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

Akutan učinak antioksidantnog lijeka U-74389G kod srednjih razina crvenih krvnih tjelešaca za vrijeme hipoksije i ponovljene oksigenacije kod štakora

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Summary

Aim: 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 protocol. The beneficial effect or non-effectiveness of that molecule was studied hematologically using blood mean corpuscular hemoglobin (MCH) levels.

Methods: 40 rats of mean weight 231.875 gr were used in the study. MCH levels were measured 60 min after reperfusion (groups A and C) and 120 min after reperfusion (groups B and D) with the administration of drug U-74389G in groups C and D.

Results: The results were that U-74389G administration significantly increased the MCH levels by 2.40% ± 0.57% (p = 0.0001). Reoxygenation time non-significantly decreased the MCH levels by 0.48% ± 0.69 (p = 0.4103). However, U-74389G administration and reoxygenation time together significantly increased the MCH levels by 1.33% ± 0.36% (p = 0.0005).

Conclusion: The results of this study indicate that U-74389G administration either alone or interacted with reoxygenation time has significant increasing short – term effects on the pathophysiology recovery of MCH values.

Key words: hypoxia, U-74389G, meann corpuscular hemoglobin levels, reperfusion

Sažetak

Cilj: Cilj ove eksperimentalne studije je ispitati učinak antioksidantnog lijeka "U-74389G", na model štakora, a posebno u protokolu hipoksije i ponovljene oksigenacije. Blagotvoran učinak ili neučinkovitost navedene molekule ispitani su hematološki korištenjem srednjih razina crvenih krvnih tjelešaca (MCH).

Metode: U studiji je korišteno 40 štakora srednje težine od 231,875 g. MCH razine izmjerene su 60 min nakon reperfuzije (skupine A i C) i 120 min nakon reperfuzije (skupine B i D) davanjem lijeka U-74389G skupinama C i D.

Rezultati: Davanje lijeka U-74389G značajno je povećalo razine MCH za 2,40% ± 0,57% (p = 0,0001). Ponovljena oksigenacija smanjila je beznačajno razine MCH za 0,48% ± 0,69 (p = 0,4103). Davanje U-74389G tijekom ponovljene oksigenacije međutim, značajno je povećalo MCH razine za 1,33% ± 0,36% (p = 0,0005).

Zaključak: Rezultati ove studije pokazuju da zasebno davanje U-74389G ili u interakciji s ponovljenom oksigenacijom, značajno povećava kratkotrajne učinke na oporavak patofiziološke razine MCH.

Ključne riječi: hipoksija U-74389G, srednja razina crvenih krvnih tjelešaca, reperfuzija

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Introduction

Circulatory hypoxia and reoxygenation (HR) remain one of the main causes of permanent or transient damage with serious implications on adjacent organs and certainly on patients' health. The use of antioxidant substances has been a research subject for many years. However, even if important progress has been made, satisfactory answers have not been given yet to fundamental questions such as how much powerful should an antioxidant be, when it should be administered, and in which dosage. The particularly satisfactory action of the antioxidant U-74389G in tissue protection has been noted in several performed experiments. After a careful literature search (PubMed – Medline) was conducted, it was realized that this certain antioxidant has been tried in HR experiments. However, just few relative reports were found, not covering completely this particular matter. Also, a lot of publications addressed trials of other similar molecules of aminosteroids (lazaroids) to which the studied molecule also belongs. U-74389G or better 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 ishemia referfusion (IR) 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. The same authors found the influence of U-74389G as depicted in Table 1 on some biochemic variables serum levels in related IR injury experi-ments, 1h, 1.5h, 2h and interaction of U-74389G and reoxygenation time after reperfusion in rats.[2]

The aim of this experimental study was to examine the effect of the antioxidant drug "U-74389G" on rat model and particularly in an HR protocol. The beneficial effect or non-effectiveness of that molecule was studied by measuring blood mean corpuscular hemoglobin concentration (MCH) levels. This variable has great diagnostic value in anemia investigation.[3]

Materials and methods

Animal preparation

This experimental study was laid out at the Exprerimental Research Center of ELPEN Pharmaceuticals Co. Inc. S.A. at Pikermi, Attiki and by Veterinary Address of East Attiki Prefecture under 3693/12-11-2010 & 14/10-1-2012 decisions. All settings needed for the study including consumables, equipment and substances used, were a courtesy S. A. Albino female Wistar rats used in accordance with the accepted standards of humane animal care. They were housed in a laboratory 7 days before the experiment, having easy access to water and food. The experiment was acute, that is, the animal usage was completed by following experimental set of times without awakening and preservation of the rodents. They were randomly assigned to four experimental groups (10 animals in each group).

