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# Comparison of the Hypervolemic Capacities of Erythropoietin and U-74389G Concerning Mean Platelet Volumes Levels

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## ABSTRACT

**Aim:** *This study calculated the hypervolemic 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 mean platelet volumes (MPV) levels augmentation, after the respective drug usage in an induced hypoxia reoxygenation animal experiment.*

**Materials and methods:** *The 2 main experimental endpoints at which the MPV levels were evaluated was the 60$^{th}$ reoxygenation min (for the groups A, C and E) and the 120$^{th}$ 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 increased the MPVl by 1.60%±2.14% (p-value=0.4430). Also, the second preliminary study of U-74389G significantly increased the MPVl by 6.69%±1.81% (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 4.164431-fold more hypervolemic potency than Epo (p-value=0.0000).*

**Conclusions:** *The anti-oxidant capacities of U-74389G enhance the acute hypervolemic properties; presenting 4.164431-fold more hypervolemia on MPVl than epo (p-value=0.0000).*

**Keywords:** *hypoxia; erythropoietin; U-74389G; mean platelets volumes levels; reoxygenation*

## INTRODUCTION

U-74389G is not famous for its hypervolemic[1] capacity (p-value=0.0005). 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 hypervolemic action (p-value=0.4430), it can be used as a reference drug in order a

hypervolemic capacity of U-74389G to become comprehensible. Although Epo is met in over 29,852 published biomedical studies, only a 10.50% of them negotiate the known type of HR experiments. Nevertheless, Epo as a cytokine, never goes out of the jurisdiction of the mean platelet volumes study.

This experimental work tried to compare the hypervolemic effects of the above drugs on a rat induced HR protocol. They were tested by calculating the serum MPV levels augmentation.

## 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 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 45 min hypoxia was common for all 6 groups. Afterwards, reoxygenation of 60 min was followed in group A; reoxygenation of 120 min in group B; immediate Epo intravenous (IV) administration and reoxygenation of 60 min in group C; immediate Epo IV administration and reoxygenation of 120 min in group D; immediate U-74389G IV administration and reoxygenation of 60 min in group E; and immediate U-74389G IV administration and reoxygenation of 120 min in group F. The dose height assessment for both drugs are described at preliminary studies as 10 mg/Kg body mass.

Hypoxia 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 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 MPV levels (MPVl) 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).However, the predicted MPV values were used since a very powerful relation was rised with animals' mass (p-value=0.0005).

### Statistical Analysis

Table 1 presents the (%) hypervolemic influence of Epo regarding reoxygenation time. Also, Table 2 presents the (%) hypervolemic 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, StataCorp LP, Texas, USA].

**Table1.** *The (%) hypervolemic influence of erythropoietin in connection with reoxygenation time.*

| Hypervolemia | ±SD | Reoxygenation time | p-value |
|---|---|---|---|
| 0.06% | ±14.74% | 1h | 0.9904 |
| 2.96% | ±12.92% | 1.5h | 0.3549 |
| 5.87% | ±10.17% | 2h | 0.1509 |
| -2.96% | ±13.90% | reperfusion time | 0.3721 |
| 1.60% | ±2.14% | interaction | 0.4430 |

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

| Hypervolemia | ±SD | Reoxygenation time | p-values |
|---|---|---|---|
| 8.76% | ±13.32% | 1h | 0.0663 |
| 12.03% | ±11.53% | 1.5h | 0.0001 |
| 15.30% | ±8.77% | 2h | 0.0003 |
| -2.40% | ±10.93% | reperfusion time | 0.4103 |
| 6.69% | ±1.81% | interaction | 0.0005 |

## RESULTS

The successive application of chi-square tests revealed that U-74389G accentuated the hypervolemia by 145.8532-fold [144.4617-147.258] than Epo at 1h, by 4.053619-fold [4.051612-4.055626] at 1.5h, by 2.603947-fold [2.600845-2.607053] at 2h, by 1.2334644-fold [1.2309333-1.2360010] without drugs and by 4.164431-fold [4.155693-4.173187] whether all

variables have been considered (p-value=0.0000).

