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JOURNAL OF SCIENTIFIC ACHIEVEMENTS

RESEARCH ARTICLE

VOLUME 2, ISSUE 11, NOVEMBER 2017, PAGE: 18-21

Comparison of the Attenuating Capacities of Erythropoietin and U-74389G Concerning Blood Platelet Counts

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Abstract

Aim: This study compared the attenuatin effects of erythropoietin (Epo) and antioxidant drug U-74389G based on 2 preliminary studies. The provided results on blood platelet counts were co-evaluated in a hypoxia reoxygenation protocol of an animal model.

Materials and methods: Blood platelet counts 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 recommended a non-significant attenuating effect of Epo (p-value=0.9725). The second preliminary study also recommended a more powerful attenuating effect of U-74389G (p-value=0.0857). 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 at least 37-fold more attenuating action than Epo (p-value=0.0000).

Conclusions: The hematologist must be informed about the effective attenuating potencies of U-74389G in related coagulant and clotting situations.

Keywords: *hypoxia; erythropoietin; U-74389G; platelet count; reoxygenation*

Citation to This Article: Tsompos C, Panoulis C, Toutouzas K, Triantafyllou A, Zografos GC, Papalois A. Comparison of the Attenuating Capacities of Erythropoietin and U-74389G Concerning Blood Platelet Counts. Journal of Scientific Achievements, November 2017; 2 (11): 18-21.

1. INTRODUCTION

The short-term attenuating[1] action of U-74389G is not significant (p-value=0.0857). U-74389G is a novel antioxidant factor. It implicates just only 255 known biomedical studies at present. 4.31% 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 organs as heart, liver and kidneys. These membrane-associating antioxidants are particularly effective in preventing permeability changes in brain microvascular endothelial cells monolayers. The biochemical properties of U-74389G were reviewed as complete or partial lipid peroxidation (LPO) products attenuation and lung DNA synthesis reduction, consistent with a role for hydroxyl radicals (HO^{-}) or lipid hydroperoxides as second messengers in normal regulation of lung growth. U-74389G up-regulates CYP3A6 but inhibits its catalytic activity, prevents the hepatic malondialdehyde (MDA) enhancement and prevents CYP1A1/2 down-regulation and activity decrease by a double mechanism: hindering the release of serum mediators and by averting intracellular events. The action mechanism of U-74389G can be linked by its $O^{=}$ concentration decline. GS-Pt complex as a major metabolite from loss of both protein –SH and the thiol groups of GSH, in platelet cytosol, was found to induce the very active platelet LPO, measured as $O^{=}$ generation. It accelerates the clearance of $O^{=}$ by the increased superoxide dismutase (SOD) and sulfhydryl group which inhibit platelet aggregation by TXA2/PGI2 ratio regulation in plasma. It can effectively scavenge superoxide radicals in a surrounding of increased spontaneous platelet aggregation with elevated blood reactive oxygen species

(ROS) and OH^- levels and increased superoxide production of leukocytes ($p < 0.05$). It rivals the $O^=$ reaction products (MDA), the red cell superoxide dismutase, the collagen levels increase, the spontaneous whole blood platelet aggregation ($p < 0.05$) and the plasma thiol levels reduce. It effectively scavenges $O^=$ by SOD. It resembles tPA which rivals the activated increased neutrophil count (aggregation, $O^=$). It decreases the plasma matrix metalloproteinase (MMP) –9 activities. Platelet disaggregation and inactivation are accompanied by a decrease in OH^- levels formation, by TxB2 degradation and protein kinase C (PKC) translocation from cell membrane to the cytosol. However, the attenuating capacity of U-74389G gets more comprehensible whether is compared with the same capacity of a standard known drug. Such one of the more well studied drug; whereas without significant attenuating[2] action (p-value=0.9725) is erythropoietin (Epo). Actually, Epo implicates over 29,754 known biomedical studies at present. 10.47% at least of these studies concern tissue hypoxia reoxygenation experiments. Since the concept has been moved away from the original action of Epo in stem red blood cells recovery, just few related reports were found.

The special aim of this experimental work was to compare the attenuating effects of U-74389G and Epo on a rat model and mainly in an HR protocol. Their effects were tested by measuring the blood platelet counts.

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 the experiment are mentioned in related references[1,2]. Accepted standards of human animal care were adopted for Albino female Wistar rats. 7 days pre-experimental normal housing included *ad libitum* diet in laboratory. Continuous intra-experimental anesthesiologic techniques, oxygen supply, electrocardiogram and acidometry were provided. Euthanasia did not permit post-experimental awakening and preservation of animals. 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. Reoxygenation was induced by removing the clamp and restoration the inferior aorta patency. 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 reoxygenation; through catheterized inferior vena cava. The blood platelet counts 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).

2.2 Statistical analysis

Table 1 presents the (%) attenuating influence of Epo regarding reoxygenation time. Also, Table 2 presents the (%) attenuating influence of U-74389G regarding reoxygenation 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 blood platelet counts used were not adjusted for rats' mass since a powerless relation was invented between them (p-value=0.1200). The statistical analysis was performed by Stata 6.0 software [Stata 6.0, StataCorp LP, Texas, USA].

