ORIGINAL ARTICLE

Comparison of the Hypocalcemic Effects of Erythropoietin and U-74389G

Constantinos Tsompos[1], Constantinos Panoulis[2], Konstantinos Toutouzas[3], Aggeliki Triantafyllou[4], C. George Zografos[3], Kalliopi Tsarea[5], Maria Karamperi[5], Apostolos Papalois[6]

[1]Department of Gynecology, General Hospital of Thessaloniki "St. Dimitrios" Thessaloniki, Hellas, [2]Department of Obstetrics and Gynecology, Aretaieion Hospital, Athens University, Athens, Attiki, Hellas, [3]Department of Surgery, Ippokrateion General Hospital, Athens University, Athens, Attiki, Hellas, [4]Department of Biologic Chemistry, Athens University, Athens, Attiki, Hellas, [5]Experimental Research Centre ELPEN Pharmaceuticals, S.A. Inc., Co., Pikermi, Attiki, Hellas, [6]Experimental, Educational and Research Center ELPEN, School of Medicine, European University Cyprus, Engomi, Cyprus

ABSTRACT

Aim: This study calculated the effects on serum calcium (Ca) levels, after treatment with either of two drugs: The erythropoietin (Epo) and the antioxidant lazaroid (L) drug U-74389G. The calculation was based on the results of two preliminary studies, each one of which estimated the certain influence, after the respective drug usage in an induced ischemia-reperfusion animal experiment. **Materials and Methods:** The two main experimental endpoints at which the serum Ca levels were evaluated were the 60th reperfusion min (for the Groups A, C, and E) and the 120th reperfusion min (for the Groups B, D, and F). Especially, the Groups A and B were processed without drugs, Groups C and D after Epo administration, whereas Groups E and F after the L administration. **Results:** The first preliminary study of Epo presented a non-significant hypocalcemic effect by 0.34% ± 0.68% (P = 0.6095). However, the second preliminary study of U-74389G presented a non-significant hypercalcemic effect by 0.14% ± 0.66% (P = 0.8245). These two studies were coevaluated since they came from the same experimental setting. The outcome of the coevaluation was that L is 2.3623042-fold (2.3482723–2.3764196) more hypercalcemic than Epo (P = 0.0000). **Conclusions:** The antioxidant capacities of U-74389G ascribe 2.3623042-fold more hypercalcemic effects than Epo (P = 0.0000).

Key words: Erythropoietin, ischemia, reperfusion, serum calcium levels, U-74389G

INTRODUCTION

The lazaroid U-74389G (L) maybe not famous for its hypercalcemic[1] capacity (P = 0.8245). U-74389G, as a novel antioxidant factor, implicates exactly only 261 published studies. The ischemia-reperfusion (IR) type of experiments was noted in 19.15% of these studies. A tissue-protective feature of U-74389G was obvious in these IR studies. The U-74389G chemically 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 complex, which prevents the lipid peroxidation either iron-dependent or arachidonic acid-induced one. Animal kidney, liver, brain microvascular endothelial cells monolayers, and heart models were protected by U-74389G after IR injury. U-74389G also attenuates the leukocytes; downregulates the pro-inflammatory gene; treats the endotoxin shock; produces cytokine; enhances

Address for correspondence:
Constantinos Tsompos, Department of Gynecology, General Hospital of Thessaloniki "St. Dimitrios," 2 Elenis Zografou Street, Thessaloniki 54634, Hellas. Tel.: 00302313322171/00306946674264. Fax.: 00302106811215.
E-mail: tsomposconstantinos@gmail.com

the mononuclear immunity; protects the endothelium; and presents antishock property.

Erythropoietin (Epo), even if it is not famous for its hypocalcemic[2] action (P = 0.6095), it can be used as a reference drug for comparison with U-74389G. Although Epo is met in over 31,236 published biomedical studies, only 3.66% of them negotiate the known type of IR experiments. Nevertheless, Epo as a cytokine, it is worth of being studied about its effects on serum calcium (Ca) levels too. This experimental work tried to compare the effects of the above drugs on a rat induced IR protocol. They were tested by calculating the serum Ca levels alterations.

