The effect of the antioxidant drug "U-74389G" on serum calcium during ischemia reperfusion injury in rats.

Tsompos C[1], Panoulis C[2], Toutouzas K[3], Zografos G[4], Papalois A[5]

Abstract

The aim of this experiment was to study the effects of U-74389G on rat model, particularly in ischemia reperfusion (IR) protocol. The beneficial or other effects of that molecule were studied estimating the mean blood calcium level.

Material and methods: Forty rats were used. Mean weight 231.875 g. Serum calcium was measured at these time points: 60 min after reperfusion (groups A and C), and 120 min after reperfusion (groups B and D). Groups A and B without the drug and C and D with U-74389G administration.

Results: 1) U-74389G administration non significantly decreased the calcium levels by 0.015 mg/dl [-0.2473879 mg/dl - 0.2173882 mg/dl] (P= 0.8967), in accordance also with paired t-test (P= 0.8598). 2) Reperfusion time non significantly increased the calcium levels by 0.145 mg/dl [-0.0825105 mg/dl - 0.3725103 mg/dl] (P= 0.2048), in accordance also with paired t-test (P= 0.1379). 3) Interaction of U-74389G administration and reperfusion time non significantly increased the calcium levels by 0.0154546 mg/dl [-0.1246202 mg/dl - 0.1555294 mg/dl] (P= 0.8245).

Conclusion: U-74389G administration, reperfusion time and their interaction have no significant alternation of calcium levels within a time period of 2 hours. Perhaps, longer experiment times may reveal any possible significant effect of U-74389G on blood calcium.

Key words: *U-74389G, calcium, reperfusion*

Introduction

Tissue ischemia and reperfusion (IR) remain one of the main causes of permanent or transient damage with serious implications on patients' health. The use of antioxidant substances has been a subject of research for a long time. However despite important progress made, satisfactory answers have not been obtained yet to the fundamental questions: How "powerful" should an antioxidant be, when should it be administered, and in what dosage. The particularly satisfactory action of the antioxidant, U-74389G, in tissue protection was noted in various experiments. It was noted that this antioxidant has been used in IR experiments. Review of international literature reveals just a few reports. A lot of publications are present on trials of similar other molecules of aminosteroids (lazaroids) supprimer. U-74389G belongs to this group.

1. Constantinos Tsompos: Consultant A, Department of Obstetrics & Gynecology, Mesologi County Hospital, Etoloakarnania, Greece
2. Constantinos Panoulis: Assistant Professor, Department of Obstetrics & Gynecology, Aretaieion Hospital, Athens University, Attiki, Greece
3. Konstantinos Toutouzas: Assistant Professor, Department of Surgery, Ippokrateion General Hospital, Athens University, Attiki, Greece
4. George Zografos: Professor, Department of Surgery, Ippokrateion General Hospital, Athens University, Attiki, Greece
5. Apostolos Papalois: Director, Experimental Research Center ELPEN Pharmaceuticals, S.A. Inc., Co.

Aim of the experiment

The effect of U-74389G on serum calcium levels in rats animal models in IR protocol.

Experimental groups

This experimental study was carried out by Exprerimental Research Center of ELPEN Pharmaceuticals Co. Inc. S.A. at Pikermi, Attiki, and all consumables, equipment and substances used, were provided by them. Wistar rats were used in accordance with accepted standards of humane animal care. They spent 7 days in laboratory before experimentation with easy access to water and food. They were randomly assigned into the following experimental groups (10 animals in each group).

Group A: Ischemia for 45 min followed by reperfusion for 60 min.

Group B: Ischemia for 45 min followed by reperfusion for 120 min.

Group C: Ischemia for 45 min followed immediately by U-74389G intravenous (IV) administration and reperfusion for 60 min.

Group D: Ischemia for 45 min followed immediately by U-74389G IV administration and reperfusion for 120 min.

The molecule U-74389G was administered in a dose of: 10 mg/Kg body weight of the animal.

The experiment started with prenarcosis and general anesthesia administration to the animals. Their electrocardiogram and acidometry were continuously monitored. The inferior vena cava was prepared so as its blood flow could be excluded by forceps. After exclusion, the protocol of IR was applied (described in detail below). The molecules were administered at the time of reperfusion, through inferior vena cava catheterization, which had been carried out after general anesthesia.

The serum calcium measurements were performed at these time points:

1. after 60 min of reperfusion (groups A and C),

2. after 120 min of reperfusion (groups B and D).

