

# Investigation of the Opposite Capacities of Erythropoietin and U-74389G on Platelet Crit Levels

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## Abstract

**Aim:** This study calculated the opposite capacities of 2 drugs: the erythropoietin (Epo) and the antioxidant drug U-74389G. The calculation was based on the results of 2 preliminary studies, each one of which estimated the platelet crit (PCT) levels alterations, after the respective drug usage in an induced hypoxia reoxygenation animal experiment.

**Materials and methods:** The 2 main experimental endpoints at which the PCT levels (PCTl) were evaluated were the 60th reoxygenation min (for the groups A, C and E) and the 120th reoxygenation 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 U-74389G administration.

**Results:** The first preliminary study of Epo non significantly decreased the PCTl by  $6.88\% \pm 3.69\%$  ( $p$ -value=0.0615). Also, the second preliminary study of U-74389G non-significantly increased the PCTl by  $6.73\% \pm 37.45\%$  ( $p$ -value=0.0712). 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 opposite acute effect than Epo ( $p$ -value=0.0000).

**Conclusion:** The anti-oxidant capacities of U-74389G enhance the acute increasing properties on PCTl than epo ( $p$ -value=0.0000).

**Keywords:** Hypoxia; Erythropoietin; U-74389G; Platelet crit; Reoxygenation

## Introduction

U-74389G is not famous for its increasing [1] capacity ( $p$ -value=0.0712). U-74389G as a novel antioxidant factor, implicates exactly only 255 published studies. The hypoxia reoxygenation (HR) type of experiments was noted in 4.31% of these studies. A tissue protective feature of U-74389G was obvious in these HR 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 HR 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 acute decreasing action ( $p$ -value=0.0615), it can be used as a reference drug in order a related capacity of U-74389G to become comprehensible. Although Epo is met in over 29,645 published biomedical studies, only a 10.48% of them negotiate the known type of HR experiments. Nevertheless, Epo as a cytokine, never goes out of the jurisdiction of the PCTl study. This experimental work tried to compare the kind of effects of the above drugs on a rat induced HR protocol. They were tested by calculating the serum PCTl 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 anaesthesiology techniques, the acidometry, the electrocardiogram and 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 45min hypoxia was common for all 6 groups. Afterwards, reoxygenation of 60min was followed in group A; reoxygenation of 120min in group B; immediate Epo intravenous (IV) administration and reoxygenation of 60min in group C; immediate Epo IV administration and reoxygenation of 120min in group D; immediate U-74389G IV administration and reoxygenation of 60min in group E; and immediate U-74389G IV administration and reoxygenation of 120min in group F. The dose height assessments for both drugs are described at preliminary studies as 10mg/Kg body mass.

Hypoxia was caused by laparotomic clamping the inferior aorta over renal arteries by forceps for 45min. 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 PCT levels (PCTI) 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). No relation was found between PCTI with animal's mass (p-value=0.5401).

### Statistical analysis

Table 1 presents the (%) declining influence of Epo regarding reoxygenation time. Also, Table 2 presents the (%) increasing 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 statistical analysis was performed by Stata 6.0 software [Stata 6.0, Stata Corp LP, Texas, USA].

**Table 1:** The (%) declining influence of erythropoietin in connection with reoxygenation time.

Decrease	±SD	Reoxygenation Time	p-Value
-16.47%	±26.13%	1h	0.0921
-13.74%	±21.07%	1.5h	0.0158
-11.01%	±15.50%	2h	0.0882
+0.67%	±24.05%	reperfusion time	0.9083
-6.88%	±3.69%	interaction	0.0615

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

Increase	±SD	Reoxygenation Time	p-Values
+3.80%	±21.58%	1h	0.6373
+9.23%	±21.89%	1.5h	0.1064
+14.66%	±22.31%	2h	0.0833
+3.76%	±20.02%	reperfusion time	0.4803
+6.73%	±37.45%	interaction	0.0712

**Table 3:** The U-74389G/erythropoietin efficacies ratios on PCTI after chi-square tests application.

Odds Ratio	[95% Conf. Interval]		p-Values	Endpoint
-0.2312044	-2.667818	-.200372	0.0000	1h
-0.6719365	-6.726743	-.6711995	0.0000	1.5h
-1.330756	-1.848376	-.9580512	0.0886	2h
5.620077	5.601274	5.638943	0.0000	Reperfusion time
-0.9771515	-.9784991	-.9758057	0.0000	Interaction

### Results

The successive application of chi-square tests revealed that U-74389G accentuated the PCTI by -.2312044-fold [-2.667818 - -.200372] than Epo at 1h, by -.6719365-fold [-6.726743 - -.6711995] at 1.5h, by -1.330756-fold [-1.848376 - -.9580512] at 2h, by 5.620077-fold [5.601274 - 5.638943] without drugs and by -0.9771515-fold [-.9784991 - -.9758057] whether all variables have been considered (p-value=0.0000).

