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Comparison of the Acute Erythropoietic Capacities of Erythropoietin and U-74389G Concerning Mean Corpuscular Hemoglobin Levels

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Abstract

Aim: This study compared the erythropoietic capacities of erythropoietin (Epo) and antioxidant drug U-74389G based on 2 preliminary studies. The provided results at mean corpuscular hemoglobin (MCH) levels augmentation were co-evaluated in a hypoxia reoxygenation protocol of an animal model.Materials and methods: MCH levels (MCHl) were evaluated at the 60th reoxygenation min (for groups A, C and E) and at the 120th reoxygenation min (for groups B, D and F) in 60 rats. Groups A and B received no drugs, rats from groups C and D were administered with Epo; whereas rats from groups E and F were administered with U-74389G. **Results:** The first preliminary study of Epo non significantly increased the MCHl by 0.31%$\pm$0.16% (p-value=0.4430). However, the second preliminary study of U-74389G significantly rised the MCHl by 1.37%$\pm$0.37% (p-value=0.0005). 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 4.362893-fold erythropoietic potency than Epo (p-value=0.0000). **Conclusions:** The anti-oxidant capacities of U-74389G accelerate the acute erythropoietic properties; presenting 4.362893-fold erythropoietic rise than epo (p-value=0.0000).

Keywords: Hypoxia; Erythropoietin; U-74389G; Mean corpuscular hemoglobin levels; Reoxygenation.

1. Introduction

The acute erythropoietic capacity [1] of U-74389G is thus sagnificant (p-value=0.0005). U-74389G is a novel antioxidant factor. It implicates just only 256 known biomedical studies at present. 4.29% of these studies concern tissue hypoxia and reoxygenation (HR) experiments. The promising effect of U-74389G in tissue protection has been noted in these HR studies. U-74389G or also 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 which prevents both arachidonic acid-induced and iron-dependent lipid peroxidation. It protects against HR injury in animal heart, liver and kidney models. These membrane-associating antioxidants are particularly effective in preventing permeability changes in brain microvascular endothelial cells monolayers. Lazaroids, a novel series of glucocorticoid compounds 21-aminosteroids have the properties of free radical scavenging. U-74389G is one of the 132 similar lazaroid compounds. It has a molecular weight of 726.90406 g/mol; it has a selective action on vascular endothelium with vitamin E-like properties.

However, the erythropoietic capacity of U-74389G gets more comprehensible whether is compared with the same capacity of a standard known drug. Such one of the most well studied drug; actually with original erythropoietic capacity (p-value=0.4430) is erythropoietin (Epo). Indeed, Epo implicates over 30,063 known

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biomedical studies at present. 10.55% at least of these studies concern tissue hypoxia and reoxygenation (HR) experiments. Certainly, the concept has been moved away from the original action of Epo in stem blood cells recovery. However, just few related reports were found, not covering completely the specific matter with antioxidant factors.

The special aim of this experimental work was to compare the acute erythropoietic capacities of U-74389G and Epo on a rat model and mainly in an HR protocol. Their effects were tested by measuring the mean corpuscular hemoglobin (MCH) levels.

2. Materials and Methods

2.1. Animal Preparation

The Vet licenses of the research were provided under 3693/12-11- 2010 & 14/10-1-2012 decisions. The granting company and the place of experiment are mentioned in related references [1, 2]. Appropriate humanistic care were adopted for Albino female Wistar rats. 7 days pre-experimental normal housing included *ad libitum* diet in laboratory. Continuous intra-experimental general anesthesia, oxygen supply, electrocardiogram, acidometry and post-experimental euthanasia were provided. Rats 16 – 18 weeks old were randomly delivered to six (6) groups (n=10), using the following protocols of HR: Hypoxia for 45 min followed by reoxygenation for 60 min (group A); hypoxia for 45 min followed by reoxygenation for 120 min (group B); hypoxia for 45 min followed by immediate Epo intravenous (IV) administration and reoxygenation for 60 min (group C); hypoxia for 45 min followed by immediate Epo IV administration and reoxygenation for 120 min (group D); hypoxia for 45 min followed by immediate U-74389G intravenous (IV) administration and reoxygenation for 60 min (group E); hypoxia for 45 min followed by immediate U-74389G IV administration and reoxygenation for 120 min (group F). The dose height selection criteria of Epo and U-74389G were assessed at preliminary studies as 10 mg/Kg body mass of animals for both drugs.

