International Journal of Research Studies in Medical and Health Sciences
Volume 3, Issue 6, 2018, PP 14-18
ISSN : 2456-6373

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

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ABSTRACT

***Aim:** This study calculated the hypalbuminemic capacities 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 hypalbuminemic influence, after the respective drug usage in an induced ischemia reperfusion animal experiment.*

***Materials and methods:** The 2 main experimental endpoints at which the serum albumins 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 significant hypalbuminemic effect by 5.37%±1.95% (p-value=0.0072). The second preliminary study of U-74389G presented a non significant hypalbuminemic effect by 2.68%±1.68% (p-value=0.1069). These 2 studies were co-evaluated since they came from the same experimental setting. The outcome of the co-evaluation was that U-74389G has half hypalbuminemic potency than Epo (p-value=0.0000).*

***Conclusions:** The anti-oxidant capacities of U-74389G restrict the acute hypalbuminemic properties by half than Epo (p-value=0.0000).*

Keywords: *ischemia; erythropoietin; U-74389G; serum albumin levels; reperfusion*

INTRODUCTION

The lazaroid U-74389G (L) is not famous for its hypalbuminemic[1] capacity (p-value=0.1069). U-74389G as a novel antioxidant factor, implicates exactly only 258 published studies. The ischemia reperfusion (IR) type of experiments was noted in 18.60% 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; 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 hypalbuminemic action (p-value=0.0072), it can be used as a reference drug for comparison with U-74389G. Although Epo is met in over 30,384 published biomedical studies, only a 3.56% of them negotiate the known type of IR experiments. Nevertheless, Epo as a cytokine, it is worth of being studied about serum albumin levels too.

This experimental work tried to compare the hypalbuminemic effects of the above drugs on a rat induced IR protocol. They were tested by calculating the serum albumin (Al) levels declines.

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. 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 inferior vena cava catheter. The Al levels (All) 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). However, the predicted All values were not used since a weak relation was rised with animals' mass (p-value=0.1163).

Statistical Analysis

Table 1 presents the (%) hypalbuminemic influence of Epo regarding reperfusion time. Also, Table 2 presents the (%) hypalbuminemic 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. The statistical analysis was performed by Stata 6.0 software [Stata 6.0, StataCorp LP, Texas, USA].

Table1. *The (%) hypalbuminemic influence of erythropoietin in connection with reperfusion time*

Hypalbuminemia	±SD	Reperfusion time	p-value
-4.61%	+11.85%	1h	0.2530
-9.28%	+13.39%	1.5h	0.0054
-13.96%	+13.81%	2h	0.0095
+3.09%	+13.29%	reperfusion	0.3405
-5.37%	+1.95%	interaction	0.0072

Table2. *The (%) hypalbuminemic influence of U-74389G in connection with reperfusion time*

Hypalbuminemia	±SD	Reperfusion time	p-value
-1.13%	+9.83%	1h	0.7377
-4.92%	+12.45%	1.5h	0.0832
-8.71%	+14.23%	2h	0.0616
+3.83%	+12.22%	reperfusion	0.1723
-2.68%	+1.68%	interaction	0.1069

Table3. *The U-74389G / erythropoietin efficacies ratios on serum hypalbuminemia after chi-square tests application*

Odds ratio	[95% Conf. Interval]	p-values	Endpoint
0.2457507	0.0854016 - 0.7082004	0.0073	1h
0.5303472	0.5293544 - 0.531342	0.0000	1.5h
0.6243052	0.3926271 - 0.9928358	0.0465	2h
1.237477	1.234338 - 1.240624	0.0000	reperfusion
0.5000416	0.4988042 - 0.501282	0.0000	interaction

RESULTS

The successive application of chi-square tests revealed that U-74389G was less hypalbuminemic by 0.2457507-fold [0.0854016 - 0.7082004] than Epo at 1h; by 0.5303472-fold [0.5293544 - 0.531342] at 1.5h, by 0.6243052-fold [0.3926271 - 0.9928358] at 2h, hyperalbuminemic by 1.237477-fold [1.234338 - 1.240624] without drugs and less hypalbuminemic by half whether all variables have been considered (p-value=0.0465).

