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

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Abstract

Aim: This study calculated the effects on serum sodium (Na) 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 an induced ischemia reperfusion (IR) animal experiment.

Materials and methods: The 2 main experimental endpoints at which the serum Na 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). Specially, 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 hyponatremic effect by 0.11% ± 0.38% (p-value = 0.7531). The second preliminary study of U-74389G presented a non significant hyponatremic effect by 0.32% ± 0.36% (p-value = 0.3693). These 2 studies were co-evaluated since they came from the same experimental setting. The outcome of the co-evaluation was that L is 2.74914-fold [2.74424-2.754048] more hyponatremic than Epo (p-value = 0.0000).

Conclusions: The anti-oxidant capacities of U-74389G ascribe 2.74914-fold more hyponatremic effects than Epo (p-value = 0.0000).

Keywords

Ischemia, Erythropoietin, U-74389G, Serum sodium levels, Reperfusion

Introduction

The lazaroid U-74389G (L) may be not famous for its hyponatremic [1] capacity (p-value = 0.3693). U-74389G as a novel antioxidant factor, implicates exactly only 260 published studies. The ischemia reperfusion (IR) type of experiments was noted in 18.84% 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 male ate 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 proinflammatory gene; treats the endotoxin shock; produces cytokine; enhances the mononuclear immunity; protects the endothelium and presents antishock property.

Erythropoietin (Epo) even if is not famous for its hypona-

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tremic [2] action (p-value = 0.7531), it can be used as a reference drug for comparison with U-74389G. Although Epo is met in over 30,844 published biomedical studies, only a 3.62% 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 sodium (Na) 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 Na levels alterations.

Materials 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 in 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 (Table 1). 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 the blood flow interruption, 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 Na 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, a weak relation was rised between Na values with animals' mass (p-value = 0.2366).

Table 1: Serum sodium levels at the rest endpoints of 1 h, 1.5 h, and 2.0 h.

Sodium levels	Obs	Mean	Std. Dev.	Min	Max
1 h placebo	10	137.8	2.299758	135	141
1.5 h placebo	10	137.6	2.157674	134.5	140.5
2 h placebo	10	137.4	2.75681	133	141
1 h after Epo	10	138.8	1.988858	135	141
1.5 h after Epo	10	137.9	2.024846	135	141.5
2 h after Epo	10	137	3.496029	133	143
1 h after L	10	139.5	1.433721	138	142
1.5 h after L	10	137.85	2.147996	135	141.5
2 h after L	10	136.2	3.119829	131	141

Statistical Analysis

Table 2 presents the (%) hyponatremic influence of Epo regarding reoxygenation time. Also, Table 3 presents the (%) hyponatremic 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 4. The statistical analysis was performed by Stata 6.0 software [Stata 6.0, StataCorp LP, Texas, USA].

Results

The successive application of chi-square tests revealed that U-74389G enhanced the hyponatremia by 1.695709-fold [1.323912-2.171914] more than Epo at 1 h (p-value = 0.0000), by 0.8085706-fold [0.7951-0.8222694] more than Epo at 1.5 h (p-value = 0.0000), by 3.008772-fold [1.02035-8.866639] more than Epo at 2 h (p-value = 0.0455), more by 1.631842-fold [1.619651-1.644125] (p-value = 0.0000) without drugs and by 2.74914-fold [2.74424-2.754048] more than Epo whether all variables have been considered (p-value = 0.0000).

Discussion

The unique available study investigating the hyponatremic effect of U-74389G was the preliminary one [1]. Al-

Table 2: The (%) hyponatremic influence of erythropoietin in connection with reperfusion time.

Hyponatremia	± SD	Reperfusion time	p-value
0.72%	± 2.07%	1 h	0.3054
0.21%	± 2.45%	1.5 h	0.7136
-0.29%	± 2.85%	2 h	0.7415
-0.79%	± 2.43%	Reperfusion	0.1800
-0.11%	± 0.38%	Interaction	0.7531

Table 3: The (%) hyponatremic influence of U-74389G in connection with reperfusion time.

Hyponatremic	± SD	Reperfusion time	p-value
1.22%	± 1.95%	1 h	0.0707
0.17%	± 2.74%	1.5 h	0.7753
-0.87%	± 3.31%	2 h	0.3995
-1.29%	± 1.97%	Reperfusion	0.0176
-0.32%	± 0.36%	Interaction	0.3693

Table 4: The U-74389G/erythropoietin hyponatremic efficacies after chi-square tests application.

