THE EFFECT OF THE ANTIOXIDANT DRUG "U-74389G" ON CHLORIDE LEVELS DURING ISCHEMIA-REPERFUSION INJURY IN RATS

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ЕФЕКТЪТ НА АНТИОКСИДАНТА "U-74389G" ВЪРХУ ХЛОРИДНИТЕ НИВА ПО ВРЕМЕ НА ИСХЕМИЯ-РЕПЕРФУЗИЯ УВРЕДА ПРИ ПЛЪХОВЕ

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Summary:

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 (IR) protocol. The beneficial effect or non-effectiveness of that molecule was studied biochemically using mean blood chloride levels. Materials and methods: 40 rats of mean weight 231.875 g were used in the study. Chloride levels were measured 60 min (groups A and C) and 120 min (groups B and D) after reperfusion. The drug U-74389G was administered only in groups C and D. Results: U-74389G administration non-significantly decreased the chloride levels by 1 mmol/l (–2.074727 mmol/l ÷ –0.0747266 mmol/l, p = 0.0673). This finding was in accordance with the results of paired t-test (p = 0.1085). Reperfusion time non-significantly decreased the chloride levels by 0.9 mmol/l (–1.984218 mmol/l ÷ –0.1842178 mmol/l, p = 0.1011), also in accordance with paired t-test (p = 0.1105). However, U-74389G administration and reperfusion time together produced a significant combined effect in decreasing the chloride levels by 0.7818182 mmol/l (–1.408963 mmol/l ÷ –0.1546735 mmol/l, p = 0.0159). Conclusion: Results of this study indicate that U-74389G administration, reperfusion time and their interaction have a decreasing short–term effect on chloride levels within the context of a 2-hour experimental period. Either significant or not, this decrease has an immediate impact on serum osmotic pressure, blood pH and other physiological states.

Key words: U-74389G, chloride levels, reperfusion

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Резюме:

Целта на проучването е да се изследва ефектът на антиоксиданта „U-74389G" върху експериментален модел при плъхове, като се приложи протоколът „исхемия-реперфузия". Полезният ефект на проучваната молекула или липсата на такъв бяха изследвани биохимично чрез измерване на средната концентрация на хлоридите в кръвта. В проучването бяха използвани 40 плъха със средно тегло 231,875 г. Хлоридната концентрация беше определена на 60-ата (групи А и С) и на 120-ата минута (групи В и D) след реперфузията. Лекарственият продукт „U-74389G" беше приложен само в групи С и D. Приложението на „U-74389G" доведе до несигнификантен спад в нивото на хлоридите с 1 mmol/l (–2,074727 mmol/l ÷ –0,0747266

mmol/l, p = 0,0673). Този резултат съвпадна и с получения при статистическия анализ на данните с помощта на Т-тест за двойки извадки (p = 0,1085). Времето за тъканна реперфузия беше свързано с несигнификантно понижение на нивата на хлоридите с 0,9 mmol/l (–1,984218 mmol/l ÷ –0,1842178 mmol/l, p = 0,1011), в синхрон с резултатите от Т-теста за двойки извадки (p = 0,1105). При съчетаване на ефектите на U-74389G и времето на реперфузия се установи значимо понижаване на хлоридните нива с 0,7818182 mmol/l (–1.408963 mmol/l ÷ –0.1546735 mmol/l, p = 0,0159). Резултатите от проучването показват, че приложението на U-74389G, времето на реперфузия и тяхното комбинирано действие имат понижаващ ефект върху концентрацията на хлоридите в кръвта. Това понижение, значимо или не, оказва незабавно влияние върху серумното осмотично налягане, pH на кръвта и други физиологични състояния.

Ключови думи: U-74389G, хлоридни нива, реперфузия

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INTRODUCTION

Tissue 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 a lot of years. However, even if important progress has been made, satisfactory answers have not been given yet to fundamental questions, 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 (Pub Med – 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. Also, a lot of publications addressed trials of other similar molecules of aminosteroids (lazaroids) to which the studied molecule also belongs to. U-74389G, whose formal name is 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 iron-dependent lipid peroxidation. It protects against ischemia-reperfusion injury in animal heart, liver, and kidney models [1]. Also, this membrane-associating antioxidant is particularly effective in preventing both arachidonic acid-induced lipid peroxidation and permeability changes in brain microvessel endothelial cells monolayers [2].

