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

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Abstract

Aims: This study calculated the effects on serum chloride (Cl) levels, after treatment with either of 2 drugs: the erythropoietin (Epo) and the antioxidant lazaroid (L) drug U-74389G. The calculation was based on the results of 2 preliminary studies, each one of which estimated the certain influence, after the respective drug usage in induced ischemia-reperfusion (IR) animal experiment.

Materials & Methods: The 2 main experimental endpoints at which the serum Cl levels were evaluated was 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.

Findings: The first preliminary study of Epo presented a non-significant hypochloremic effect by 0.74%+0.55% (p-value=0.1701). However, the second preliminary study of U-74389G showed a significant hypochloremic effect by 0.75%+0.34% (p-value=0.0310). These two studies were co-evaluated since they came from the same experimental setting. The outcome of the co-evaluation was that L is 1.012762-fold [1.011746 - 1.01378] more hypochloremic than Epo (p-value=0.0000).

Conclusion: The antioxidant capacities of U-74389G ascribe 1.012762-fold more hypochloremic effects than Epo (p-value=0.0000).

Keywords: Ischemia; Erythropoietin; U-74389G; Serum Chloride Levels; Reperfusion

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Introduction

The lazaroid U-74389G (L) maybe not famous for its hypochloremic1 capacity (p-value=0.0310). 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 evident 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; down-regulates the preinflammatory gene; treats the endotoxin shock; produces cytokine; enhances the mononuclear immunity; protects the endothelium and presents antishock property.

Erythropoietin (Epo), even if it is not famous for its hypochloremic2 action (p-value=0.1701), it can be used as a reference drug for comparison with U-74389G. Although Epo is met in over 31,147 published biomedical studies, only 3.66% of them negotiate the known type of IR experiments. Nevertheless, Epo, as a cytokine, it is worthy of being studied about its effects on serum chloride (Cl) levels too. This experimental work tried to compare the impact of the above drugs on a rat induced IR protocol. They were tested by calculating the serum Cl levels alterations.

Material and Methods

Animal preparation

The Vet licenses under 3693/12-11- 2010 & 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 (6) groups consisted of N=10. The stage of 45 min hypoxia was common for all 6 groups. Afterwards, 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 are 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 an inferior vena cava catheter. The Cl 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, non relation was raised between Cl values with animals' mass (p-value=0.2175).

Statistical analysis

Table 1 presents the (%) hypochloremic influence of Epo regarding reoxygenation time. Also, Table 2 shows the (%) hypochloremic 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 at Table 3.

Findings

The successive application of chi-square tests revealed that U-74389G caused hypochloremia by 0.5544784-fold [0.392656 - 0.7829949] less than Epo at 1h (p-value=0.0007), by 0.8643683-fold [0.8568604 - 0.8719421] less than Epo at 1.5h (p-value=0.0000), by 1.07745-fold [0.8477728 - 1.36935] more than Epo at 2h (p-value=0.5428), by 1.358293-fold [1.345045 - 1.371671] more (p-value=0.0000) without drugs and by 1.012762-fold [1.011746 - 1.01378] more than Epo whether all variables have been considered (p-value=0.0000).

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

Hypochloremia	±SD	Reperfusion time	p-value
-0.87%	±2.98%	1h	0.3637
-1.07%	±4.42%	1.5h	0.2635
-1.27%	±5.70%	2h	0.4633
-0.68%	±3.86%	reperfusion	0.4457
-0.74%	±0.55%	interaction	0.1701

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

Hypochloremia	±SD	Reperfusion time	p-value
-0.48%	±3.04%	1h	0.6063
-0.92%	±2.87%	1.5h	0.1402
-1.36%	±2.77%	2h	0.1113
-0.92%	±2.87%	reperfusion	0.1402
-0.75%	±0.34%	interaction	0.0310

Table 3) The U-74389G/erythropoietin hypochloremic efficacies after chi-square tests application

Odds ratio	95% Conf.	Interval	p-values	Endpoint
0.5544784	0.392656	0.7829949	0.0007	1h
0.8643683	0.8568604	0.8719421	0.0000	1.5h
1.07745	0.8477728	1.36935	0.5428	2h
1.358293	1.345045	1.371671	0.0000	reperfusion
1.012762	1.011746	1.01378	0.0000	interaction

