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Original article

The acute effect of the antioxidant drug "U-74389G" on mean platelet volume levels during hypoxia reoxygenation injury in rats

C. Tsompos[a,*], C. Panoulis[b], K. Toutouzas[c], A. Triantafyllou[d], G. Zografos[c], A. Papalois[e]

[a] Department of Obstetrics & Gynecology, Mesologi County Hospital, Etoloakarnania, Greece
[b] Department of Obstetrics & Gynecology, Aretaieion Hospital, Athens University, Attiki, Greece
[c] Department of Surgery, Ippokrateion General Hospital, Athens University, Attiki, Greece
[d] Department of Biologic Chemistry, Athens University, Attiki, Greece
[e] Experimental Research Centre ELPEN Pharmaceuticals, S.A. Inc., Co., Pikermi, Attiki, Greece

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ABSTRACT

Background: This experimental study examined the effect of the antioxidant drug "U-74389G", on a rat model and particularly in a hypoxia – reoxygenation protocol. The effects of that molecule were studied hematologically using blood mean platelets volume (MPV) levels.
Methods: 40 rats of mean weight 231.875 g were used in the study. MPV levels were measured at 60 min of reoxygenation (groups A and C) and at 120 min of reoxygenation (groups B and D). The drug U-74389G was administered only in groups C and D.
Results: U-74389G administration kept significantly increased the predicted MPV levels by 12.77 ± 3.07% ($p = 0.0001$). Reoxygenation time non-significantly decreased the predicted MPV levels by 2.55 ± 3.71% ($p = 0.4103$). However, U-74389G administration and reoxygenation time together kept significantly increased the predicted MPV levels by 7.09 ± 1.91% ($p = 0.0005$).
Conclusions: U-74389G administration whether it interacted or not with reoxygenation time kept significantly increased the predicted MPV levels. This finding has great clinical interest in blood clotting and coagulation pathophysiology.

Introduction

Transient or permanent damage with serious implications on adjacent organs and systems may be due to tissue hypoxia – reoxygenation (HR). The use of U-74389G in HR has been a challenge for many years. However, although the progress was significant, several practical affairs have not been clarified. They include: (a) how potent U-74389G should be (b) when should it be administered and (c) at what optimal dose U-74389G should be administered. The promising effect of U-74389G in tissue protection has been noted in several 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.[1] It protects against ischemia – reperfusion (IR) injury in organs such as animal hearts, livers and kidneys. These membrane-associating antioxidants are particularly effective in preventing permeability changes in brain microvascular endothelial cells monolayers.[2] A meta-analysis of 25 published seric variables, as WBC, RBC and platelet counts, hematocrit, hemoglobin, MCH, MCHC, RbcDW, platelet-crit, PDW, glucose, creatinine, uric acid, total protein, γGT, ALP, ACP, CPK, LDH, sodium, potassium, chloride, calcium, phosphorus and magnesium levels coming from the same experimental setting, enhanced by 0.48 ± 17.28% (p-value = 0.2377) their total catabolism after U-74389G administration for the same endpoints.[7,8] The studied molecule U-74389G belongs to a wider family of other similar antioxidant molecules; all used in several published trials.

Mean platelet volume (MPV) is a machine-calculated measurement of the average size of platelets found in blood and is typically included in blood tests as part of the complete blood count one. Since the average platelet size is larger when the body is producing increased numbers of platelets, the MPV test results can be used to make inferences about platelet production in bone marrow or platelet destruction problems. MPV is higher when there is destruction of platelets. This may be seen in inflammatory bowel disease,[3] immune thrombocytopenic purpura (ITP),

* Corresponding author at: Department of Obstetrics & Gynecology, Mesologi County Hospital, Nafpaktou Street, Mesologi 30200, Etoloakarnania, Greece.
E-mail address: Tsomposconstantinos@gmail.com (C. Tsompos).

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myeloproliferative diseases and Bernard–Soulier syndrome. It may also be related to pre-eclampsia and recovery from transient hypoplasia.[4] Abnormally low MPV values correlate with thrombocytopenia when it is due to impaired production as in aplastic anemia. In addition, low MPV, can correlate with abnormally small platelet size, sometimes a symptom of a spectrum referred to as Wiskott–Aldrich Syndrome,[5] caused by a genetic mutation of the WAS gene. A typical range of platelet volumes is 9.7–12.8 femtolitre (fL), equivalent to spheres 2.65–2.9 μm in diameter. Normal range is given as 7.5–11.5 fL.[6] The **aim** of this experimental study was to examine the effect of the antioxidant drug "U-74389G" on rat model and particularly in a hypoxia-reoxygenation HR protocol. The effects of that molecule were studied by measuring blood MPV levels.