1) Hypoxemia for 45 min followed by reoxygenation for 60 min (group A).
2) Hypoxemia for 45 min followed by reoxygenation for 120 min (group B).
3) Hypoxemia for 45 min followed by immediate U-74389G intravenous (IV) administration and reoxygenation for 60 min (group C).
4) Hypoxemia for 45 min followed by immediate U-74389G IV administration and reoxygenation for 120 min (group D). The molecule U-74389G dose was 10 mg/Kg body weight of animals.

Table 1: The U-74389G influence (±SD) on the levels of some biochemic variables concerning reperfusion (rep) time
Tablica 1. (±SD) utjecaj U-74389G na razine nekih biokemijskih varijabli povezane s vremenom reperfuzije

Variable *Varijabla*	1h rep	p	1.5h rep	p	2h rep	p	Interaction of U-74389G and rep *Interakcija*	p
alkaline phosphatase[20]	+22.66%±12.37%	0.0663	+31.91%±7.69%	0.0001	+41.16%±9.65%	0.0003	+17.75%±4.79%	0.0005
sodium[21]	+1.22%±0.66%	0.0707	+0.17%±0.61%	0.7714	-0.87%±1.03%	0.3995	-0.32%±0.36%	0.3693
chloride[22]	-0.58%±0.77%	0.4533	-0.97%±0.53%	0.0879	-1.36%±0.76%	0.1113	-0.75%±0.38%	0.0159
phosphorus[23]	-2.23%±5.51%	0.7966	-1.61%±3.32%	0.5789	-1%±4.48%	0.8129	-1.09%±2%	0.5771

The experiment started with animals submitted to prenarcosis followed by general anesthesia. Their electrocardiogram and acidometry were continuously monitored. Their inferior aorta was prepared so as their flow to be excluded by forceps. After exclusion, the protocol of HR was applied, exactly as described in experimental groups. The molecules were administered at the time of reoxygenation, through inferior vena cava after catheterization had been achieved. The MCH levels measurement was performed at 60 min of reoxygenation (groups A and C) and 120 min of reoxygenation (groups B and D).

Rats were submitted to general anesthesia by initial intramuscular (IM) administration of 0.5 cc compound, which constituted of 0.25 cc xylazine, [25 cc, 20mg/cc] and 0.25 cc ketamine hydrochloride [1000, 100mg/cc, 10cc]. Before the initiation of laparotomy, 0.03 cc butorphanol [10mg/cc, 10cc] an anesthetic agent was administered subcutaneously (SC). Continuous oxygen supply was administered during the whole experiment performance. Hypoxemia was caused by clamping inferior aorta over renal arteries for 45 min after laparotomic access was achieved. Reoxygenation was induced by removing the clamp and reestablishing the inferior aorta patency. Forty (40) female Wistar albino rats were used of mean weight 231.875 gr [Std. Dev: 36.59703 gr], with min weight $\geq$ 165 gr and max weight $\leq$ 320 gr. The rats' weight could potentially be a confusing factor, e.g. fatter rats to have greater blood MCH levels. This suspicion will be investigated.

Control groups

20 control rats of mean weight 252.5 gr [Std. Dev: 39.31988 gr] were subjected to hypoxemia for 45 min followed by reoxygenation.

Group A

Reoxygenation which lasted 60 min concerned 10 controls rats of mean weight 243 gr [Std. Dev: 45.77724 gr] and post-experimental mean MCH levels 19.92 pgr [Std. Dev: 0.9635586 pgr] (Table 2).

Group B

Reoxygenation which lasted 120 min concerned 10 controls rats of mean weight 262 gr [Std. Dev: 31.10913 gr] and post-experimental mean MCH levels 19.8 pgr [Std. Dev: 0.8273115 pgr] (Table 2).

Lazaroid (L) group

20 rats of mean weight 211.25 gr [Std. Dev: 17.53755 gr] suffered hypoxemia for 45 min followed by reoxygenation in the beginning of which 10 mg U-74389G/kg body weight were IV administered.

Group C

Reoxygenation which lasted 60 min concerned 10 L rats of mean weight 212.5 gr [Std. Dev: 17.83411 gr] and post-experimental mean MCH levels 20.6 pgr [Std. Dev: 0.5715475 pgr] (Table 2).

Group D

Reoxygenation which lasted 120 min concerned 10 L rats of mean weight 210 gr [Std. Dev: 18.10463 gr] and post-experimental mean MCH levels 20.29 pgr [Std. Dev: 0.6332459 pgr] (Table 2).