Discussion

The unique available study investigating the hyperkalemic effect of U-74389G was the preliminary one [1]. Although the most popular 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 an immunosuppressant in flap survival. It prevents the learning impairments and it delays the early synaptic transmission decay during hypoxia improving 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 hypochloremic effect of Epo preparations in non-iron-deficient individuals. Román-Anguiano NG et al. have shown [3] that NO inhibits the activity of caspases and calpains through S-nitrosylation of a cysteine located in their catalytic site since the independent cGMP pathway involves post-translational modification of proteins by S-nitrosylation. Infarct size was measured with 2,3,5-triphenyl tetrazolium chloride stain. S-nitrosylation of caspase-3 and calpain-1 was evaluated by labeling S-nitrosylated cysteines. Their results showed that both Prolame and SNAP increased NO content and improved functional recovery in post-ischemic hearts. Liu K et al. showed [4] that mangiferin (MAF) could significantly reduce myocardial injury, inhibited myocardial oxidative stress and preinflammatory cytokines and resumed the ST segment, after triphenyl tetrazolium chloride (TTC) staining and pathological analysis in H/R-induced rats H9c2 cells. Wang Y et al. suggested [5] that Wnt/β-catenin signaling is correlated with intermedin induced angiogenesis and neovascularization an in vitro model established adding CoCl2 HUVECs. Shu L et al. suggested [6] that troxerutin decreased neonatal rat cardiomyocyte apoptosis and alleviated myocardial I/R injury in rats via inhibition or downregulating miR-146a-5p in vivo. Infarct size was examined by 2,3,5-triphenyl tetrazolium chloride staining. Yin B et al summarized [7] that astragaloside IV attenuates myocardial I/R injury via inhibition of CaSR/ERK1/2 and the related apoptotic signaling pathways after MI/R injury by co-treatment with a calcium-sensing receptor CaSR agonist, gadolinium chloride (GdCl3) or with a specific extracellular signal-regulated kinase 1/2 (ERK1/2) inhibitor U0126 in rats. Lou Z et al. demonstrated [8] that transforming growth beta (TGF-β) signaling activation is involved in the regulation of NADPH oxidase 2 / NADPH oxidase 4 expression and exacerbates cerebral I/R injury in PC-12 cells. Rao H et al. revealed [9] that special AT-rich sequence binding protein 1 (binds to nuclear matrix/scaffold-associating DNA) (SATB1) is a tumor promoter that participates in cancer cell migration and invasion. H/R-treated trophoblasts with lower SATB1 levels exhibited weaker invasive and growth capacities, whereas up-regulation of the SATB1 level with recombinant SATB1 restored these impairments. Moreover, the elevated concentration of SATB1 also increased the expression of β-catenin, which is involved in human placental trophoblast invasion and differentiation is down regulated in PE. However, a specific activator, namely, lithium chloride (LiCl), increased β-catenin expression but had no evident influence on SATB1 expression. Together, these data show that SATB1expression in the human placenta is affected by oxidative stress and might regulate the migration and invasion of trophoblasts via β-catenin signaling. Mohamed AS et al. isolated [10] cardiomyocytes from newborn rats (0-2 days), and hypoxia was induced by using cobalt chloride (CoCl2). Their findings showed that ursodeoxycholic acid (UDCA) counteracted the effects of CoCl2 on cell viability, beating frequency, HIF-1α, and p53 protein expression. Furthermore, they observed that UDCA protects cardiomyocytes (CMs) against CoCl2-induced [Ca2+]i dynamic alteration. Pharmacological inhibition of the Gαi -sensitive receptor did not abolish the cardioprotection of UDCA against CoCl2 detrimental effects, except for cell viability and [Ca2+]i. Pertussis toxin is partially effective in inhibiting UDCA protection against CoCl2 effects on CM cell viability. Interestingly, PTX fully inhibits UDCA cardioprotection on CoCl2 -induced [Ca2+]i dynamic changes. They concluded that UDCA cardioprotection against CoCl2 -induced hypoxia is similar to FTY720, and its actions are not fully mediated by the Gαi -coupled protein sensitive pathways. The current data generated were the first to show that UDCA can inhibit the activation of HIF-1α and p53 protein during CoCl2 -induced hypoxia in cardiomyocytes. Qiu LY et al. acknowledged [11] that anion exchanger 3 (AE3) serves crucial roles in maintaining intracellular chloride homeostasis by facilitating the reversible electroneutral exchange of Cl for HCO3 across the plasma membrane. Their previous studies reported that sasanquasaponin (SQS) could inhibit hypoxia/reoxygenation (H/R) induced elevation of intracellular Cl concentration ([Cl]i) and elicit cardioprotection by favoring Cl /HCO3 exchange of AE3. Additionally, both inhibition of PKCε by εV1 2 and S67A mutation of AE3 eradicated the SQS induced increase of AE3 activity, reversed the inhibitory effect of SQS on H/R induced elevation of [Cl]i, Ca++ overload and generation of reactive oxygen species and eliminated SQS induced cardioprotection. They