### Discussion

The unique available study investigating the increasing effect of U-74389G on PCTI 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  $\gamma$ GT, 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 confirmed [2] the short-term declining effect of Epo preparations in non iron deficient rats. Rajajee et al. [3] considered acute liver failure patients typically coagulopathic and at risk for intracranial hemorrhage. Fealy et al. [4] associated the decreased platelet count (hazards ratio, 1.19; p = 0.03) with a reduced likelihood of circuit clotting repeated events in critically ill adults requiring continuous renal replacement therapy. Muzaffar et al. [5] could differentiate among normo coagulant, hypo coagulant, hyper coagulant states by thromboelastography unlike conventional coagulation assay which estimated the platelet count at  $153.96 \pm 99.16 (\times 10^3/\text{mm}^3)$ . Htet et al. [6] estimated almost 20% of critically ill patients having moderate-to-severe coagulopathy (platelets < 50k/uL). Shaver et al. [7] associated the elevated micro particle (MP) concentrations in the airspace during ARDS with activation of coagulation, reduced risk of ARDS and contribute to acute lung injury in critically ill patients. Etchill et al. [8] associated

the high platelets-to-packed RBCs ratio (>1:2) with decreased 48hour mortality 54.4% (p=0.032) in trauma patients. Mundt et al. [9] showed that differences in platelet counts are not formaldehyde exposure dependent since it induces damage to hematopoietic cells. Francischetti et al. [10] found that post-cannulation platelet counts among other variables also have discriminative power within 30days in surviving patients on ECMO. Jones et al. [11] claimed that packed platelets break down and undergo chemical changes during storage (known as the storage lesion) that lead to an inflammatory response once the blood components are transfused to patients deteriorating their outcome. Bruchim et al. [12] significantly (P <0.05) associated the haemostatic derangements of lower tPCA at 12hours post presentation with the 60% mortality in dogs with naturally occurring heatstroke. Pannu et al. [13] reversed rapidly and safely severe thrombocytopenia ( $\leq 20,000/mm^3$ ) secondary to dengue virus (DEV) infection by administration of a single dose of 50 $\mu g/kg$  (25 IU/kg) anti-D IV. Patients in the intervention group were achieving 9.85-fold more frequently the platelet count of  $\geq 50,000/mm^3$  than control group (P = 0.0019) at the end of 48h. Mohammed et al. [14] significantly increased PLTs intracellular ascorbic acid levels by 2.66-fold (Lo Vitamin C) and by 13.08-fold (Hi Vitamin C, P<0.05) after Vitamin C supplementation. Also, Vitamin C at the higher dose (3mmol/L) also induced the release of several eicosanoids including thromboxane B<sub>2</sub> and prostaglandin E<sub>2</sub>, as well as products of arachidonic acid metabolism via the lipoxygenases pathway such as 11-/12-/15-hydroxyicosatetraenoic acid (P <0.05). Marly Voquer et al. [15] ascribed the stronger clot in

the FIBTEM assay of rotational thromboelastometry at a stronger contribution of platelets in feline blood of people than cats. Aubron et al. [16] independently associated platelet transfusion with 5.5-fold more hospital-acquired infections in the ICU (p <0.01), with 2.56%-fold more infections (p <0.001). Navas Carrillo et al. [17] described the formation of luminal thrombi secondary to platelet activation and the release of thrombogenic elements within the atherosclerotic lesions in acute coronary syndromes. Appiah Kubi et al. [18] detected 36 oncogenic platelet-derived growth factor receptor (PDGFR) ETV6 (TEL)-PDGFRB and FIP1L1-PDGFRB fusions genes. 33 were as a result of chromosomal translocation, FIP1L1-PDGFRB and EBF1-PDGFRB were the result of chromosomal deletion and CDK5RAP2- PDGFRB was the result of chromosomal insertion in hematological malignancies. Asanuma et al. [19] estimated the mean values of PCT significantly lower and the TPO levels significantly higher in hemodialysis patients (HD) patients undergoing treatment with rHuEPO at 9000 IU/week than those in healthy controls. Haddad et al. [20] examined the effect of intra peritoneal injections of 40mg/kg of the U-74389G every 12 hours, on acute otitis media in guinea pigs. Streptococcus pneumonia 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 accentuated by -.9771515-fold [-.9784991 - -.9758057] the PCTI than Epo (p-value=0.0000); a trend accentuated along time, in Epo non-deficient rats. A meta-analysis of these ratios from the same experiment, for 4 other seric variables, provides comparable results (Table 4).

**Table 4:** A U-74389G / erythropoietin efficacies ratios meta-analysis on 4 hematologic variables (3 variables with balancing efficacies and 1 variable with opposite efficacies) [21].

Endpoint \ Variable	1h	p-Value	1.5h	p-Value	2h	p-Value	Reperfusion Time	p-Value	Interaction	p-Value
Hematocrit	38.424	0	9.076658	0	6.222898	0	1.001356	0.2184	12.66419	0
Haemoglobin	1.268689	0	1.839035	0	13.1658	0	1.252422	0	1.94889	0
Creatinine	168.9034	0	4.872332	0	3.039572	0	1.0262016	0	5.005523	0
Mean	20.1929009	0	4.33262345	0	6.29145057	0	1.08773713	0.0728	4.98048231	0

Endpoint Variable	1h	p-Value	1.5h	p-Value	2h	p-Value	Reperfusion Time	p-Value	Interaction	p-Value
Mean corpuscular haemoglobin concentrations	-0.2774225	0	-0.55047	0	-0.85224	0	+3.044774	0	-0.7793243	0

### Conclusion

The anti-oxidant capacities of U-74389G accentuated by -.9771515-fold [-.9784991 - -.9758057] the PCTI than Epo (p-value=0.0000) in rats. Also, this trend is accentuated along the short term time frame of the experiment.

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