74389G administration can indirectly cure ischemic cases. The following clinical situations can show this more clearly. Alhan *et al.*[15] inhibited the significant increase in urea levels induced due to acute necrotizing pancreatitis in rats treated by the use of U-74389G. Hori *et al.*[16] designed the present study in order to investigate whether U-74389G has a protective effect on 0.9 mg/kg IV cisplatin (CDDP)-induced nephrotoxicity in Fisher rats. No significant difference in serum blood urea nitrogen level between control

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

Change	95% c. in.	Reperfusion time	P-values t-test	P-values glm
–4.828179 mg/dL	–9.995043 mg/dL - 0.3386853 mg/dL	1 h	0.0674	0.0653
–6.529913 mg/dL	–9.615044 mg/dL - –3.444781 mg/dL	1.5 h	0.0002	0.0001
–8.231647 mg/dL	–12.01713 mg/dL - –4.446163 mg/dL	2 h	0.0004	0.0002
+1.305982 mg/dL	–2.426665 mg/dL - 5.038628 mg/dL	Reperfusion time	0.4831	0.3375
–3.633726 mg/dL	–5.559681 mg/dL - –1.707771 mg/dL	Interaction		0.0005

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

Change	±standard deviation	Reperfusion time	P-values
–8.51%	±4.64%	1 h	0.0663
–11.35%	±2.73%	1.5 h	0.0001
–14.19%	±3.32%	2 h	0.0003
+2.26%	±3.29%	Reperfusion time	0.4103
–6.31%	±1.70%	Interaction	0.0005

and case groups was noted. U-74389G was found not to ameliorate cisplatin CDDP-induced nephrotoxicity.

Conclusions

U-74389G administration whether it interacted or not with reperfusion time had significant decreasing effect on the serum urea levels, reflecting a respective renal urea excretion augmentation.

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