Statistical analysis

Every rat weight group initially was compared with each one of the 3 remaining groups by applying statistically paired t-test (Table 3).

Table 2: Weight and mean MCH levels and Std. Dev. of groups
Tablica 2. Težina i prosječna MCH razina kod standardnih devijacija skupina

Group / *Skupina*	Variable / *Varijabla*	Mean/ *Prosjek*	SD*
A	Weight */težina*	243 gr	45.77724 gr
	MCH	19.92 pgr	0.9635586 pgr
B	Weight */težina*	262 gr	31.10913 gr
	MCH	19.8 pgr	0.8273115 pgr
C	Weight */težina*	212.5 gr	17.83411 gr
	MCH	20.6 pgr	0.5715475 pgr
D	Weight */težina*	210 gr	18.10463 gr
	MCH	20.29 pgr	0.6332459 pgr

*Standard deviation / *Standardna devijacija*

Table 3: Statistical significance of mean value difference for groups after statistical paired t test application.
Tablica 3. Statistička značajnost prosječne vrijednosti razlike kod skupina nakon primjene parnog t-testa

DG*	Variable / *Varijabla*	Difference / *Razlika*	p-value / *p-vrijednost*
A-B	Weight */težina*	-19 gr	0.2423
	MCH	0.1199995 pgr	0.3732
A-C	Weight */težina*	30,5 gr	0.0674
	MCH	-0.6800003 pgr	0.1167
A-D	Weight */težina*	33 gr	0.0574
	MCH	-0.3700003 pgr	0.3522
B-C	Weight */težina*	49.5 gr	0.0019
	MCH	-0.4899998 pgr	0.2042
B-D	Weight */težina*	52 gr	0.0004
	MCH	-0.4899998 pgr	0.2042
C-D	Weight */težina*	2,5 gr	0.7043
	MCH	0.31 pgr	0.0986

* Difference for groups/*Razlika za skupine*

Table 4: The increasing influence of U-74389G in connection with reoxygenation time.
Tablica 4. Rastući utjecaj U-74389G povezan s vremenom ponovljene oksigenacije

Increase / *Povećanje*	95% c. in.*	Reoxygenation time / *Vrijeme ponov. oksigen*	p-values * *P-vrijednosti*	
			t-test	glm
0.6800003 pgr	-0.064305 pgr - 1.424306 pgr	1h	0.1167	0.0709
0.58500005 pgr	0.1021569 pgr - 1.067843 pgr	1.5h §	0.0360	0.0189
0.4899998 pgr	-0.2021718 pgr - 1.182171 pgr	2h	0.2042	0.1542

*95% confidence interval / *Razmak povjerljivosti*
§ Reoxygenation endpoint representative of the total experiment / *Predstavnik krajnje točke cijelog eksperimenta ponovljene oksigenacije*

Any emerging significant difference among MCH levels will be investigated whether owed to the above mentioned probable significant weight correlation. Every rat MCH group was initially compared with another from the 3 remaining groups by applying statistically paired t-test (Table 3). The application of generalized linear models (glm) with dependant variable of MCH levels and independent variables of U-74389G administration or not, reoxygenation time and their interaction, resulted in: U-74389G administration significantly increased the MCH levels by 0.58500005 pgr [0.1021569 pgr - 1.067843 pgr] (p = 0.0189). This finding was in accordance with the result of paired t-test (p = 0.0360). Reoxygenation time non-significantly increased the MCH levels by 0.2149998 pgr [-0.7298397 pgr - 0.2998401 pgr] (p = 0.4032), also in accordance with paired t-test (p = 0.0551). However, U-74389G administration and reoxygenation time together non-significantly increased the MCH levels by 0.2627273 pgr [-0.038527 pgr - 0.5639815 pgr] (p = 0.0855). Reviewing the above and Table 3, Table 4 sums up concerning the increasing influence of U-74389G in connection with reoxygenation time. Inserting the rats' weights also as an independent variable at generalized linear models analysis, a significant relation results in (p = 0.0004), so further investigation is needed among predicted values of MCH corrected for rats' weights. The predicted MCH values were calculated for every rat (Table 5). The statistically paired t-test appliance among all groups for predicted MCH values was revealed in Table 6 and also glm with dependant variable number of predicted MCH levels and independent variables of U-74389G administration or none, reoxygenation time and their interaction were again applied.