MATERIALS AND METHODS

Animal preparation

The Vet licenses under 3693/12-11-2010 and 14/10-1-2012 numbers, the granting company and the experiment location are mentioned in preliminary references.[1,2] The human animal care of albino female Wistar rats, the 7 days pre-experimental *ad libitum* diet, the non-stop intra-experimental anesthesiologic techniques, the acidometry, the electrocardiogram, the oxygen supply, and post-experimental euthanasia are also described in preliminary references. Rats were 16–18 weeks old. They were randomly assigned to six groups consisted of n = 10. The stage of 45 min hypoxia was common for all six groups. Afterward, reperfusion of 60 min was followed in Group A; reperfusion of 120 min in Group B; immediate Epo intravenous (IV) administration and reperfusion of 60 min in Group C; immediate Epo IV administration and reperfusion of 120 min in Group D; immediate U-74389G IV administration and reperfusion of 60 min in Group E; and immediate U-74389G IV administration and reperfusion of 120 min in Group F. The dose height assessment for both drugs is described at preliminary studies as 10 mg/kg body mass.

Ischemia was caused by laparotomic clamping the inferior aorta over renal arteries with forceps for 45 min. The clamp removal was restoring the inferior aorta patency and reperfusion. After exclusion of the blood flow, the protocol of IR was applied, as described above for each experimental group. The drugs were administered at the time of reperfusion; through inferior vena cava catheter. The Ca levels were determined at 60th min of reperfusion (for A, C, and E groups) and at 120th min of reperfusion (for B, D, and F groups). Along, nonrelation was raised between Ca values with animals' mass (P = 0.1689).

Statistical analysis

Table 1 presents the (%) hypocalcemic influence of Epo regarding reoxygenation time. Furthermore, Table 2 presents the (%) hypercalcemic influence of U-74389G regarding reperfusion time. Chi-square tests were applied using the ratios which produced the (%) results per endpoint. The outcomes of Chi-square tests are depicted in Table 3.

Table 1: The (%) hypocalcemic influence of erythropoietin in connection with reperfusion time

Hypocalcemia±SD (%)	Reperfusion time	*P*-value
+0.28±3.66	1 h	0.8065
−0.56±4.05	1.5 h	0.5761
−1.41±4.46	2 h	0.4100
+0.65±4.31	Reperfusion	0.5281
−0.34±0.68	Interaction	0.6095

Table 2: The (%) hypocalcemic influence of U-74389G in connection with reperfusion time

Hypocalcemia±SD (%)	Reperfusion time	*P*-value
+0.00±2.23	1 h	1.0000
−0.14±3.50	1.5 h	0.8782
−0.28±4.59	2 h	0.8492
+1.35±3.92	Reperfusion	0.1713
+0.14±0.66	Interaction	0.8245

RESULTS

The successive application of Chi-square tests revealed that U-74389G caused hypercalcemia by 0.00000334-fold (0–0.00001891) less than Epo at 1 h (P = 0.0000), hypocalcemia by 0.2490068-fold (0.2476003–0.2504212) less than Epo at 1.5 h (P = 0.0000), hypocalcemia by 0.1988753-fold (0.1980955–0.1996583) less than Epo at 2 h (P = 0.0000), and hypercalcemia by 2.063208-fold (2.057305–2.069128) more than Epo (P = 0.0000) without drugs and hypercalcemia by 2.3623042-fold (2.3482723–2.3764196) more than Epo whether all variables have been considered (P = 0.0000).