Materials and methods

Animal preparation

This biomedical research was licensed by Veterinary Address of East Attiki Prefecture based on 3693/12-11-2010 & 14/10-1-2012 decisions. All substances, consumables and equipment were a grant of Experimental Research Centre of ELPEN Pharmaceuticals Co. Inc. S.A. at Pikermi, Attiki. Appropriate humanistic care was adopted for Albino female Wistar rats. Pre-experimental normal housing included ad libitum diet in laboratory for one week. Animals' pre-narcosis was preceded of continuous intra-experimental general anesthesia,[7,8] oxygen supply, electrocardiogram and acidometry. Euthanasia did not permit post-experimental awakening and preservation of animals. Rats were randomly delivered to four experimental groups by 10 animals in each one, using 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 U-74389G intravenous (IV) administration and reoxygenation for 60 min (group C); hypoxia for 45 min followed by immediate U-74389G IV administration and reoxygenation for 120 min (group D). The dose of U-74389G was 10 mg/kg body mass of animals. Hypoxia was caused by laparotomic clamping inferior aorta over renal arteries with forceps for 45 min. The clamp removal re-established the inferior aorta patency and restored reoxygenation. U-74389G was administered at the time of reperfusion; through catheter of inferior vena cava. The MPV levels were determined at 60th min of reoxygenation (for A and C groups) and at 120th min of reoxygenation (for B and D groups). The sampling was performed after vena cava venipuncture by filling a 2 cc dimpled control stroke syringe with pre-set volume for withdrawal and sterile insulin needle. Then, the sample was transferred to vacuum blood collection tube (disposable vacutainer) of 2 ml containing K_2EDTA, the "anticoagulant of choice in specimen collection and blood cell features counting" according to both NCCLS and the International Council for Standardization in Hematology. MPV levels measurements were performed by Nihon Kohden celltac a MEK-6450 K automatic hematology analyzer with pre-set rat type and cyanide-free reagents. Fourty female Wistar albino rats were used (mean weight 231.875 g [Standard Deviation (SD): 36.59703 g], with minimum weight 165 g and maximum weight 320 g. Rats' weight could be potentially a confusing factor, e.g. more obese rats to have higher MPV levels. This assumption was also investigated.

Control groups

20 control rats (mean mass 252.5 g [SD: 39.31988 g]) experienced hypoxia for 45 min followed by reoxygenation.

Table 1
Weight and mean platelet volume levels and Std. Dev. of groups.

Groups	Variable	Mean	Std. dev
A	Weight	243 g	45.77724 g
A	MPV	6.15 fl	0.347211 fl
B	Weight	262 g	31.10913 g
B	MPV	6.51 fl	0.4433459 fl
C	Weight	212.5 g	17.83411
C	MPV	8.78 fl	2.078889 fl
D	Weight	210 g	18.10463 g
D	MPV	8.92 fl	1.917638 fl

MPV, mean platelet volume; Std. Dev, standard deviation.

Table 2
Statistical significance of mean values difference for groups (DG) after statistical standard *t* test application.

	Variable	Difference	*p*-value
A–B	Weight	−19 g	0.2423
A–B	MPV	−0.36 fl	0.0820
A–C	Weight	30.5 g	0.0674
A–C	MPV	−2.63 fl	0.0049
A–D	Weight	33 g	0.0574
A–D	MPV	−2.77 fl	0.0024
B–C	Weight	49.5 g	0.0019
B–C	MPV	−2.27 fl	0.0080
B–D	Weight	52 g	0.0004
B–D	MPV	−2.41 fl	0.0045
C–D	Weight	2.5 g	0.7043
C–D	MPV	−0.14 fl	0.6091

MPV, mean platelet volume; DG, difference for groups.

Group A

Reoxygenation lasted for 60 min ($n = 10$ controls rats) mean mass 243 g [SD: 45.77724 g], mean MPV levels 6.15 fl [SD: 0.347211 fl] (Table 1).

Group B

Reoxygenation lasted for 120 min ($n = 10$ controls rats) mean mass 262 g [SD: 31.10913 g], mean MPV levels 6.51 fl [SD: 0.4433459 fl] (Table 1).

Lazaroid (L) group

20 L rats (mean mass 211.25 g [SD: 17.53755 g] experienced hypoxia for 45 min followed by reoxygenation in the beginning of which 10 mg U-74389G/kg body weight were IV administered.

Group C

Reoxygenation lasted for 60 min ($n = 10$ L rats) mean mass 212.5 g [SD: 17.83411 g], mean MPV levels 8.78 fl [SD: 2.078889 fl] (Table 1).

Group D

Reoxygenation lasted for 120 min ($n = 10$ L rats) mean mass 210 g [SD: 18.10463 g], mean MPV levels 8.92 fl [SD: 1.917638 fl] (Table 1).

Statistical analysis

Every weight and MPV level group was compared with each other by statistical standard *t*-tests (Table 3). Any significant difference among MPV levels, was investigated whether owed in any potent significant weight one. The application of generalized linear models (glm) with dependant variable the MPV levels was followed. The 3 independent variables were the U-74389G or no drug administration, the reoxygenation time and both variables in combination. Inserting the rats' weight also as an independent variable at glm analysis, a significant relation resulted in ($p = 0.0033$), so as