Hypoxia was caused by laparotomic clamping inferior aorta over renal arteries with forceps for 45 min. The clamp removal was restoring the inferior aorta patency and reoxygenation. After exclusion of the blood flow, the protocol of HR was applied, as described above for each experimental group. The drugs were administered at the time of reperfusion; through inferior vena cava catheter. The MCH levels (MCHl) were determined at 60th min of reoxygenation (for A, C and E groups) and at 120th min of reoxygenation (for B, D and F groups).The animals' mass was a confusing factor for MCHl presenting a very powerful relation (p-value=0.0000).

3. Statistical Analysis

Table 1 presents the (%) augmentation influence of Epo regarding reoxygenation time. Also, Table 2 presents the (%) augmentation influence of U-74389G regarding reoxygenation time. Chi-square tests was 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].

4. Results

The successive application of chi-square tests revealed that U-74389G accelerated erythropoiesis by 151.125-fold [149.2245-153.0498] than Epo at 1h, by 4.246814-fold [4.238199-4.255447] at 1.5h, by 2.709729-fold [2.7056-2.713864] at 2h, by 1.177347-fold [1.17419-1.180513] without drugs and by 4.362893-fold [4.350927-4.374891] whether all variables have been considered (p-value=0.0000).

5. Discussion

The unique available study investigating the rising effect of U-74389G on MCHl 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, SOD, and 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 generally confirmed [2] the short or long-term erythropoietic effect of various Epo preparations in 29 IR laboratory or clinical biomedical studies on human individuals or animals. Karalyan, *et al.* [3] observed that the MCHl were significantly decreased than control, whereas the levels of Epo were significantly increased in African swine fever infected pigs. Sharma JB et al found serum Epo levels [4] significantly 2.56-fold higher in severe anemia, 1.51-fold higher in moderate anemia and 1.25-fold higher in mild anemia in anemic pregnant patients (p=0.064). Ichii, *et al.* [5] observed chronic anemia without any changes in the MCHl in female cotton rats (Sigmodon hispidus) 5 - 9 months old. Zubrikhina, *et al.* [6] noticed that the Epo level corresponded to the degree of anemia and was 4-5-fold the normal values; whereas, the Epo did not correspond to the degree of anemia in 74% of anemia of chronic diseases patients. Noguchi-Sasaki M et al showed high Epo levels and lower values of MCHl in LC-06-JCK-bearing mice than non-tumor-bearing (NTB) ones [7] through the overproduction of IL-6 which elevate the serum hepcidin levels assumption. Wu and Tsai [8] showed that the mean blood test values were significantly increased after switching oral ferric (139.02±49.39 mg) to ferrous (96.34±23.43 mg) ($p<0.01$) with the exception of MCHl in all iron deficiency anemia patients. Ito, *et al.* [9] calculated the MCHl in group continuous