DISCUSSION

The unique available study investigating the hypercalcemic effect of U-74389G was the preliminary one.[1] Although the most famous activities of neuroprotection and membrane-stabilization properties, it accumulates in the cell membrane, protecting vascular endothelium from peroxidative damage but hardly penetrates the blood-brain barrier. It elicits a beneficial effect in ototoxicity and Duchenne muscular dystrophy. It increases γGT, superoxide dismutase (SOD), and glutathione (GSH) levels in oxygen-exposed cells. It treats septic states and acts as immunosuppressant in flap survival. It prevents the learning impairments; it delays the early synaptic transmission decay during hypoxia improving

Table 3: The U-74389G/erythropoietin hypocalcemic efficacies after Chi-square tests application

Odds ratio	(95% Confidence interval)		Endpoint	*P* value
0.00000334	0	0.00001891	1 h	0.0000
0.2490068	0.2476003	0.2504212	1.5 h	0.0000
0.1988753	0.1980955	0.1996583	2 h	0.0000
2.063208	2.057305	2.069128	Reperfusion	0.0000
2.3623042	2.3482723	2.3764196	Interaction	0.0000

energetic state of neurons. It shows antiproliferative properties on brain cancer cells and is considered as a new promising anti-inflammatory drug for the treatment of reperfusion syndrome in IR injuries.

The same authors confirmed[2] the short-term hypocalcemic effect of Epo preparations in non-iron-deficient individuals. Wei *et al*. led to reduced arrhythmia events and apoptosis rates since the enhanced[3] L-type Ca^{2+} current in human-induced pluripotent stem cell-derived cardiomyocytes (CMs) after IR injury also significantly decreased by Danshen. Fazal *et al.* demonstrated[4] that mitochondrial exchange protein directly activated by cAMP produced within mitochondria (1Epac1) favors Ca^{2+} exchange between the endoplasmic reticulum and the mitochondrion, by increasing interaction with a macromolecular complex, leading to mitochondrial Ca^{2+} overload and opening of the mitochondrial permeability transition pore (mPTP) and inhibiting isocitrate dehydrogenase two through the mitochondrial recruitment of Ca^{2+}/calmodulin-dependent protein kinase II in CMs. Mofid *et al.* remarked that CM-specific transgenic mice overexpress S100A6, a member of the family of EF-hand Ca^{2+} - binding proteins, which improved[5] Ca transients and protected against apoptosis induced by H/R through enhanced calcineurin activity after acute myocardial infarction. Hu *et al*. demonstrated[6] that liraglutide significantly lowered Ca^{2+} overload, improved Ca transient compared with H/R group, directly protected CMs against reperfusion injury, possibly through modulation of intracellular Ca homeostasis in H9c2 cells in patients with acute myocardial infarction undergoing percutaneous coronary intervention. Guan *et al*. concluded[7] that the intracellular Ca^{2+} release was markedly decreased associated with the decrease of nuclear translocation of NFATc4 and inhibition of extracellular signal-regulated kinase (ERK)/AKT phosphorylation; thus, CD38 inhibited SIRT3 expression and activation of Ca^{2+} – NFAT signaling pathway in CD38 knockout mice H9c2 cells. Oropeza-Almazán *et al*. considered[8] intracellular Ca^{2+} mishandling as an