## DISCUSSION

The unique available study investigating the hypervolemic effect of U-74389G on MPVl 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 confirmed[2] the short-term hypervolemic effect of Epo preparations in non iron deficient individuals. Hong F et al significantly increased mean platelet volume after 9 consecutive months exposure to titanium dioxide nanoparticles ($TiO_2$ NPs)[3] in mice. Choi S et al suggested[4] diffuse white matter abnormalities in sickle cell disease (SCD) patients, especially in the frontal, parietal and temporal lobes, that are associated with low mean platelet volume levels. Yilmaz Avci A et al associated[5] hypoxia parameters with MPV and the severity of obstructive sleep apnea (OSA) ($P < 0.05$). Above the hypoxia threshold ($CT_{90} \geq 10\%$), MPV was increased significantly. Tekin M et al calculated the mean MPV values of the infants with meconium-stained amniotic fluid (MSAF)[6] statistically significantly lower than control ($p < 0.001$) healthy infants. The optimal cut-off value for the MPV was 9.90 fl (area under the curve [AUC: 0.788]) in infants with MSAF, with a sensitivity of 78.1% and a specificity of 74.3% possibly associated with a hypoxic process. Zicari AM et al provided evidence of higher MPV levels in children with Primary Snoring as well as in children with Obstructive Sleep Apnea Syndrome than healthy control ones. Poorey VK et al consider[8] MPV as one of the platelet activation index which reflects the platelet production rate and reemphasized the concept that MPV is increased in chronic nasal obstruction due to deviated nasal septum (DNS) and this increase is in accordance with the severity of deviated nasal septum. Hou J et al found that[9] only females with low MPV were at greater risk for having low MPV (OR=1.33, 95% CI=1.10, 1.62 ptrend<0.001) in the highest BMI groups than those who had low MPV in the corresponding lowest BMI group. Simsek G et al Adenotonsillar hypertrophy (ATH) is[10] the most common cause of obstructive sleep apnea in children. Mean platelet volume values were decreased remarkably in groups that underwent adenotonsillectomy. Unlu I et al[11] determined that as the duration of nasal obstruction was elongated the mean platelet volume values were increased. However, Tekin YB et al detected[12] no difference in mean platelet volume and SCUBE1 levels in adolescents with primary dysmenorrheal than normal group. Cengiz C et al associated[13] obstructive sleep apnea (OSA) caused by chronic tonsillitis -adenoid hypertrophy (CT-AH) which is one of the most common reasons of nocturnal hypoxia with low MPV values in childhood. Ulu S et al[14] found MPV levels significantly higher in patients with nasal septal deviation (NSD) than the control group. Moreover, this increase was found in relation with the severity of nasal airway obstruction. Dogan NO et al found[15] significantly higher the mean platelet volume levels in patients with cortical infarction and transient ischemic attack ($p < 0.001$ and $p < 0.002$), previous stroke ($p < 0.005$) and may be considered as an atherosclerotic risk factor. Sagit M et al[16] remarked that chronic hypoxia and hypercarbia show tendency for hypercoagulopathy. Mean platelet volume (MPV), the most commonly used measure of platelet size, is a potential marker of platelet reactivity. Large platelets that contain more dense granules are enzymatically and metabolically more active and have greater prothrombotic potential. Since the MPV levels were significantly higher in the marked (MNSD) group, it was also found that MPV levels were significantly decreased in MNSD group after septoplasty operation. Increased platelet activation may be related with increased cardiovascular risk in patients with MNSD. Haddad J Jr et al examined[17] the effect of intraperitoneal injections of 40 mg/kg of the 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.

**Table3.** *The U-74389G / erythropoietin efficacies ratios on MPV levels hypervolemia after chi-square tests application*

| Odds ratio | [95% Conf. Interval] | p-values | Endpoint |
|---|---|---|---|
| 145.8532 | 144.4617 147.258 | 0.0000 | 1h |
| 4.053619 | 4.051612 4.055626 | 0.0000 | 1.5h |
| 2.603947 | 2.600845 2.607053 | 0.0000 | 2h |
| 1.2334644 | 1.2309333 1.2360010 | 0.0000 | reperfusion time |
| 4.164431 | 4.155693 4.173187 | 0.0000 | interaction |

**Table4.** *A U-74389G / erythropoietin efficacies ratios meta-analysis on 9 hematologic variableç (7 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 |
|---|---|---|---|---|---|---|---|---|---|---|
| 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 count | 2.42839 | 0.0000 | 6.00238 | 0.0000 | 6.1333429 | 0.0000 | 3.939027 | 0.0000 | 37.62979 | 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** | 3.86005598 | 0.0540 | 2.85823064 | 0.0000 | 4.57821524 | 0.0000 | 1.53777117 | 0.0312 | 4.22373897 | 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 |

According to above, table 3 shows that U-74389G accentuated by 4.164431-fold [4.155693-4.173187] the hypervolemic potency on MPVl 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 9 other seric variables, provides comparable results (table 4).

## CONCLUSION

The anti-oxidant capacities of U-74389G accentuated by 4.164431-fold [4.155693-4.173187] the hypervolemic potency on MPVl than Epo (p-value=0.0000) in rats. However, this trend is attenuated along the short term time frame of the experiment.

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