The **aim** of this experimental study was to examine the effect of the antioxidant drug "U-74389G" on rat model and particularly in an intravascular IR protocol. The impacts of this ischemia on serum and interstitial fluid were studied. Trying a circulatory ischemia by clapping inferior aorta, it inevitably ended in tissue spaces and multiorgan ischemia. The beneficial effect or non-effectiveness of that molecule was studied by measuring blood chloride (Cl) levels.

MATERIALS AND METHODS

Animal preparation

This experimental study was laid out at the Experimental Research Center of ELPEN Pharmaceuticals Co. Inc. S.A. at Pikermi, Attiki, and all settings needed for the study including consumables, equipment and substances used, were a courtesy of that S. A. Wistar albino rats were used in accordance with accepted standards of humane animal care. They spent in laboratory 7 days before the experiment with easy access to water and food. The experiment was acute, that is, the animal usage was completed 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 and afterwards reperfusion for 60 min (group A); 2) Ischemia for 45 min and afterwards reperfusion for 120 min (group B); 3) Ischemia for 45 min and afterwards immediate U-74389G intravenous (IV) administration and reperfusion for 60 min (group C); 4) Ischemia for 45 min and afterwards immediate U-74389G IV administration and reperfusion for 120 min (group D).

The molecule U-74389G dose was 10 mg/Kg body weight of animals. The experiment started with prenarcosis and general anesthesia administration in animals. Their electrocardiogram and acidometry were continuously monitored. The vessels providing blood supply were prepared so as their flow to be excluded by forceps. After exclusion, the protocol of IR

was applied, described more in detail in the description of experimental groups. The molecules were administered at the time of reperfusion, through inferior vena cava, which has been catheterized in the beginning of the experiment, after general anesthesia establishment.

The Cl measurement was performed at 60 min of reperfusion (groups A and C) and at 120 min of reperfusion (groups B and D).

Rats underwent general anesthesia by initial intramuscular (IM) administration of 0.5 cc compound, which constituted of 0.25 cc xylazine, [25 cc, 20 mg/cc] and 0,25 cc ketamine hydrochloride [1000, 100 mg/cc, 10 cc]. Before initiation of laparotomy, 0.03 cc butorphanol [10 mg/cc, 10 cc] anesthesia was administered subcutaneously (s.c.). Continuous oxygen supply was administered during whole experiment performance. Ischemia was caused by clapping inferior aorta for 45 min, after laparotomic access was achieved. Reperfusion was induced by removing the clapping and reestablishment of inferior aorta patency. Forty (40) Wistar albino rats of mean weight 231.875 g [SD: 36.59703 g] were used, with min weight ≥ 165 g and max weight < 320 g.

Model of ischemia-reperfusion injury

Control groups

20 control rats of mean weight 252.5 g [SD: 39.31988 g] were subjected to ischemia for 45 min and then reperfusion was induced.

Group A

Reperfusion which lasted 60 min was induced in 10 control rats of mean weight 243 g [SD: 45.77724 g], mean Cl levels 103.4 mmol/l [SD: 2.1187 mmol/l] (Table 1).

Group B

Reperfusion which lasted 120 min was induced in 10 control rats of mean weight 262 g [SD: 31.10913 g], mean Cl levels 102.9 mmol/l [SD: 1.911951 mmol/l] (Table 1).

Lazaroid (L) group

20 rats of mean weight 211.25 g [SD: 17.53755 g] were subjected to ischemia for 45 min and then reperfusion in the beginning of which 10 mg/kg body weight U-74389G were IV administered.

Group C

Reperfusion which lasted 60 min was induced in 10 L rats of mean weight 212.5 g [SD: 17.83411 g], mean Cl levels 102.8 mmol/l [SD: 1.032796 mmol/l] (Table 1).