underlying mechanism in H/R injury that results in mitochondrial dysfunction and CMs death. These events are mediated by mitochondrial Ca^{2+} (mCa^{2+}) overload that is facilitated by the mitochondrial Ca uniporter (MCU) channel. First, CMs treated with siRNA demonstrated a reduction of mitochondrial Ca^{2+} transport. siRNA treated CMs showed decreased mitochondrial permeability pore opening and oxidative stress trigger by Ca^{2+} overload. Kapelko *et al*. prevented[9] hypoxia-induced elevation of diastolic Ca^{2+} level and eliminated Ca^{2+} transport alterations manifested by slow Ca^{2+} removal from the sarcoplasm and delay in CMs relaxation mainly due to preservation of Ca^{2+} transport after Oxacom administration in isolated rat hearts. Tahrir *et al*. showed.[10] that the presence of Tat impairs the uptake of mitochondrial Ca^{2+} ($[Ca^{2+}]_m$) and the electrophysiological activity resulting in dysregulation of CMs health and homeostasis in neonatal rat ventricular CMs (NRVMs). He *et al*. identified[11] canonical transient receptor potential channels (TRPCs) three and six as the cation channels through which most of the damaging Ca enters cells to trigger their death, consistent with activation of a positive-feedback loop in which Ca entering through TRPCs activates calcineurin-mediated NFATc3-directed transcription of TRPC genes, leading to more Ca^{2+} entry *in vitro* in H9c2 CMs. Wu *et al*. induced[12] Ca^{2+} influx in CMs, with subsequent reactive oxygen species (ROS) release, depolarizing of $\Delta\psi m$, opening mPTP, inducing injury and transient receptor potential vanilloid 4 (TRPV4) has key roles during IR through these pathways after activation of TRPV4 in H9c2 cells and NRVMs *in vitro*. Consolini *et al*. associated that free Ca concentration ($[Ca^{2+}]m$) mainly depends[13] on mitochondrial entrance through the uniporter (UCam) and extrusion in exchange with Na^+ (mNCX) driven by the electrochemical gradient ($\Delta\Psi m$). Contrarily, relaxation was slowed by cardioplegia (high K-low Ca Krebs) and by inhibition of UCam. Thus, Mit regulates the cytosolic $[Ca^{2+}]$ and SR Ca^{2+} content in I/R rat hearts. Panel *et al.* treated[14] cells either with Ca^{2+} ionophores (A23187, ionomycin, and ETH129) or subjected to hypoxia followed by reoxygenation. Thus, Ca^{2+} ionophores are not suitable to induce CypD-dependent mPTP opening in adult isolated murine CMs. Qiu *et al*. reversed[15] the inhibitory effect of sasanquasaponin (SQS) on H/Rinduced elevation of $[Cl]_i$, Ca^{2+} overload and generation of ROS, and eliminated SQSinduced cardioprotection after both inhibitions of PKCε by εV12 and S67A mutation of AE3 in H9c2 cells. Luo *et al*. prevented arrhythmias since hypoxia increased[16] I_{NaL}, $I_{NCX,}$ and diastolic intracellular $[Ca^{2+}]_i$ through the stimulated reverse Na^{+y}-Ca^{2+} exchange (NCX) and decreased amplitude of $[Ca^{2+}]_i$ transients in ventricular myocytes through inhibiting I_{NaL} and I_{CaL}. Maiolino *et al*. demonstrated[17] that the Na^+/Ca^{2+} exchanger 1 (NCX1) provides functional support for both glutamate uptake and use for ATP synthesis. Mohamed *et al*. observed[18] that ursodeoxycholic acid (UDCA) activates cell

