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The Hypoglucemic Effect of the Antioxidant Drug "U-74389g" during Ischemia Reperfusion Injury in Rats

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Abstract

Aim: The aim of this experimental study was to examine the effect of the antioxidant drug "U-74389G", on rat model and particularly in an ischemia – reperfusion protocol. The beneficial effects or non-effectiveness of that molecule were studied biochemically using blood mean glucose levels.

Materials and methods: 40 rats of mean weight 231.875gr were used in the study. Glucose levels were measured at 60 min of reperfusion (groups A and C) and 120 min of reperfusion (groups B and D), A and B without but C and D with U-74389G administration.

Results: Results were that U-74389G administration significantly decreased the predicted glucose levels by 8.57% ± 2.06% (p=0.0001). Reperfusion time non-significantly increased the predicted glucose levels by 1.71% ± 2.49% (0.4103). However, U-74389G administration and reperfusion time together significantly decreased the predicted glucose levels by 4.76% ± 1.28% (p=0.0005).

Conclusion: Conclusions are that U-74389G administration interacted or not with reperfusion time has significant decreasing effect on the glucose serum levels, enabling consideration of it as a potential hypoglucemic factor.

Keywords

Ischemia, U-74389G, Glucose, Reperfusion

Introduction

Ischemia and reperfusion (IR) remain one of the main causes of permanent or transient damage with serious implications on adjacent organs and certainly on patients' health. The use of antioxidant substances has been a research subject for many years. However, even if important progress has been made, satisfactory answers have not been given yet to fundamental questions, such as, how much powerful should an antioxidant be, when should it be administered, and in which dosage. The particularly satisfactory action of the antioxidant U-74389G in tissue protection has been noted in several performed experiments. Since a careful literature search (PubMed - Medline) was conducted, it was realized that this certain antioxidant has been tried in IR experiments. However, just few relative reports were found, not covering completely this particular matter. Luo X et al. completely or partially attenuated [1] lipid peroxidation products and reduced lung DNA synthesis, consistent with a role for hydroxyl radicals or lipid hydroperoxides as second messengers in normal regulation of lung growth, by U74389G administration after exposure to 95% O_2 in 4-7-days old rats lungs and serum. Pulmonary O_2 toxicity is thought to be a major contributor to the development of bronchopulmonary dysplasia of preterm infants and antioxidant interventions hold significant promise for therapy, although U74389G did not improve the survival rate. Taherzadeh M et al. concluded [2] that U74389G up-regulates CYP3A6 but inhibits its catalytic activity, prevents the hepatic malondialdehyde (MDA) enhancement and prevents CYP1A1/2 down-regulation and decrease in activity by a double mechanism: hindering the release of serum mediators and by averting intracellular events, effect possibly associated with its antioxidant activity in rabbits after induced inflammatory reaction. Also, a lot of publications addressed trials of other similar molecules of aminosteroids (lazaroids) to which the studied molecule also belongs to. 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 [3] 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. These membrane-associating antioxidants [4] are particularly effective in preventing permeability changes in brain microvascular endothelial cells monolayers. The same authors found the influence of U-74389G as depicted at table 1 on some biochemical variables serum levels in related IR injury experiments, 1h, 1.5h, 2h and interaction of U-74389G with reperfusion time after reperfusion in rats.

The aim of this experimental study was to examine the effect of the antioxidant drug "U-74389G" on rat model and particularly in a pancreas and liver IR protocol. The beneficial effects or non-effectiveness of that molecule were studied by measuring blood mean glucose (gl) levels.

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Table 1: The U-74389G influence (± SD) on the levels of some seric variables [5] 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
RBC	+1.39% ± 0.71%	0.7161	+0.64% ± 0.32%	0.8106	-0.10% ± 0.05%	0.9762	+1.05% ± 0.53%	0.4911
alkaline phosphatase	+22.66% ± 12.37%	0.0663	+31.91% ± 7.69%	0.0001	+41.16% ± 9.65%	0.0003	+17.75% ± 4.79%	0.0005
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[6]	0% ± 1.75%	01/01/00	-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

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

Groups	Variable	Mean	Std. Dev
A	Weight	243g	45.77724g
A	Glucose	23.343mmol/l	3.840mmol/l
B	Weight	262 g	31.10913g
B	Glucose	23.509mmol/l	5.716mmol/l
C	Weight	212.5 g	17.83411g
C	Glucose	21.511mmol/l	3.987mmol/l
D	Weight	210g	18.10463g
D	Glucose	22.294mmol/l	6.244mmol/l