Group D

Reperfusion which lasted 120 min was induced in 10 L rats of mean weight 210 g [SD: 18.10463 g], mean Cl levels 101.5 mmol/l [SD: 1.269296 mmol/l] (Table 1).

Table 1: Weight and chloride (Cl) mean levels and SD of groups

Groups	*Variable*	*Mean*	*SD*
A	Weight	243 g	45.77724 g
	Cl	103.4 mmol/l	2.1187 mmol/l
B	Weight	262 g	31.10913 g
	Cl	102.9 mmol/l	1.911951 mmol/l
C	Weight	212.5 g	17.83411 g
	Cl	102.8 mmol/l	1.032796 mmol/l
D	Weight	210 g	18.10463 g
	Cl	101.5 mmol/l	1.269296 mmol/l

Abbr: SD – standard deviation

STATISTICAL ANALYSES

At baseline all rats from the investigated and control groups were individually measured and weight-matched group by group. Comparison between groups was performed using paired t-tests (Table 2). Any emerging significant difference among Cl levels was investigated whether owed to the above mentioned significant weight correlations. Additionally, Cl level comparison between rat groups was also performed at baseline, applying statistical paired t-tests (Table 2). Generalized linear models (glm) were applied with dependant variable being the chloride levels and independent variables being the absence or presence of U-74389G administration, the reperfusion time and their interaction. Inserting the rats' weight also as an independent variable at generalized linear model analysis, a non-significant relation to chloride levels was found (p = 0.4699), therefore further investigation was not needed regarding the influence of rats' weight into the study.

Table 2: Statistical significance of mean values difference for groups (DG) after statistical paired t test application

DG	*Variable*	*Difference*	*p-value*
A-B	Weight	-19 g	0.2423
	Cl	0.5 mmol/l	0.6262
A-C	Weight	30.5 g	0.0674
	Cl	0.6 mmol/l	0.4754
A-D	Weight	33 g	0.0574
	Cl	1.9 mmol/l	0.0101
B-C	Weight	49.5 g	0.0019
	Cl	0.1 mmol/l	0.8793
B-D	Weight	52 g	0.0004
	Cl	1.4 mmol/l	0.1530
C-D	Weight	2.5 g	0.7043
	Cl	1.3 mmol/l	0.0176

RESULTS

U-74389G administration non-significantly decreased the chloride levels by 1 mmol/l (–2.074727 mmol/l ÷ –0.0747266 mmol/l, p = 0.0673). This finding was in accordance with the results of paired t-

test (p = 0.1085). Reperfusion time non-significantly decreased the chloride levels by 0.9 mmol/l (–1.984218 mmol/l ÷ –0.1842178 mmol/l, p = 0.1011), also in accordance with paired t-test (p = 0.1105). However, U-74389G administration and reperfusion time together significantly decreased the chloride levels by 0.7818182 mmol/l (–1.408963 mmol/l ÷ –0.1546735 mmol/l, p = 0.0159). Reviewing the above and table 2, table 3 sums up the results concerning the influence of U-74389G in decreasing the chloride levels in connection with reperfusion time.

Table 3: The decreasing influence of U-74389G in connection with reperfusion time

Decrease	*95% c. in*	*p-values*		
		Reperfusion time	*t-test*	*glm*
0.6 mmol/l	–2.165935 mmol/l – 0.9659348 mmol/l	1 h	0.4754	0.4313
1 mmol/l	–2.074727 mmol/l – 0.0747266 mmol/l	1.5 h	0.1085	0.0673
1.4 mmol/l	–2.924677 mmol/l – 0.124677 mmol/l	2 h	0.1530	0.0696