Table 4: A U-74389G/erythropoietin efficacies ratios meta-analysis on 27 hematologic variables (23 variables with balancing efficacies and four variables with opposite efficacies)[52,53]

Endpoint variable	1 h	*P*-value	1.5 h	*P*-value	2 h	*P*-value	Reperfusion time	*P*-value	Interaction	*P*-value
WBC	0.957451	0.3782	1.396122	0.0000	1.918237	0.0000	1.71622	0.0000	1.601887	0.0000
RBC count	0.961059	0.0000	1.733395	0.0000	6.519657	0.0000	1.039524	0.0000	1.309673	0.0000
Hematocrit	38.424	0.0000	9.076658	0.0000	6.222898	0.0000	1.001356	0.2184	12.66419	0.0000
Hemoglobin	1.268689	0.0000	1.839035	0.0000	13.1658	0.0000	1.252422	0.0000	1.94889	0.0000
MCH	151.125	0.0000	4.246814	0.0000	2.709729	0.0000	1.177347	0.0000	4.362893	0.0000
MCV	150.8518	0.0000	4.236722	0.0000	2.704247	0.0000	1.180156	0.0000	4.352528	0.0000
RbcDW	3.306773	0.0000	3.023389	0.0000	2.655885	0.0000	0.2259914	0.0000	2.370353	0.0000
Platelet count	2.42839	0.0000	6.00238	0.0000	6.1333429	0.0000	3.939027	0.0000	37.62979	0.0000
MPV	145.8532	0.0000	4.053619	0.0000	2.603947	0.0000	1.2334644	0.0000	4.164431	0.0000
Platelet DW	0.6940233	0.0000	1.319118	0.0000	2.206972	0.0000	2.2484006	0.0000	2.458888	0.0000
Glucose	156.4991	0.0000	4.53659	0.0000	2.81397	0.0000	0.9073196	0.0000	4.660603	0.0000
Urea	158.4209	0.0000	4.50889	0.0000	2.850291	0.0000	0.9017775	0.0000	4.632148	0.0000
Creatinine	168.9034	0.0000	4.872332	0.0000	3.039572	0.0000	1.0262016	0.0000	5.005523	0.0000
Total proteins	155.9562	0.0000	4.421079	0.0000	2.803573	0.0000	0.8842162	0.0000	4.541934	0.0000
Albumins	0.2457507	0.0073	0.5303472	0.0000	0.6243052	0.0465	1.237477	0.0000	0.5000416	0.0000
AST	1.149264	0.0391	0.9347365	0.0000	0.6695775	0.0000	0.7631082	0.0000	0.8224656	0.0000
ALP	134.0033	0.0000	3.602703	0.0000	2.349961	0.0000	0.7205412	0.0000	3.701187	0.0000
ACP	2.774031	0.0000	5.450674	0.0000	7.86942	0.0000	0.121724	0.0000	8.011334	0.0000
CPK	144.0769	0.0000	3.987264	0.0000	2.567192	0.0000	0.7974539	0.0000	4.09626	0.0000
CK-MB	141.313	0.0000	3.883186	0.0000	2.509108	0.0000	1.2876033	0.0000	3.989339	0.0000
LDH	142.9228	0.0000	3.944068	0.0000	2.543149	0.0000	1.2677226	0.0000	4.051881	0.0000
Sodium	1.695709	0.0000	0.8085706	0.0000	3.008772	0.0455	1.631842	0.0000	2.74914	0.0000
Potassium	1.640618	0.0000	0.968488	0.0000	3.346145	0.0000	2.414214	0.0000	11.4937	0.0000
Mean	14.431358	0.0181	2.765383	0.0000	2.944445	0.0037	1.039175	0.0092	3.693471	0.0000
Mean corpuscular hemoglobin concentrations	−0.2774225	0.0000	−0.5504722	0.0000	−0.8522433	0.0000	+3.044774	0.0000	−0.7793243	0.0000
Plateletcrit	−0.2312044	0.0000	−0.6719365	0.0000	−1.330756	0.0886	+5.620077	0.0000	−0.9771515	0.0000
ALT	+0.5955473	0.0000	−1.157335	0.0000	+7.967324	0.0000	+0.4734427	0.0000	−0.6208232	0.0000
γGT	1	1.0000	+0.5367033	0.0000	−0.9428571	0.8982	+2.146813	0.0000	−0.2683513	0.0000
Mean	−0.4757810	0.0250	−0.9450332	0.0000	−0.6052695	0.2467	+2.0421598	0.0000	−0.5968125	0.0000

ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, ALP: Alkaline phosphatase, ACP: Acid phosphatase, CPK: Creatinine phosphokinase, CK-MB: Creatinine kinase muscle-brain, LDH: Lactic dehydrogenase, MPV: Mean platelet volume, MCV: Mean corpuscular volume, WBC: White blood cell, RBC: Red blood cell, MCH: Mean corpuscular hemoglobin