Materials and Methods

Animal preparation

This experimental study was laid out at the Exprerimental Research Center of ELPEN Pharmaceuticals Co. Inc. S.A. at Pikermi, Attiki and by Veterinary Address of East Attiki Prefecture under 3693/12-11-2010 & 14/10-1-2012 decisions. All settings needed for the study including of consumables, equipment and substances used, were a courtesy of that S. A. Albino female Wistar rats were used in accordance with accepted standards of humane animal care. They were housed in laboratory 7 days before the experiment, having easy access to water and food. The experiment was acute, that is, the animal usage was completed by following experimental set of times without awakening and preservation of the rodents. They were randomly assigned to four experimental groups (10 animals in each group). 1) Ischemia for 45 min followed by reperfusion for 60 min (group A). 2) Ischemia for 45 min followed by reperfusion for 120 min (group B). 3) Ischemia for 45 min followed by immediate U-74389G intravenous (IV) administration and reperfusion for 60 min (group C). 4) Ischemia for 45 min followed by immediate U-74389G IV administration and reperfusion for 120 min (group D). The molecule U-74389G dose was 10mg/Kg body weight of animals.

The animals were submitted into prenarcosis at first of experiment followed by general anesthesia. Their electrocardiogram and acidometry were continuously monitored. Their inferior aorta flow was excluding by forceps. After exclusion, the protocol of IR was applied, exactly as described in experimental groups. The molecules were administered at the time of ischemia removal through inferior vena cava after catheterization had been achieved. The gl levels measurement was performed at 60 min of reperfusion (groups A and C) and 120 min of reperfusion (groups B and D).

The detailed anesthesiologic technique is described in related references [5,6]. Continuous oxygen supply was administered during whole experiment performance. Hypoxemia was caused by clamping inferior aorta over renal arteries for 45 min after laparotomic access was achieved. Reperfusion was induced by removing the clamp and reestablishment of inferior aorta patency. Forty (40) female Wistar albino rats were used of mean weight 231.875gr [Std. Dev: 36.59703gr], with min weight ≥ 165gr and max weight ≤ 32 gr. Rats weight could be potentially a confusing factor, e.g. fatter rats to have greater blood gl levels. This suspicion was investigated.

Control groups

20 control rats of mean weight 252.5gr [Std. Dev: 39.31988gr] were subjected to ischemia for 45 min followed by reperfusion.

Group A: Reperfusion which lasted 60 min concerned 10 controls rats of mean weight 243gr [Std. Dev: 45.77724gr] and mean gl levels 23.343mmol/l [Std. Dev: 3.840mmol/l] (Table 2).

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	-19g	0.2423
A-B	Glucose	-0.166mmol/l	0.9165
A-C	Weight	30.5 g	0.0674
A-C	Glucose	1.831mmol/l	0.3277
A-D	Weight	33g	0.0574
A-D	Glucose	1.048mmol/l	0.7192
B-C	Weight	49.5g	0.0019
B-C	Glucose	1.997mmol/l	0.3962
B-D	Weight	52 gr	0.0004
B-D	Glucose	1.215mmol/l	0.7279
C-D	Weight	2.5g	0.7043
C-D	Glucose	-0.782mmol/l	0.6765

Group B: Reperfusion which lasted 120 min concerned 10 controls rats of mean weight 26gr [Std. Dev: 31.10913gr] and mean gl levels 23.509mmol/l [Std. Dev: 5.716mmol/l] (Table 2).

Lazaroid (L) group: 20 rats of mean weight 211.25gr [Std. Dev: 17.53755gr] suffered by hypoxemia for 45 min followed by reoxygenation in the beginning of which 10 mg U-74389G /kg body weight were IV administered.

Group C: Reoxygenation which lasted 60 min concerned 10 L rats of mean weight 212.5gr [Std. Dev: 17.83411gr] and mean gl levels 21.511mmol/l [Std. Dev: 3.987mmol/l] (Table 2).

Group D: Reperfusion which lasted 120 min concerned 10L rats of mean weight 210gr [Std. Dev: 18.10463gr] and mean gl levels 22.294mmol/l [Std. Dev: 6.244mmol/l] (Table 2).