Odds ratio	[95% Conf. Interval]		p-values	Endpoint
1.695709	1.323912	2.171914	0.0000	1 h
0.8085706	0.7951	0.8222694	0.0000	1.5 h
3.008772	1.02035	8.866639	0.0455	2 h
1.631842	1.619651	1.644125	0.0000	Reperfusion
2.74914	2.74424	2.754048	0.0000	Interaction

though 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 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 hyponatremic effect of Epo preparations in non iron deficient individuals. Serum sodium levels at baseline are not available for many reasons: The first is that the baseline blood sampling of 2 cc might be ominous for the small rats so as the experiment jeopardize not to proceed. The second is that we should alike to receive baseline histologic samples, as the experiment ended up to internal genitalia study, something which was impossible. The third is that such baseline values would be deprieved by the "IR event" which we wished to investigate whether and how much would be reverted by the drugs. These are the reasons we used as baseline values those of placebo groups.

Liu L, et al. [3] identified Epo as the predominant treatment component; with sodium selenite serving as an adjuvant, and combination treatment was markedly more effective, compared with either treating drug alone. The optimal ratio of treatment was 10: 1 (10 IU EPO: 1 μg sodium selenite). Together, the results suggested that co-administration of EPO and sodium selenite effectively ameliorates IRI-induced renal injury by reducing oxidative stress and activating the PI3K/NO signaling pathway in renal IRI. Jeong JH, et al. [4] confirmed the neuroprotective effects of recombinant human erythropoietin (rhEPO)-loaded poly (lactic-co-glycolic acid) (PLGA) nanoparticles stabilized by sodium cholate (rhEPO-Ch-NP) and compared their effects with those of rhEPO. Notably, it was demonstrated that rhEPO-Ch-NPs were safer at any concentration investigated and rescued more neuronal cells in an *in vitro* model of cerebral ischemia. Deliyanti D, et al. [5] found that a low-salt (LS) diet reduced erythropoietin, mineralocorticoid receptor, angiotensin type 1 receptor and renin mRNA levels in retina, whereas, as expected, plasma levels of aldosterone and renin were increased. In cultured Müller cells, high salt increased epithelial sodium channel alpha (ENaCα), which was prevented by mineralocorticoid receptor and angiotensin type 1 receptor blockade in pregnant Sprague Dawley rats. Kang J, et al. [6] significantly up-regulated the expression of protein and mRNA levels of HIF-1α, VEGF and EPO in the β-sodium aescinate (SA) group ($P < 0.05$) after return of spontaneous circulation in rat cerebral cortex. Bimpis A, et al. [7] revealed an injury specific type of behavior after U-74389G administration that could be considered as neuroprotective since Na (+), K (+)- and Mg (2+)-ATPase inhibition might in this case diminish the local ATP consumption in a spontaneous intracerebral hemorrhage (ICH) porcine model. Vignes JR, et al. [8] found that lazaroid compounds and certainly U-74500A decreased neuronal death to 37-23.5%, U-74389G to 37-32%, and U-83836E to 42-33% compared with exposed primary cultures of cortical neurons in rats exposed to 0.5 mM sodium cyanide for 6 h. Washo-Stultz D, et al. [9] assumed that damage to mitochondria is an upstream event in sodium deoxycholate (NaDOC)-induced apoptosis and that a pro-oxidant state of the cell favors survival. Lazaroid pre-treatment caused a marked decrease in NaDOC-induced activation of the anti-apoptotic transcription factor, NF-κB, which may provide the basis for the sensitization to apoptosis caused by these antioxidants. Horáková L, et al. [10] found that U 74389G decreased the preventive effect concerning lipid peroxidation by (160 IC50 in μmol/l) in brain homogenate. Stanimirovic DB, et al. [11] implicated both the inhibition of Na, K-ATPase and membrane lipid peroxidation in the presence of the steroid antioxidants U-74500A and U-74389G (5-20 μM) in rat cerebromicrovascular endothelial cells.

According to above, Table 4 shows that U-74389G has 2.74914-fold [2.74424-2.754048] more hyponatremic effect than Epo (p-value = 0.0000) whether all variables have been considered (p-value = 0.0000); a trend accentuated along time, in Epo non-deficient rats.

Conclusion

The anti-oxidant agent U-74389G was proved having 2.74914-fold [2.74424-2.754048] more hyponatremic 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. The mechanism of hyponatremia is impossible by the current knowledge. It seems that sodium somewhere is consumed; however, further assumptions about the hyponatremic trend is high risky. This is the reason that this study is statistical and not molecular, nevertheless, the authors finally recommend for a further molecular elucidation of hyponatremia.

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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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