signaling pathways such as p53, intracellular calcium ($[Ca^{2+}]_i$), and sphingosine-1-phosphate (S1P)-receptor through $G\alpha_i$-coupled-receptor, thus UDCA protects CMs against $CoCl_2$-induced $[Ca^{2+}]_i$ dynamic alteration in hepatocytes. Pahlitzsch *et al*. noted that HR significantly increased[19] the ANG II response compared with control, although calcium transients remained similar, perhaps due to increased oxidative stress and increased Ca sensitivity of the contractile apparatus in live slices and in isolated afferent arterioles in C57Bl6 mice. He *et al*. found the intracellular Ca^{2+} accumulation significantly increased[20] compared with control group, along with enhanced mPTP opening and elevated ROS generation. However, suppression of stromal interaction molecule 1 by siRNA significantly decreased apoptosis and intracellular Ca^{2+} accumulation induced by H/R in H9c2 CMs. Nwankwo *et al*. provided[21] the first evidence that calpain-1 reduces the platelet hyperactivity in sickle cell disease (SCD) mice. He *et al*. have shown[22] that: Tris (1, 3-dichloro-2-propyl) phosphate could decrease store-operated calcium entry, restore H9c2 cell viability, mitigate Ca^{2+}-overload in H/R injury, and reduce the mitochondrial membrane potential in H/R injury. O'Shea *et al*. concluded[23] that the binding of the receptor for advanced glycation end-products cytoplasmic domain to the diaphanous-related form in, DIAPH1, led to increased expression of sarcoplasmic reticulum Ca^{2+} ATPase and reduced expression of the sodium Ca exchanger in experimental myocardial IR in mice. Liu and Dong ameliorated the overproduction of intracellular ROS and intracellular[24] Ca overload in the presence of carnosic acid for myocardial infarction in H9c2 CMs. Chang *et al*. decreased[25] mitochondrial Ca overload and inhibited the opening of mPTP after exenatide a glucagon-like peptide-1 pretreatment in H9c2 cells subjected to HR. Hu *et al*. were showed[26] that melatonin reduced Ca overload, inhibited IP3R expression, and promoted SERCA2a expression through ERK1 pathway in CMs against H/R. Skyschally *et al*. reduced infarct size (by 45% with polylactic acid plasma, $P < 0.05$) and improved mitochondrial function (e.g., increased respiration, ATP formation, and Ca retention capacity and decreased ROS formation) after remote ischemic[27] perconditioning RPER plasma/plasma dialysate in rat. Lopez *et al.* found[28] that pGz ameliorated the $[Ca^{2+}]_i$, and $[Na^+]_i$ elevation and ROS overproduction and further increased the activities of SOD, and GSH peroxidase and reduced the malondialdehyde and calpains. pGz diminished cell damage and elevated $[Ca^{2+}]_i$ during human resources (HR) and improved cognitive function in mdx mice. Qian *et al*. noticed that the downregulation[29] of IP3 receptors by IL-1ra attenuates Ca^{2+} overload versus the dimethyl sulfoxide group and the systolic and diastolic dysfunctions of HR-injured CMs, which contributes to inhibition of apoptosis in IR-injured CMs and reduction of myocardial infarct size *in vivo*. Braun *et al*. provided new evidence that blocking[30] the Ca activated potassium channels $K_{Ca3.1}$ and $K_{Ca2.1}$, the main mediators of the endothelium-derived hyperpolarizing factor, with TRAM34 and UCL1684, respectively, showed similar effects in HR and control impaired renal artery relaxation. Yin *et al*. significantly improved[31] the cell viability and decreased lactate dehydrogenase release, attenuated myocyte apoptosis, decreased $[Ca^{2+}]_i$ and Ca-sensing receptor (CaSR) expression, increased the ERK1/2 phosphorylation levels and inhibited the related apoptotic signaling pathways after pretreatment with Astragaloside IV (60 μmol/L) in rats with MIR injury. Li *et al*. protected[32] hippocampal neurons from IR injury through two independent signaling pathways, the one including the calcineurin/FKBP12.6-RyR/Ca overload pathway after propofol administration. Zheng *et al*. concluded that miR-148a may mitigate hepatic IR injury by ameliorating toll-like receptor 4 (TLR4)-mediated inflammation through targeting CaMKIIα. Positive crosstalk with the Ca^{2+}/CaMKII pathway is required[33] for complete activation of the TLR4 pathway and inflammation *in vitro* and *in vivo* IR-treated mice. Zeng *et al.* indicated[34] that CaSR may modulate T lymphocytes to release cytokines through mitogen-activated protein kinase pathways and affect CM injury. Kleinbongard *et al*. associated[35] cardioprotection by remote ischemic preconditioning (26% decrease in the area under the curve of troponin I/T) with greater mitochondrial ADP-stimulated complex I respiration (+10%), ATP production (+46%), and Ca retention capacity (+37%), whereas ROS production (−24%) was less than placebo. Li *et al*. showed[36] that dichloroacetate treatment ameliorated contractile dysfunction and improved the intracellular Ca signal of isolated CMs under HR conditions in mice. Gu *et al*. used chronic intermittent hypobaric hypoxia treatment to reduce[37] the Ca overload and cTnI protein expression ($P < 0.01$) by upregulating the expression of PGC-1α and regulating the energy metabolism of glucose and lipid in CMs after HR. Li *et al*. investigated[38] the role of a circular RNAs (circRNA) transcribed from the sodium/Ca exchanger 1 (ncx1) gene, named circNCX1, in oxidative stress-induced CM apoptosis during ischemic myocardial injury. Bai and Han detected[39] mitochondrial viability, cellular apoptosis, ROS production, and Ca overloading in H9c2 cells that exposed to HR-induced cytotoxicity. Further, nicorandil decreased the production of ROS and alleviated Ca overloading in these HR-induced cells. Cheng *et al*. showed[40] that tetrahydroxystilbene glucoside enhanced the cardioprotective effect of transient hypoxia on HR by reducing excessive ROS production and Ca overloading. Ke *et al.* showed[41] that S1P prevented loss of $\Delta\Psi m$, relieved mitochondrial Ca overload, inhibited opening of the mPTP, and release of cytochrome C in H9c2 cells. Woods *et al.* found[42] that mitochondrial Ca^{2+} ($_mCa^{2+}$) uptake mediated by the MCU plays a critical role in signal transduction, bioenergetics, and cell death, and its dysregulation is linked with several human diseases. Xing *et al.* decreased[43] p21-activated kinase 2 (Pak2) associated with oxidative stress, Ca overload and caspase-12-mediated apoptosis activation in HR-treated N2a cells on ER stress. Harhous *et al*. proposed[44] that STAT3 had a weak effect on