Every rat's weight group initially was compared with each one from 3 remained groups applying statistical paired t-test (Table 3). Any emerging significant difference among gl levels was investigated whether owed in the above mentioned probable significant weight correlation. Every rat's gl group initially was compared with other one from 3 remained groups applying statistical paired t-test (Table 3). Appliance of generalized linear models (glm) with dependant variable the gl levels and independent variables the U-74389G administration or no, the reperfusion time and their interaction was followed. Statistical software package STATA 6.0 was used for all calculations. Inserting the rats weight also as an independent variable at glm analysis, a significant relation resulted in (p=0.0265), so as to further investigation was needed (Table 4). The predicted values of glucose levels, adjusted for weight were calculated and are depicted at table 5. Predicted glucose values comparison of each one from 4 rats groups initially was performed with other one from 3 remained groups applying statistical paired t-test (Table 6). Glm with dependant variable the predicted gl levels and independent variables the U-74389G administration or no, the reperfusion time and their interaction was iterated.

Results

U-74389G administration non-significantly increased the gl levels by 0.474mmol/l [-2.717mmol/l–3.666mmol/l] (P=0.7651). This finding was in accordance with the results of paired t-test (p=0.6875). Reperfusion time non-significantly decreased the gl levels by 1.523mmol/l [-4.680mmol/l–1.633mmol/l] (P=0.3348), also in accordance with paired t-test (p=0.4231). However, U-74389G

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

			p-values	
Decrease	95% c. in.	Reperfusion time	t-test	glm
1.831mmol/l	-5.509mmol/l – 1.846 mmol/l	1h	0.3277	0.3094
1.523mmol/l	-4.680mmol/l – 1.633mmol/l	1.5h	0.4231	0.3348
1.215mmol/l	-6.839mmol/l – 4.408mmol/l	2h	0.7279	0.6552
1.523mmol/l	-4.680mmol/l – 1.633mmol/l	reperfusion time	0.4231	0.3348
0.688mmol/l	-2.602mmol/l – 1.225mmol/l	interaction	-	0.4708

Table 5: Mean predicted glucose levels and Std. Dev. of groups

Groups	Mean	Std. Dev
A	23.190mmol/l	2.162mmol/l
B	24.087mmol/l	1.469mmol/l
C	21.749mmol/l	0.842mmol/l
D	21.631mmol/l	0.855mmol/l

Table 6: Statistical significance of mean predicted gl values difference for groups (DG) after statistical paired t test application

DG	Difference	p-value
A-B	-0.897mmol/l	0.2423
A-C	1.440mmol/l	0.0674
A-D	1.558mmol/l	0.0574
B-C	2.337mmol/l	0.0019
B-D	2.455mmol/l	0.0004
C-D	0.118 mmol/l	0.7043

administration and reperfusion time together non-significantly decreased the gl levels by 0.688mmol/l [-2.602mmol/l–1.225mmol/l] (P=0.4708). Reviewing the above and table 3, table 4 sums up concerning the alteration influence of U-74389G in connection with reperfusion time. The iteration of above calculation with predicted gl values is quoted: U-74389G administration significantly decreased the predicted gl levels by 1.948mmol/l [-2.868mmol/l –1.027mmol/l] (P=0.0001). This finding was in accordance with the results of paired t-test (p=0.0002). Reperfusion time non-significantly increased the predicted gl levels by 0.389mmol/l [-0.724mmol/l –1.503mmol/l] (P=0.4831), also in accordance with paired t-test (p=0.3375). However, U-74389G administration and reperfusion time together significantly decreased the predicted gl levels by 1.084mmol/l [-1.658mmol/l–0.509mmol/l] (P=0.0005). Reviewing the above and table 6, tables 7 and 8 sum up concerning the alteration influence of U-74389G in connection with reperfusion time.

Discussion

The following situations show how gl levels influence ischemia since special references concerning how ischemia can influence gl levels does not exist. Gąsecki D et al. scored at 7.1 ± 6.5 units one week after stroke onset by National Institutes of Health Stroke Scale (NIHSS) measuring [7] carotid-femoral (CF) pulse wave velocity (PWV) and central augmentation index (cAIx) on admission in patients with acute ischemic stroke. Low CF-PWV (P=0.001) was significantly associated with early favorable outcome at hospital discharge. CF-PWV >9.0m/s remained significantly associated with favorable early outcome after adjustment for blood glucose level on admission, measured at day 7 (P=0.006). Nardi K et al found [8] median admission glucose levels being 6.271 (5.383-8.602)mmol/l. Patients with diabetes had significantly higher median glucose levels by 52,33% than patients without diabetes [p<0.001]. The only significant predictive value of glycemia was ≥7.936mmol/l for 72-hour fatality especially in non-diabetics. This cut-off point was an independent predictor for 72-hour fatality (overall: OR=4.0, p=0.003; without diabetes: OR=4.9, p=0.004). Admission hyperglycemia (≥143mg/dL) is a strong and an independent predictor for 72-hour fatality, especially in patients with no prior history of diabetes mellitus. Admission hyperglycemia increases the risk of death in first-ever acute ischemic stroke patients. Yang YM et al. compared [9] the impact of the first 24 hours mean blood gl levels (at admission, 6 and 24 hours after admission) and admission glucose (AG) during hospitalization with the short term mortality and combined end

