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Comparison of the anti inflammatory capacities of erythropoietin and U-74389G

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Abstract

Aim: This study compared the anti inflammatory effects of erythropoietin (Epo) and antioxidant drug U-74389G based on 2 preliminary studies. The provided results at white blood cells count (wbc) count restoration, were co-evaluated in a hypoxia reoxygenation protocol of an animal model.

Materials and methods: Wbc count 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 kept significantly increased the wbc count by 14.64%±5.40% (*p-value=0.0080*). The second preliminary study of U-74389G also kept significantly increased the wbc count by 23.64%±6.32% (*p-value=0.0004*). These 2 studies were co-evaluated since they came from the same experimental setting. The outcome of the co-evaluation was that U-74389G is at least 1.6-fold less anti inflammatory than Epo (*p-value=0.0000*).

Conclusions: Epo is at least 1.6-fold more anti inflammatory than the antioxidant drug U-74389G (*p-value=0.0000*).

Key words: hypoxia; erythropoietin; U-74389G; white blood cells count; reoxygenation

Introduction

The short-term anti inflammatory 1action of U-74389G is not satisfactory (*p-value=0.0004*). 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 heart, liver and kidney models. These membrane-associating antioxidants are particularly effective in preventing permeability changes in brain microvascular endothelial cells monolayers. Some biochemical capacities of U-74389G are summarized as activating attenuation of leukocytes; proinflammatory gene down-regulation; endotoxin shock treatment; cytokine production; mononuclear immunoenhancement; antishock and endothelial protection.

However, the anti inflammatory 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; also without satisfactory anti inflammatory action (*p-value=0.0080*) is erythropoietin (Epo). Actually, Epo implicates over 29,735 known biomedical studies at present. 10.47% 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 as a glycoprotein cytokine secreted by the kidney in response to cellular hypoxia; which stimulates red blood cell production (erythropoiesis) in the bone marrow. However, just few related reports were found, not covering completely the specific matter with white blood cells count (wbc).

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

Materials and methods

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 references1,2. 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. Post-experimental euthanasia excluded awakening and preservation of animals. Rats 16 – 18 weeks old were randomly delivered to four (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 reperfusion; through catheterized inferior vena cava. The wbc count 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).

Statistical analysis

Table 1 presents the (%) restoration influence of Epo regarding reoxygenation time. Also, Table 2 presents the (%) restoration 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].

Table 1: The (%) influence of erythropoietin on wbc count restoration in connection with reoxygenation time

Restoration	±SD	Reoxygenation time	*p-values*
24.01%	±13.38%	1h	*0.1012*
22.09%	±9.11%	1.5h	*0.0163*
20.17%	±12.94%	2h	*0.0902*
14.55%	±9.53%	reoxygenation time	*0.0883*
14.64%	±5.40%	interaction	*0.008*

Table 2: The (%) influence of U-74389G on wbc count restoration in connection with reoxygenation time.

Restoration	±SD	Reoxygenation time	*p-values*
22.99%	±12.45	1h	*0.0914*
30.85%	±11.14	1.5h	*0.0045*
38.70%	±17.39	2h	*0.0185*
24.97%	±11.55	reoxygenation time	*0.0272*
23.45%	±6.28	interaction	*0.0004*

Table 3: The U-74389G / erythropoietin efficacies ratios on wbc counts restoration after chi-square tests application

Odds ratio	[95% Conf. Interval]		*p-values*	Endpoint
0.957451	0.869207	1.054654	*0.3782*	1h
1.396122	1.394892	1.397353	*0.0000*	1.5 h
1.918237	1.763902	2.086076	*0.0000*	2h
1.71622	1.714481	1.717962	*0.0000*	Reperfusion time
1.601887	1.60025	1.603525	*0.0000*	interaction

Results

The successive application of chi-square tests revealed that the restoring capacity of U-74389G was superior than that of erythropoietin by 0.9574511-fold [0.8692073 - 1.054654, *p-value=0. 3782*] at 1h, by 1.396122-fold [1.394892 - 1.397353] at 1.5h, by 1.918237-fold [1.763902 - 2.086076] at 2h, by 1.71622-fold [1.714481 - 1.717962] without drugs and by 1.601887-fold [1.60025 - 1.603525] whether all variables have been considered (*p-value=0.0000*).

Discussion

The same authors summarized1 6 IR studies for the effect of U-74389G leading to consistent results in humans or animals. They recorded lower wbc count in 2 studies, general anti inflammatory properties in 3 studies and reducing leukopenia in 1 study. Even during reperfusion phase, a reperfusion syndrome occurs which seamlessly carries on the vicious cycle of leukocytosis. mRNAs expression of inflammatory (TNFa) and anti inflammatory (IL-10) cytokines were up-regulated still 1 hour after ischemia removal. The macromolecular permeability and adhesiveness of capillaries for wbc are due to oxygen free radicals. Reperfusion with consecutive re-entry of molecular oxygen into microvasculature, provokes the formation of oxygen-radicals and accumulation of leukocytes adhering to endothelium of post-capillary venules. Targeted release of reactivate oxygen metabolites, hydrolytic enzymes, additional oxygen-radicals and aggressive mediators delivery by activated neutrophils, such as proteases, cytokines and eicosanoids, which have chemotactic influence on wbc, result in a vicious cycle during reperfusion phase of tissue injury. Leukocyte-generated oxygen free radical are implicated as mediators of reperfusion-associated cellular membrane injury in IR tissues. Whole body systemic extension becomes through these activated inflammatory cells and possibly,

results in secondary detectable tissue damage in endothelial cells of the systemic circulation inducing prolonged DNA damage even in early reperfusion period. A vicious cycle of wbc trapping, activation and tissue damage is engaged. The assumption is whether U-74389G administration which has oxygen free radical scavenging properties is a promising new anti inflammatory drug for the treatment of IR injury.