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

Decrease	±SD	Reoxygenation time	p-values
7.32%	±13.11%	1h	0.52
2.13%	±8.04%	1.5h	0.75
-3.04%	±10.78%	2h	0.72
-0.31%	±7.89%	Reperfusion time	0.96
0.16%	±4.76%	interaction	0.97

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

Decrease	±SD	Reoxygenation time	p-values
17.79%	±9.40%	1h	0.064
12.83%	±5.79%	1.5h	0.030
7.88%	±7.83%	2h	0.29
0.16%	±6.07%	Reoxygenation time	0.97
6.12%	±3.58%	interaction	0.085

Table 3. The U-74389G / erythropoietin attenuating efficacies ratios on blood platelet counts after chi-square tests application

Odds ratio	[95% Conf. Interval]	P-values	Endpoint
2.42	2.35-2.50	<0.0001	1h
6.00	5.99-6.01	<0.0001	1.5h
6.13	6.12-6.14	<0.0001	2h
3.93	3.93-3.94	<0.0001	Reperfusion time
37.62	37.57-37.68	<0.0001	interaction

Table 4. A U-74389G / erythropoietin efficacies ratios meta-analysis on 6 variables with balancing efficacies.[11,12,13]

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
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
Mean	4.17003275		2.52576476	0.0000	4.36043049	0.0000	1.31464605		2.93353507	0.0000

Table 5. A U-74389G / erythropoietin efficacies ratios meta-analysis on 2 hematologic variables with opposite efficacies.

Endpoint Variable	1h	p-value	1.5h	p-value	2h	p-value	Reperfusion time	p-value	interaction	p-value
Mean corpuscular hemoglobin concentrations	-0.27	<0.0001	-0.55	<0.0001	-0.85	<0.0001	+3.04	<0.0001	-0.77	<0.0001
Platelet crit	-0.23	<0.0001	-0.67	<0.0001	-1.33	0.08	5.62	<0.0001	-0.97	<0.0001
Mean	-0.25	<0.0001	-0.60	<0.0001	-1.06	0.04	4.13	<0.0001	-0.87	<0.0001

3. RESULTS

The successive application of chi-square tests revealed that the attenuating capacity of U-74389G was superior than that of erythropoietin by 2.42-fold [2.3-2.50] at 1h, by 6.00-fold [5.99-6.01] at 1.5h, by 6.13-fold [6.12-6.14] at 2h, by 3.93-fold [3.93-3.94] without drugs and by 37.62-fold [37.57-37.68] whether all variables have been considered (p-value<0.0001).

4. DISCUSSION

The same authors reviewing[2] 11 clinical trials, found 3 trials in which Epo had attenuating effect and 8 trials with increasing effect on platelet counts. The majority of these trials concerned long-term study times. The hypothesis of competition between precursor cells of the erythrocytic and megakaryocytic cell lines (stem-cell competition) as the cause of thrombocytopenia in Epo-treated individuals is valid for short-term study times until 5 days. Furthermore, Hache G et al inhibited[3] the ARA290 [a specific agonist of EPOR/CD131 complex, on a subpopulation of endothelial progenitor cells named endothelial colony-forming cells (ECFCs) after peripheral ischemia] -induced improvement of homing prior neutralization of platelet-endothelial cell adhesion molecule-1 (CD31) expressed by the transplanted cells. Fauchère JC et al found[4] significantly a lower platelet count at day 7-10, in the high dose recombinant human erythropoietin (rhEpo) group given shortly after birth for neuroprotection and subsequently over the first 2 days in very preterm infants. Benders MJ et al found the platelet counts in the normal range[5] after recombinant human erythropoietin (rhEPO) 3000 IU/kg administration given in total during a 3-day period in neonates with perinatal arterial ischemic stroke. Siasios I. et al associated[6] the reversible autoregulatory vasculopathy of aneurysmal subarachnoid hemorrhage vasospasm with hypovolemia including platelet count. Yanagawa T et al confirmed[7] that epo induce hypertension, polycythemia, and platelet activation in patients with cardiovascular diseases. Gan Y et al completely abolished[8] the erythropoietic and platelet-stimulating activity of EPO after the serine to isoleucine mutation at position 104 (S104I-EPO) of Epo. Ulusoy S et al classified[9] the signal peptide-CUB (complement C1r/C1s, Uegf, and Bmp1)-EGF (epidermal growth factor)-domain-containing protein 1 (SCUBE1) as a cell surface protein belonging to the SCUBE gene family and rising in parallel with platelet activation in acute ischemic events.

The same authors noted that the attenuating effect of U-74389G on platelet count could exert a potent beneficial effect on a range of diseases such as vascular thrombosis, myocardial cell membrane, attacks of angina pectoris, myocardial ischemia, atherosclerosis and coronary artery disease, percutaneous transluminal coronary angioplasty, spinal cord tissue and brain injuries, diabetic neuropathy, skin flap and renal transplants, glomerulonephritis, bronchopulmonary dysplasia, inflammations, even in mental stress. Pratt MF et al demonstrated[10] the mechanisms of skin flap failure including the alteration of platelet function with resultant accumulation of damaging oxygen-free radicals in a pig model. Random skin flap survival was improved significantly after U-74389G administration.

According to above, table 3 shows that U-74389G has at least 37-fold attenuating capacity than Epo (p-value=0.0000). A more detailed molecular and biochemical investigation of this attenuating potency must be hold in order to elucidate the U-74389G

action mechanism. A meta-analysis of these ratios from the same experiment, for 8 other hematologic variables, provides comparable results (tables 4,5).

4.1 Conclusion

The hematologist must be informed about the effective attenuating potency of U-74389G in related coagulant and clotting situations.

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