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

			p-values	
Alteration	95% c. in.	Reperfusion time	t-test	glm
-1.440mmol/l	-2.982mmol/l – 0.101mmol/l	1h	0.0674	0.0653
-1.948mmol/l	-2.868mmol/l - -1.027mmol/l	1.5h	0.0002	0.0001
-2.455mmol/l	-3.585mmol/l - -1.326 mmol/l	2h	0.0004	0.0002
+0.389mmol/l	-0.724mmol/l – 1.503mmol/l	reperfusion time	0.3375	0.4831
-1.084mmol/l	-1.658mmol/l - -0.509mmol/l	interaction	-	0.0005

Table 8: The (%) decreasing influence of U-74389G in connection with reperfusion time

Alteration	± SD	Reperfusion time	p-values
-6.41%	± 3.50%	1h	0.0663
-8.57%	± 2.06%	1.5h	0.0001
-10.74%	± 2.52%	2h	0.0003
+1.71%	± 2.49%	reperfusion time	0.4103
-4.76%	± 1.28%	interaction	0.0005

point events in patients with ST-segment elevation acute myocardial infarction (STEMI). 7-day and 30-day mortality and combined end point events were increased in proportion to plasma gl level increase. Elevated gl (equal or greater than 7.1-8.5mmol/l) level is an independent predictor and superior to AG (P<0.001) on predicting 7-day and 30-day mortality and combined end point events on predicting short-term prognosis in this patient cohort.

The following situations show the inhibiting role of U-74389G in gl production. Vlkolinský R et al exposed [10] rat hippocampal slices to reversible in vitro ischemia - hypoxia (HYP) combined with lowered D-glucose concentration to induce synaptic transmission (ST) failure, which turned out to be irreversible in approximately 80%-100% of slices during reoxygenation (ROX). The amplitude of population spikes (PoS) evoked trans-synaptically recorded was the parameter of ST. Pretreatment of slices with pyridoindole stobadine improved ST recovery after 20-min tissue ROX, decreased the number of irreversibly damaged slices, increased the average amplitude of PoS during tissue ROX, delayed the half-time of PoS decayed (t1/2) during HYP significantly. 21-aminosteroid U-74389G (10 microM) revealed more powerful protective activity on ST recovery and on t1/2 during HYP. Stobadine as well as U-74389G antioxidants with remarkably different chemical structures, exerted neuroprotective activity, probably determined by antioxidative properties of these compounds. Moreover, both were able to delay the early ST decay during HYP, which might indicate improved energetic state of neurons in the treated tissue. Astrocytes are the site of bioactivation of the parkinsonism-inducing agent 1-methyl-4-phenyl-1,2,3, 6-tetrahydropyridine (MPTP) into its toxic 1-methyl-4-phenylpyridinium (MPP(+)) metabolite which is capable of decreasing astrocytic glutamate uptake. Indeed, the spin trapping agent α-phenyl-tert-butyl nitrone, the lazaroid antioxidant U-74389G, was not capable of restoring glutamate net uptake by the extracellular space in astrocytes preincubated with MPP(+). The effect of MPP(+) on glutamate clearance: (i) was accompanied by a decrease in cellular ATP; (ii) could be enhanced by withdrawing glucose from the incubation medium or by inhibiting glycolysis and (iii) could be reproduced using the mitochondrial complex I inhibitor rotenone. Di Monte DA et al. [11] indicated that, by acting as a mitochondrial poison, MPP(+) impairs energy metabolism of astrocytes and significantly reduces their ability to maintain low levels of extracellular glutamate.

Conclusion

Conclusions are that U-74389G administration interacted or not with reperfusion time, have significant decreasing effects on gl levels. U-74389G is proved a clear hypoglycemic factor even in absolute insulin lack. The usefulness of this information must be investigated in clinical practice.

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Statement of Animal Rights

All procedures followed were in accordance with the ethical standards of the responsible committee on Helsinki Declaration of 1975, as revised in 2008.

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