the Ca retention capacity after IR in various cell types. Li *et al*. accompanied[45] the inhibited Ca^{2+} overload and the upregulation of miR-202-5p and also upregulation or downregulation of its downstream TRPV2, with the increase of SERCA2a and suppression of IP3R presenting the cardioprotective effects in MIR rats CMs. Li *et al*. indicated[46] that pre-treatment with YiqiYangyinHuoxue activated the PI3K/AKT and ERK1/2 signaling pathways, which reduces mPTP opening, overproduction of ROS and Ca overload in isolated rat hearts. Sadler *et al*. found voltage-gated calcium channel $\alpha_2\delta_1$ subunit expression[47] similar in sciatic nerve, dorsal root ganglia, and lumbar spinal cord tissue from SCD and control mice. Wang *et al*. attenuated[48] HR-mediated ER stress, redox imbalance, Ca overload and caspase-12-related CMs apoptosis, dependent on Pak2 upregulation after melatonin treatment in CMs. Yuan *et al*. indicated[49] the pathophysiologic mechanisms associated with ROS generation, calcium overload, energy metabolism disorder, neutrophil infiltration, and others in ischemic heart disease myocardial IR (MI/R) rat. Guan *et al*. concluded[50] that during I/R, MCU upregulation induces calpain activation, which downregulates OPA1 in a mouse myocardial IR model. Zeng *et al*. inhibited[51] HR-increased INaL, reversed INCX augmentation, shortened the APD, and diminished $[Ca^{2+}]_i$ overload, to maintain Ca^{2+} homeostasis through the reverse mode of the Na^+/Ca^{2+} exchange current and protected CMs after T3 administration against HR injury.

According to the above, Table 3 shows that U-74389G has 2.3623042-fold (2.3482723–2.3764196) more hypercalcemic effect than Epo whether all variables have been considered (P = 0.0000); a trend reversed along time, in Epo non-deficient rats. A meta-analysis of these ratios from the same experiment, for 27 other seric variables, provides comparable results [Table 4].[52,53]

CONCLUSIONS

The antioxidant agent U-74389G was proved to have 2.3623042-fold (2.3482723–2.3764196) more hypercalcemic effect than Epo whether all variables have been considered (P = 0.0000); a trend reversed along the short-term time frame of the experiment in rats. A biochemical investigation remains about how U-74389G mediates in these actions.

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ETHICAL APPROVAL

"All applicable international, national, and/or institutional guidelines for the care and use of animals were followed."

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