Table 5: The predicted mean MCH values for every group
Tablica 5. Predviđene prosječne MCH vrijednosti za svaku skupinu

Group *Skupina*	Mean *Prosjek*	SD*
A	20.02206 pgr	0.5367315 pgr
B	19.79929 pgr	0.3647491 pgr
C	20.37967 pgr	0.2090994 pgr
D	20.40898 pgr	0.2122739 pgr

*Standard deviation/ *Standardna devijacija*

Table 6: Statistical significance of mean predicted MCH values difference for groups after statistical paired t test application
Tablica 6. Statističko značenje prosječnih predviđenih MCH vrijednosti za skupine nakon primjene statističkih parnih t-testova

DG*	Difference *Razlika*	p-value *p-vrijednost*
A-B	0.2227711 pgr	0.2423
A-C	-0.357604 pgr	0.0674
A-D	-0.3869175 pgr	0.0574
B-C	-0.5803751 pgr	0.0019
B-D	-0.6096886 pgr	0.0004
C-D	-0.0293135 pgr	0.7042

*Difference for groups / *Razlika za skupine*

Results

Regarding predicted MCH values: U-74389G administration significantly increased the predicted MCH levels by 0.4836479 pgr [0.255143 pgr - 0.7121529 pgr] (p = 0.0001). This finding was in accordance with the results of paired t-test (p = 0.0002). Reoxygenation time non-significantly decreased the predicted MCH by 0.0967295 pgr [-0.3731936 pgr - 0.1797346 pgr] (p = 0.4831) also in accordance with paired t-test (p = 0.3375). However, U-74389G administration and reoxygenation time together significantly increased the MCH levels by 0.2691374 pgr [0.1264887 pgr - 0.4117862 pgr] (p = 0.0005). Reviewing the above and Table 6, Tables 7 and 8 sum up concerning the increasing influence of U-74389G in connection with reoxygenation time.

Table 7: The predicted increasing influence of U-74389G in connection with reoxygenation time
Tablica 7. Predviđeni rasteći utjecaj U-74389G povezan s vremenom ponovljene oksigenacije

Alteration / *Promjena*	95% c. in.*	Reoxygenation time / *Vrijeme ponov. oksigen*	p-values * *p-vrijednosti*	
			t-test	glm
+0.357604 pgr	-0.0250888 pgr - 0.7402968 pgr	1h	0.0674	0.0653
+0.4836463 pgr	0.255143 pgr - 0.7121529 pgr	1.5h §	0.0002	0.0001
+0.6096886 pgr	0.3293102 pgr - 0.8900669 pgr	2h	0.0004	0.0002
-0.0967295 pgr	-0.3731936 pgr - 0.1797346 pgr	reoxygenation time / *vrijeme ponov. oksigen*	0.4831	0.3375
+0.2691374 pgr	0.1264887 pgr - 0.4117862 pgr	interaction / *interakcija*	0.0005	

* 95% Confidence interval / *95% Razmak povjerljivosti*
§ Reoxygenation endpoint representative of the total experiment / *Krajnje točke ponovljene oksigenacije cijelog eksperimenta*

Table 8. (%) predicted increasing influence of U-74389G in connection with reoxygenation time
Tablica 8. Postotak predviđenog rastućeg utjecaja U-74389G povezan s vremenom ponovljene oksigenacije

Alteration / *Promjena*	±*SD**	*Reoxygenation time* / Vrijeme pon. oksigenacije	p-values / *p-vrijednosti*
1.77%	±0.96%	1h	0.0663
2.40%	±0.57%	1.5h §	0.0001
3.03%	±0.71%	2h	0.0003
-0.48%	±0.69%	reoxygenation time / *vrijeme ponov. oksigen*	0.4103
1.33%	±0.36%	Interaction / *interakcija*	0.0005

*Standard deviation / *Standardna devijacija*
§ Reoxygenation endpoint representative of the total experiment / *Krajnje točke ponovljene oksigenacije cijelog eksperimenta*