Abbr. glm – generalized linear models

DISCUSSION

Unpleasantly, there are no described studies in the available literature on whether ischemia can influence Cl levels. On the contrary, there are a lot of case reports on how Cl levels fluctuations affect the function of various organs. Such example was described herein. Since isolated Cl administration is impossible, it was meant that Cl was associated with another drug or a factor which influences the Cl levels when being administered. Frequently, the change in Cl levels is associated with relevant changes in sodium levels. Sodium is the main electrolyte found in extracellular fluid and is involved in fluid balance and blood pressure control. All known higher life forms require a subtle and complex electrolyte balance between the intracellular and extracellular environment. In particular, the maintenance of precise osmotic gradients of electrolytes is of major importance. Such gradients affect and regulate the hydration of the body as well as blood pH. However, when sodium chloride (NaCl) is placed in serum, the salt dissolves into its component ions, due to thermodynamic interactions between serum and solute molecules according to dissociation reaction salvation.

$$NaCl_{(s)} \rightarrow Na^{+}_{(aq)} + Cl^{-}_{(aq)}$$

Thus, the vast content of chloride in serum is as independent chloride ion $Cl^{-}_{(aq)}$, and not as salt. For this reason, an individual and autonomous consideration of chloride regardless of sodium should not be considered incomplete. Le LL et al [3] achieved brain IR injury three weeks after ischemic preconditioning (IPC) in rats. 2,3,5-triphenyltetrazolium chloride staining showed IPC significantly reduced brain infarct area and improved neurological function for 3 weeks after cerebral injury. Rehni AK et al [4] found beneficial effects of IPC on global cerebral IR-induced injury and behavioral deficits in mice. Cerebral infarct size was measured by using triphenyltetrazolium chloride staining. Hinkel R et al [5] increased the 24 hours survival of neonatal pig cardiomyocytes by 2.8-fold after control eEPCs ($5x10^6$ cells) in vivo application into coronary IR. Segmental endocardial shortening in the infarct zone and infarct size were determined by triphenyl tetrazolium chloride viability. Murlasits Z et al [6] resulted in cardioprotection as evidenced by reduced infarct size ($p<0.05$) in the exercise-trained male Sprague-Dawley rats followed by bouts (70% of O_2max). Infarct area was determined by a histological (triphenyltetrazolium chloride (TTC) method. Mozaffari MS et al [7] worsened markedly contractile function following IR injury in male rats but infarct size was reduced by high 5 weeks salt regimen (1% NaCl solution) relative to control groups. Birnbaum Y et al [8] assessed infarct size by triphenyltetrazolium chloride reduced by 39.4% in a placebo group (p = 0.503) in male Sprague-Dawley rats. Kadambi A et al [9] observed elevated XO in rat muscles subjected to IR in combination with 0.9% saline than nonischemic controls.

Heim C et al [10] impaired by IR brain the median adult rats' learning ability, injecting either 0.3 µg or 0.06 µg intrastriatally ferric chloride ($FeCl_3$), one week after a 60-minute oligemic episode. 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 is applied alone one week after the oligemic episode on its own, also impairs the animals' learning ability. Moore RM et al [11] subjected horses to 3-hour reduced by 20% of baseline colonic arterial blood flow and, afterwards, to 3 hours of reperfusion. One of 3 drug solutions was administered IV 30 minutes

prior to colonic reperfusion: group 1, 0.9% NaCl; group 2, 21-aminosteroid U-74389G: 10 mg/kg; and group 3, manganese chloride ($MnCl_2$): 10 mg/kg. Heart rate, mean arterial pressure only for 30 min, and cardiac output increased with $MnCl_2$ infusion. There were significant increases in mean pulmonary artery and mean right atrial pressures at least for 3 hours in all groups. Colonic arterial resistance decreased during IR in all groups.

CONCLUSION

U-74389G administration, reperfusion time and their interaction exhibit a decreasing, either significant or not, short–term effects on chloride levels within the time context of 2 hours. This decrease has an immediate impact on serum osmotic pressure, blood pH and other physiological states.

Acknowledgment: This study was supported by a Grant of the Experimental Research Center ELPEN Pharmaceuticals (E.R.C.E), Athens, Greece. The research facilities for this project were provided by the aforementioned institution.

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Постъпила за печат на 11 януари 2014 г.