erythropoiesis receptor activator (CERA) decreased than that in group darbepoetin$_{\alpha}$ in non-dialysis chronic kidney disease (CKD) patients. Somo, *et al.* [10] did not associate the declined MCH concentrations over the postweaning fast with their body reserves or EPO in juvenile phocid seals. Kaze, *et al.* [11] found the MCHl stable during follow-up although 29.5% of patients were receiving Epo on chronic hemodialysis. Kaliev, *et al.* [12] used Epo and its combination with hypoxic altitude chamber training in order to evaluate the efficiency of treatment for renal anemia in patients with chronic glomerulonephritis. Musalaiah, *et al.* [13] concluded that high-sensitivity C-reactive protein (hs-CRP) can depress Epo production leading to minimally lowered values in MCHl secondary to chronic inflammatory periodontal disease. Miller [14] showed increased the MCHl in the red cells of TRPC2 knockout mice since TRPC2 is highly expressed and is both activated by Epo and regulates the Epo-stimulated calcium influx in murine erythroid cells. Ribeiro, *et al.* [15] observed significant responses for the Epo TT genotype in MCHl in runners before and after 14 days of 400 mg pequi oil supplementation. Beiraghdar, *et al.* [16] showed a significant increase in MCHl during the course of a trial in both Epolyrec ($p = 0.041$) and Eprex ($p = 0.036$) groups correcting post-transplantation anemia (PTA). Zhou, *et al.* [17] decreased the MCHl after five-week treatment with total flavonoids of seabuckthorn (Hippophae rhamnoides L.) on cobalt chloride and hypobaric chamber (simulating 5 km) induced high altitude polycythemia in rats. Hirschler-Laszkiewicz, *et al.* [18] associated the reduced Epo-induced entrance in intracellular calcium (Ca^{++}_{i}) through transient receptor potential (TRP) channels TRPC2 and TRPC3 with the significantly greater MCHl in Trpc2 and Trpc2/Trpc3 double knockout mice. Haddad, *et al.* [19] examined the effect of intraperitoneal injections of 40 mg/kg of the lazaroid compound U-74389G every 12 hours, on acute otitis media in guinea pigs. Streptococcus pneumoniae organisms were inoculated into the right tympanic cavity; with the left ear served as a control one.

According to above, table 3 shows that U-74389G accelerated by 4.362893-fold [4.350927-4.374891] the erythropoietic potency 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 12 other hematologic variables, provides comparable results (table 4).

6. Conclusion

The anti-oxidant capacities of U-74389G accelerated by 4.362893-fold [4.350927-4.374891] the erythropoietic potency than Epo (p-value=0.0000) in Epo non-deficient rats. This trend is attenuated along the short term time frame of the experiment.

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Appendix

Table-1. The (%) augmentation influence of erythropoietin in connection with reoxygenation time

Augmentation	±SD	Reoxygenation time	p-value
0.01%	±0.00%	1h	0.9904
0.58%	±0.29%	1.5h	0.3549
1.15%	±0.58%	2h	0.1509
-0.58%	±0.29%	reperfusion time	0.3721
0.31%	±0.16%	interaction	0.4430

Table-2. The (%) augmentation influence of U-74389G in connection with reoxygenation time

Change	±SD	Reoxygenation time	p-values
1.82%	±1.01%	1h	0.0663
2.47%	±0.59%	1.5h	0.0001
3.12%	±0.65%	2h	0.0003
-0.49%	±0.25%	reoxygenation time	0.4103
1.37%	±0.37%	interaction	0.0005

Table-3. The U-74389G / erythropoietin efficacies ratios on RBC counts augmentation after chi-square tests application

Odds ratio	[95% Conf. Interval]	p-values	Endpoint
151.125	149.2245 153.0498	0.0000	1h
4.246814	4.238199 4.255447	0.0000	1.5h
2.709729	2.7056 2.713864	0.0000	2h
1.177347	1.17419 1.180513	0.0000	reperfusion time
4.362893	4.350927 4.374891	0.0000	interaction

Table-4. A U-74389G / erythropoietin efficacies ratios meta-analysis on 12 hematologic variableς (10 variables with balancing efficacies and 2 variables with opposite efficacies) [20]

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
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
RBC count	0.961059	0.0000	1.733395	0.0000	6.519657	0.0000	1.039524	0.0000	1.309673	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
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	7.91129943	0.0378	3.10924261	0.0000	3.90159936	0.0000	1.1749027	0.0218	4.01004182	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