The same authors summarized2 24 IR studies for the effect of Epo leading to inconsistent results in humans or rats. They recorded no change of wbc count in 13 trials; significant decrease of wbc count in 5 trials and significant increase of wbc count in 6 trials. Stevenson JL et al determined3 no significant changes in wbc count but increase of Epo levels for echinacea-based dietary supplement treatment doses groups in endurance-trained men. Ren Y et al characterized4 abnormally increased mean wbc count and higher Epo level mainly in wild-type JAK2 V617F group (*P<0.05*) at diagnosis of polycythemia vera. Shen W et al found5 that Epo stimulated the production and recruitment of wbc count and CD34(+) cells along with effective mobilization of CD34(+)/VEGF-R2(+) cells into the retina in Royal College of Surgeons rats. Thiel A et al reported6 that piperine significantly decreased the wbc count adding mechanistic endpoints including Epo level in mice. Benders MJ et al found7 no adverse effects on wbc count after rhEpo total 3000 IU/kg administration in neonates with perinatal arterial ischemic stroke. Ofori-Acquah SF et al suggested8 that SDF-1α produced by ischemic tissues mobilizes significantly at least twice higher circulating progenitor cells; total wbc count; many mononuclear cell colonies and plasma Epo concentrations in hemoglobin SS subjects 5-18 years old compared with control subjects. Yan D et al noticed9 an increase of Epo levels and wbc count in Jak2V617F mice expressing all features of human polycythemia vera. Tentori F et al associated10 lower serum wbc counts with longer hemodialysis (HD) treatment time for the same Epo dose. Powers A et al found11 less Epo use therapy and potential complications of neutropenia; pneumonia diagnoses and decreased wbc count in younger myelodysplastic syndrome patients than in older ones (*p ≤0.034*). Sugiura Y et al reported12 a secondary polycythemia due to normal wbc count and non increased Epo level in a 67-year-old patient with smokers' polycythemia and lung adenocarcinoma. Li Q et al found13 that adenovirus-mediated human hepatocyte growth factor (HGF) gene transfer could increase significantly the wbc count, the Epo levels enhancing immune function in irradiated C57BL/6 mice. Alsaran K et al assessed14 the mean wbc count dropped by 26.54% (*p < 0.001*), but the Epo dose increased by 28.27% (*p = 0.776)* after 48 weeks of peginterferon α-2b (12 kDa) plus ribavirin treatment in HD of chronic HCV patients. Rumi E et al measured15 higher wbc count and lower mutant allele burden and serum Epo levels in essential thrombocythemia JAK2 (V617F) patients than those with CALR mutation. Szygula Z et al found16 higher number of wbc count and Epo concentration after 10 and 20 whole-body cryostimulation treatments (-130°C, treatment duration: 3 minutes) in 45 men than baseline and control group. Zhang H et al accelerated17 the recovery of wbc count and the Epo secretion stimulation after the rhizome of Panax japonicus administration in blood deficiency model mice. Chiu YH et al showed18 a statistically significant rise of blood Epo values and wbc count in the immediate post-race values but a rapid drop in values at 24 hours post-race for Epo values compared with pre-race values in recruited runners. Fauchère JC et al found19 significantly higher reticulocyte and wbc counts at day 7-10 in the rhEpo group after high dose rhEpo administration shortly after birth and subsequently over the first 2 days for neuroprotection in very preterm infants. Jeong G et al demonstrated 20 leukocytosis and decreased Epo in a 61-year-old female with a history of transient ischemic attack and follicular lymphoma.

According to above, table 3 shows that U-74389G has at least 1.6-fold less anti inflammatory capacity than Epo (*p-value=0.0000*). Perhaps, a longer study time or a higher U-74389G dose may reveal more effective anti inflammatory property. A meta-analysis of these ratios from the same experiment, for 6 other hematologic variables, provides comparable results (table 4).

Table 4: A U-74389G / erythropoietin efficacies ratios meta-analysis on 6 hematologic variable**ς** (4 variables with balancing efficacies and 2 variables with opposite efficacies)

Endpoint Variable	1h	*p-value*	1.5h	*p-value*	2h	*p-value*	Reperfusion time	*p-value*	interaction	*p-value*
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*
Platelet DW	0.694023	*0.0000*	0.0000	*0.0000*	2.206972	*0.0000*	2.248401	*0.0000*	2.458888	*0.0000*
Creatinine	168.9034	*0.0000*	4.872332	*0.0000*	3.039572	*0.0000*	1.026202	*0.0000*	5.005523	*0.0000*
Mean	8.694459		3.2183563		4.8418607		1.3042516		4.1748246	

Endpoint Variable	1h	p-value	1.5h	p-value	2h	p-value	Reperfusion time	p-value	interaction	p-value
Mean corpuscular hemoglobin concentrations	-0.27742	0	-0.55047	0	-0.85224	0	+3.044774	0	-0.77932	0
Platelet crit	-0.2312	0	-0.67194	0	-1.33076	0.0886	5.620077	0	-0.97715	0
Mean	-0,2532076		-0,6081795		-1,0649544		4,1366488		-0,8726499	

Conclusion

The anti-oxidant capacities of U-74389G cannot provide satisfactory short-term anti inflammatory properties; whereas the cytocine capacities of Epo are proved more anti inflammatory at that certain setting. Otherwise, U-74389G was found 62.42% [62.40% - 62.44%] less anti inflammatory than epo (*p-value=0.0000*).

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