DISCUSSION

The unique available study investigating the hypalbuminemic effect of U-74389G on Al 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 gamma-glutamyltransferase (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 hypalbuminemic effect of Epo preparations in non iron deficient individuals. Duan LJ et al promoted[3] liver erythropoietin (EPO) expression 1246-fold by albumin Cre (Alb(Cre))-mediated, hepatocyte-specific triple disruption of prolyl hydroxylase domain Phd1, Phd2, and Phd3 (Phd(1/2/3)hKO), whereas renal EPO was down-regulated to 6.7% than normal levels in Phd(2/3)hKO mice. Aydin Z et al described for hepcidin, a small peptide hormone synthesized[4] in the liver, a central role in regulation of iron metabolism and dependent on erythropoietin levels in chronic kidney disease (CKD). A negative correlation was detected between prohepcidin levels and albumin ($r = -0.286$, $p < 0.001$) in non-diabetic uremic patients. Connolly-Andersen AM et al associated[5] the marker syndecan-1 for glycocalyx degradation significantly with the levels of both thrombocytes and albumin in hantaviral disease. Ribeiro S et al showed[6] that rHuEPO treatment corrected anaemia and improved urinary albumin excretion, particularly at lower doses in male Wistar rats with chronic renal failure (CRF) induced by 5/6 nephrectomy over a 3-week period. Meyring-Wösten A et al resembled[7] an inflammatory phenotype, with lower serum albumin levels (by -0.1 g/dl), lower hemoglobin levels (by -0.2 g/dl) and required more erythropoietin (+1374 U per hemodialysis treatment) in prolonged intradialytic hypoxemia. Schaalan MF et al noted[8] decreased levels of albumin (15.7%) and EPO levels (77.8%) in septic acute kidney injury (AKI) patients than normal subjects with control levels. Oshiro S et al evaluated[9] the therapeutic impacts of S-nitrosated human serum albumin (SNO-HSA), a long-lasting NO donor, on 2 animal models of CKD. SNO-HSA increased the expression of erythropoietin (EPO), VEGF, and eNOS by stabilizing hypoxia inducible factor-1α in HepG2 and HK-2 cells. Anusornvongchai T et al found that both endoplasmic reticulum stress and transcription factor ATF4 activation by palmitate, suppressed EPO production by the 3'-enhancer activity of the EPO gene suppression in renal erythropoietin (EPO)-producing (REP) cells in a manner independent of HIF activation in the interstitial area of inherited super-anemic mice (ISAM) mated with EPO-Cre mice. Gardner DS et al suggested[11] that preoperative erythropoietin (EPO) or remote ischemic preconditioning may have a renoprotective effect. At 2 h, a panel of biomarkers including plasma urinary albumin:creatinine could be used to predict histopathological injury in an IR porcine model. Ratilal BO et al protected[12] the brain from transient middle cerebral artery (MCA) ischemia after the administration of rhEPO 60-minutes before its onset. As supported by the lack of Akt activation, its benefits are most probably related to an indirect effect on brain edema as a consequence of blood-brain barrier preservation. Hernández-Navarrete LS et al presented[13] the hemoglobin, hematocrit, red blood cells and the glomerular filtration stable at 24 months postransplant in kidney transplants without blood transfusion but after pretransplant erythropoietin administration in all patients. Abd-Elsayed AA et al shown[14] that erythropoietin has some promise in lowering the incidence of vasospasm and delayed cerebral ischemia. Albumin is the preferred colloid in aneurysmal subarachnoid hemorrhage. Giri S et al. The differentiated hepatocyte stem cells (day 21) exhibited functional hepatic characteristics [markers] such as albumin secretion and cytochrome P450 expression. Grote Beverborg N et al showed[16] an interaction of high serum EPO levels with

urinary albumin excretion (P = 0.006); only independently associated with new-onset heart failure in subjects with albuminuria (HR 1.51, P = 0.005). Bagetta G et al delayed[17] the onset of motor and electrocortical (ECoG) seizures and reduced the number of epileptogenic discharges typically induced by an injection of dendrotoxin K (DTx-K) (35 pmol) after a latent period of approximately 5 min after systemic (i.p.) administration of U-74389G (5 mg/kg given 30 min beforehand) a scavenger of free oxygen radicals, whereas the corresponding brain regions injected with bovine serum albumin (300 ng), per se was ineffective in rats. Tasaka S et al examined[18] the effect of 21-aminosteroid (AS), 10 mg/kg of U-74389G, as a potent antioxidant on lung injury during exposure to 90% O_2 for 48 h. Thus, they measured the wet-to-dry weight ratio (W/D) as an index of lung water and the concentration ratio of I_{125}-labeled albumin in lung tissue and bronchoalveolar lavage (BAL) fluid compared with plasma (T/P and BAL/P, respectively) as indexes of pulmonary endothelial damage in guinea pigs.

According to above, table 3 shows that U-74389G was proved less hypalbuminemic by half than Epo (p-value=0.0000); a trend attenuated along time, in Epo non-deficient rats. A meta-analysis of these ratios from the same experiment, for 15 other seric variables, provides comparable results (table 4)[19-21].

Table4. *A U-74389G / erythropoietin efficacies ratios meta-analysis on 15 hematologic variableς (13 variables with balancing efficacies and 2 variables with opposite efficacies)*[19-21].

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
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
Mean	15.726282	0.0290	3.373714	0.0000	3.611258	0.0000	1.1287524	0.0167	4.128637	0.0000

Endpoint Variable	1h	p-value	1.5h	p-value	2h	p-value	Reperfusion time	p-value	interaction	p-value
Mean 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
Mean	-0.2532076	0.0000	-0.6081795	0.0000	-1.0649544	0.0443	4.1366488	0.0000	-0.8726499	0.0000

CONCLUSION

The anti-oxidant agent U-74389G was proved less hypalbuminemic by half than Epo (p-value=0.0000); a trend attenuated along the short term time frame of the experiment in rats. A biochemical investigation remains about how U-74389G is implicated in these reactions.

ACKNOWLEDGEMENT

Acknowledged in preliminary studies

Ethical approval

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

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Citation: *C. Tsompos1 et.al, "Comparison of the Hypaalbuminemic Effects of Erythropoietin and U-74389G". International Journal of Research Studies in Medical and Health Sciences. 2018; 3(6):14-18.*