Protocol of the experiment

The experimental rats were given general anesthesia by intramuscular (IM) administration of 0.5 cc of a compound, constituting 0.25 cc xylazine, [25 cc, 20mg/cc] and 0.25 cc ketamine hydrochloride [1000, 100mg/cc, 10cc]. Butorphanol [10mg/cc, 10cc] anesthetic agent was administered subcutaneous (SC) before laparotomy. Continuous oxygen supply was administered during the whole experiment. Ischemia was caused by clamping inferior aorta for 45 min after laparotomic access. Reperfusion was achieved by removing the clamp and inferior aorta patency re-establishment.

Forty Wistar rats of mean weight 231.875 g [SD: 36.59703 g] were used, min weight >165 g and max weight < 320 g.

Control groups:

Twenty control rats, mean weight 252.5 g [SD: 39.31988 g] were subjected to ischemia for 45 min followed by reperfusion.

Group A: Ten controls rats of mean weight 243 g [SD: 45.77724 g], mean calcium levels 10.53 mg/dl [SD: 0.3465705 mg/dl] were subjected to 60 min reperfusion (Table 1).

Group B: Ten controls rats of mean weight 262 g [SD: 31.10913 g], mean calcium levels 10.69 mg/dl [SD: 0.3984694 mg/dl] were subjected to 120 min reperfusion (Table 1).

Lazaroid (L) group

Table 1: Weight and calcium mean levels and SD. of groups

Groups	Variable	Mean	SD
Á	Weight	243 g	45.77724 g
	Calcium	10.53 mg/dl	0.3465705 mg/dl
B	Weight	262 g	31.10913 g
	Calcium	10.69 mg/dl	0.3984694 mg/dl
C	Weight	212.5 g	17.83411 g
	Calcium	10.53 mg/dl	0.4191261 mg/dl
D	Weight	210 g	18.10463 g
	Calcium	10.66 mg/dl	0.2796822 mg/dl

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

Group C: Ten L rats of mean weight 212.5 g [SD: 17.83411 g], mean calcium levels 10.53 mg/dl [SD: 0.4191261 mg/dl] were subjected to 60 min reperfusion (Table 1).

Group D: Ten L rats of mean weight 210 g [SD: 18.10463 g], mean calcium levels 10.66 mg/dl [SD: 0.2796822 mg/dl] were subjected to 120 min reperfusion (Table 1).

Every weight rat group was initially compared with other ones applying statistical paired t-test. (Table 2). Any emerging significant difference among calcium groups, will be investigated whether owed in the above mentioned probable significant weight correlations. Also, every calcium rat group was compared with other ones applying statistical paired t-test. (Table 2). Applying generalised linear models (glm) with dependant variable the calcium levels and independent variables the U-74389G administration or no, the reperfusion time and their interaction, results in: 1) U-74389G administration non significantly decreased the calcium levels by 0.015 mg/dl [-0.2473879 mg/dl - 0.2173882 mg/dl] (P= 0.8967), in accordance also with paired t-test (P= 0.8598). 2) Reperfusion time non significantly increased the calcium levels by 0.145 mg/dl [-0.0825105 mg/dl - 0.3725103 mg/dl] (P= 0.2048), in accordance also with paired t-test (P=

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

DG	Variable	Difference	p-value
Á-Â	Weight	-19 g	0.2423
	Calcium	-0.16 mg/dl	0.1999
Á-C	Weight	30.5 g	0.0674
	Calcium	0 mg/dl	1.0000
Á-D	Weight	33 g	0.0574
	Calcium	-0.13 mg/dl	0.3523
Â-C	Weight	49.5 g	0.0019
	Calcium	0.16 mg/d	0.0911
Â-D	Weight	52 g	0.0004
	Calcium	0.03 mg/dl	0.8508
C-D	Weight	2.5 g	0.7043
	Calcium	-0.13 mg/dl	0.4190

0.1379). 3) Interaction of U-74389G administration and reperfusion time non significantly increased the calcium levels by 0.0154546 mg/dl [-0.1246202 mg/dl - 0.1555294 mg/dl] (P= 0.8245). Reviewing the above and Table 2, the Table 3 sums up concerning the decreasing influence of U-74389G in connection with reperfusion time. Inserting the rats weight also as an independent variable at glm analysis, a non significant relation results in (p= 0.0501), so as to further investigation is not needed.