concluded that PKCε dependent phosphorylation of serine 67 on AE3 might be responsible for the increase of Cl /HCO3 exchange of AE3 and intracellular chloride efflux by SQS in H9c2 cells. Zhang X et al. found that luteolin (Lut) ameliorated [12] myocardial ischemia-reperfusion injury and H/R as evidenced by triphenyl tetrazolium chloride (TTC) staining and MTT assay, respectively. Lut also inhibited the upregulations of inflammasome components, such as NOD-like receptor 3(NLRP3), apoptosis-associated speck-like protein containing CARD(ASC) in I/R-induced rats and H/R-induced H9c2 cells. Zhang XG et al. demonstrated [13] that the isotonic substitution of extracellular chloride by gluconate (extracellular Cl--free) elicits cardioprotection by attenuating I/R induced elevation of intracellular chloride ion concentration ([Cl-]i). The results showed that extracellular Cl--free attenuated H/R-induced the elevation of [Cl-]i, accompanied by increase of cell viability and reduction of lactate dehydrogenase release. Moreover, extracellular Cl--free inhibited mPTP opening, and improved mitochondria function, as indicated by preserved mitochondrial membrane potential and respiratory chain complex activities, decreased mitochondrial reactive oxygen species generation and increased ATP content. Intriguingly, pharmacologically opening of the mPTP with Atr attenuated all the protective effects caused by extracellular Cl--free, including suppression of mPTP opening, maintenance of mitochondrial membrane potential, and subsequent improvement of mitochondrial function. These results indicated that extracellular Cl--free protects mitochondria from H/R injury in H9c2 cells and inhibition of mPTP opening is a crucial step in mediating the cardioprotection of extracellular Cl--free. Li YY et al. reported [14] that sasanquasaponin (SQS) elicits cardioprotection by suppressing H/R-induced elevation of intracellular chloride ion concentration ([Cl-]i). The increased [Cl-]i is involved in modulating the mitochondrial permeability transition pore (mPTP). The results showed that SQS attenuated H/R-induced the elevation of [Cl-]i, accompanied by reduction of lactate dehydrogenase release and increase of cell viability. Interestingly, extracellular Cl--free condition created by replacing Cl- with equimolar gluconate resulted in a decrease in [Cl-]i and induced protective effects similar to SQS preconditioning, whereas pharmacologically opening of the mPTP with ATR abolished all the protective effects induced by SQS or Cl--free, including suppression of mPTP opening, maintenance of mitochondrial membrane potential and subsequent improvement of mitochondrial function. The above results allow us to conclude that SQS-induced cardioprotection may be mediated by preserving the mitochondrial function through preventing mPTP opening via inhibition of H/R-induced elevation of [Cl-]i. Xia Y et al. reported that volume-sensitive outwardly rectifying (VSOR) Cl-channel-activated by reactive oxygen species (ROS) contributes [15] to cell apoptotic volume decrease, playing an incipient incident of cellular apoptosis in autophagy-related cell death. Interestingly, VSOR Cl-channel-blocked by VSOR Cl- channel blocker (DCPIB) could stably maintain the cell volume, intracellular pH, abundant lysosome associated membrane protein-2 (LAMP2) and autophagic intensity regardless of ROS intension derived from reoxygenation injury or adding H2O2. These results first demonstrate that VSOR Cl- channel-activated is a pivotal event to trigger autophagy-related death, which reveals a novel therapeutic target to decrease myocardial I/R injury in rats.

According to above, table 3 shows that U-74389G has 1.012762-fold [1.011746 – 1.01378] more hypochloremic effect than Epo (p-value=0.0000) whether all variables have been considered (p-value=0.0000); a trend being accentuated 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) [16, 17].

Table 4) A U-74389G / erythropoietin efficacies ratios meta-analysis on 27 hematologic variableç (23 variables with balancing efficacies and 4 variables with opposite efficacies)

Endpoint Variable	1h	p-value	1.5h	p-value	2h	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

Endpoint Variable	1h	p-value	1.5h	p-value	2h	p-value	Reperfusion time	p-value	Interaction	p-value
Corpuscular Hemoglobin concentrations	-0.2774225	0.0000	-0.5504722	0.0000	-0.8522433	0.0000	+3.044774	0.0000	-0.7793243	0.0000
Platelet crit	-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

Conclusion

The anti-oxidant agent U-74389G was proved having 1.012762-fold [1.011746 - 1.01378] more hypochloremic effect than Epo whether all variables have been considered (p-value=0.0000); a trend accentuated 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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