Discussion

Unpleasantly, situations concerning whether ischemia can influence the calcium levels are not described in bibliography. On the contrary, there are a lot of cases reporting how the calcium levels fluctuations affect the function of various organs. Such examples are described herein. Isolated calcium administration is impossible. Calcium administration is ever associated by another drug or a factor influencing the calcium levels. Assayag M et al found[1] mitochondrial Ca^{++} and function significantly lower after hypoxia/reoxygenation protocol in isolated hearts mitochondria than control ones. Gonçalves ES et al did not noted increased[2] thiobarbituric acid reactive substances levels significantly in IR small intestine and its mesentery samples in calcium carbonate (Caca)-treated rats. Yamagishi T et al noted[3] 1.33-fold higher post IR coronary flow rate in 70% reduced food fed rats group than ad-libitum ones. Pollesello P et al improved[4] acute heart failure by calcium sensitization of contractile proteins. Hale SL et al noted that decreased[5] intracellular calcium overload reduces the frequency of angina attacks and myocardial stunning ten minutes before coronary IR in rabbits. Strömer H et al associated[6] 1.6-fold intracellular Ca^{++} overload increase with left ventricular ischemia during reperfusion ($p<0.05$) in male Wistar rats. Nordlander M et al found[7] that vasoselective calcium antagonists that inhibit calcium channels, protect against IR injuries, reducing infarcts size by 40% in pigs. Riess ML et al preserved[8] cardiac function and tissue viability on reperfusion both by increasing mitochondrial Ca^{++} by cold perfusion and by preventing of its deleterious increases.

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

Decrease	95% c. in.	Reperfusion time	t-test	p-values	
				glm	
0 mg/dl	-0.3613204 mg/dl	- 0.3613208 mg/dl	1h	1.0000	1.0000
0.015 mg/dl	-0.2473879 mg/dl	- 0.2173882 mg/dl	1.5h	0.8598	0.8967
0.03 mg/dl	-0.3534328 md/dl	- 0.2934329 mg/dl	2h	0.8508	0.8477

Pang Y et al termed[9] capacitative Ca^{++} entry (CCE) the influx of extracellular Ca^{++} through non voltage-gated Ca^{++} channels. The inhibition of CCE thus explains the heart protection afforded to IR injury by limiting Ca^{++} overload. Volpe JJ et al found[10] that the perinatal accumulation of cytosolic calcium activates a variety of calcium-mediated deleterious events in fetal brain. The application of free radical production and scavengers inhibitors in human term infant is suggested. Ivanics T et al showed[11] that intracellular Ca^{++} levels are significantly increased during rat IR skeletal muscle without altering Ca^{++} homeostasis. Herzog WR et al associated[12] significantly diminished infarct size by 3.2-fold, treating myocardial IR swines by calcium-channel blockers (P=0.01). Piana RN et al improved the dyskinetic post IR heart region only minimally at the end of reperfusion

by 1 ± 2%, for the saline group ($P < .05$), whereas, arteriolar endothelium-dependent responses calcium ionophore A23187 ($P < .01$) were impaired, in pigs.

Also, calcium is perhaps influenced by U-74389G. Alhan E et al investigated[14] the influence of U-74389G on acute necrotizing pancreatitis (ANP) resulting in a significant decrease in serum calcium levels which may treat lung injury in rats. Falcone G et al indicated[15] that oxygen free radicals and the subsequent increase in intracellular calcium are only steps of doxorubicin (DXR) progressive general cardiotoxicity in rats. They propose that the 21-aminosteroids, potent inhibitors of membrane lipid peroxidation, alone delay but are not enough to protect from DXR cardiotoxic effects and mortality. Passaquin AC et al have implicated[16] increased cytosolic calcium concentrations as one of the pathological events responsible for the degeneration of dystrophic skeletal muscles on the degenerative course of Duchenne muscular dystrophy (DMD). The glucocorticoid-derived lazaroid U-74389G also elicited a decrease in Ca^{++} influx. The reduction in Ca^{++} influx was suspected to be triggered via an antioxidant mechanism. The beneficial effect of glucocorticoids in DMD could be attributed to a reduction of the pathological increase in Ca2+ influx via an effect on the sarcolemma. Corasaniti MT et al prevented either excitotoxic damage or death of rodent neuronal and human neuroblastoma cells by the HIV-1 envelope protein gp120 engenders[17] Ca^{++} mediated, by preincubating cell cultures with the 21-aminosteroid U-74389G. Vollrath B et al found that the lazaroid compound U-74389G was effective in preventing the effects of oxyhemoglobin and free radicals in canine models tested, although all effects were smaller with vessels in spasm. They resulted that the actions of hemoglobin on vascular smooth muscle are mediated by the formation of free radicals which subsequently affect intracellular calcium concentrations.

Conclusion

U-74389G administration, reperfusion time and their interaction have no significant of calcium levels within a time period of 2 hours. Perhaps, longer experiment times may reveal any possible significant effect of U-74389G on blood calcium.

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