Discussion

Low MCH production is not only being influenced by hypoxemia but also may induce it, and particularly by certain mode, as the next clinical situations show. Aikimbaev K. et al[4] significantly related the reduced ($p < 0.0001$) blood flow velocities and respective MCH levels ($p < 0.04$) of the central retinal artery and the markedly higher resistance and pulsatility index values ($p < 0.02$ and $p < 0.03$) in sickle cell patients compared with control ones. Wilke A. et al[5] removed an intestine leiomyoma which led to chronic anemia with a conspicuous MCH level of 20 pgr and additionally to angina pectoris. After the removal and normalization of hemoglobin this patient was symptom free. Anemia as possible and unique cause of angina pectoris ought to be verified. Wolf PL et al[6] found MCH levels modestly decreased over a 10-month period prior to Olympic Trials in male distance runners. The cause of runners' anemia had been demonstrated due to hematuria and ischemia of the intestinal mucosa with bleeding. Gorokhova SG et al showed[7] significantly lower indices of MCH levels, more frequent anemia by 1.25 times ($p < 0.01$), also correlated ($p < 0.01$) with depression of ST segment, more frequent ischemic changes on ECG, less exercise tolerance, high ectopic activity and more severely affected coronary circulation angiography in type 2 diabetic mellitus (DM) ischemic heart disease (IHD) patients than non-DM IHD ones. Waheed N et al found febrile seizures[8] more common between the ages of 12 months to 36 months old, with 5.3% of them being iron deficient. Mean MCH levels were 27.11 ± 3.28 and mean ferritin levels were 66.57 ± 24.7 in children of 6 months to 5 years old, admitted with fever and seizures. Iron status has no role in febrile seizures. Liu B et al treated the underground Mandarin vole *(Lasiopodomys mandarinus)* and Kunming (KM) mouse *(Mus musculus)*[9] with chronic intermittent hypoxia (10.0% oxygen), for 4 weeks showing that MCH levels in the Mandarin vole were significantly lower than those in the KM mouse. MCH content in KM mouse was increased, while only MCH was increased in the Mandarin vole suggesting that the adaptive mechanism of the blood system in the Mandarin vole responds to hypoxic conditions differently from that of the KM mouse. Liu B et al showed[10] that both rodents, the underground Mandarin vole *(Lasiopodomys mandarinus)* and Kunming mouse *(Mus musculus)* (KM) under chronic normobaric hypoxic treatment (10.0% O(2), 4 w) responded to chronic hypoxia mainly by increasing MCH levels, though the KM mouse responded more acutely. Shah SL et al caused[11] a significant increase in MCH levels administering 300/48, 30/504, 75/504 lead doses, while the 75/96 dose caused a significant decrease in Tinca tinca. These alterations were attributed to direct or feedback responses of structural damage to RBC membranes resulting in hemolysis and impairment in hemoglobin synthesis, stress related release of RBCs from the spleen and hypoxia, induced by exposure to lead. Achermann R et al significantly correlated MCH levels with several oxygen parameters like mean $pO2$[12] being 9.6 mmHg and hypoxia which exists in spontaneous canine soft tissue sarcomas. Garruto RM et al showed[13] a slight but significant increase of MCH values with age in children and adolescents in male Quechua children and adults. Armitage KB. et al determined[14] MCH levels as adaptive to environmental factors such as hypoxia of burrows, high altitude, temperature and metabolic rate in a population of Marmota flaviventris over a period of seven years. Pearson TC et al raised[15] whole-blood viscosity exponentially with decreasing MCH levels showing to be a function of it, when the samples were adjusted to a standard Hb concentration of 14 g/dl, in polycythaemia patients treated by venesection and not due to hypoxia. Hutton RD increased[16] oxygen availability to the tissues treating coexisting iron deficiency. Values for whole blood viscosity can be derived from the peripheral blood MCH in polycythemic patients secondary to hypoxia.

Unpleasantly, references relating to U-74389G and MCH were not found. So, other references showing the general role of U-74389G were retrieved. Horáková L et al[17] used brain homogenate as a model system to study antioxidant properties of U-74389G under oxidative stress. Oxidative stress was induced by Fe/ascorbate system and lipid peroxidation as well as protein modification. Thiobarbituric acid reactive substances were used as a marker of lipid peroxidation. The preventive effect concerning lipid peroxidation decreased by U-74389G (160 IC50 in μmol/l). Nediani C et al[18] proved that 21-amino-steroids (lazaroids) have beneficial effects in various pathological conditions due to their action as free radical scavengers and as membrane stabilizers. They explored the effectiveness of one of these compounds, U-74389G, in protecting pigs myocardium against ischemia reperfusion (IR) damage induced by transient coronary occlusion in a dose of (4 mg/kg) before coronary reperfusion. All these alterations were attenuated by aminosteroid treatment. The biochemical changes in adenine nucleotides and nucleosides levels, thus the reduction of energy charge, were reversed in the treated group. Myocardial concentration of malondialdehyde, which

was undetectable in the control group, was raised in all the animals after reperfusion, but this effect was significantly less marked with aminosteroid treatment. In addition, the higher myocardial content of ascorbic acid and the reduced serum potential peroxidation exhibited by the treated animals compared with untreated group indicate an enhanced antioxidant protection induced by aminosteroid administration. The conclusion is that aminosteroid treatment is effective in reducing the morphological and biochemical alterations occurring in IR myocardium. Perna AM et al[19] found lipid peroxidation as an important factor contributing to tissue damage in IR syndromes. They investigated whether the compound U-74389G, plays a significant role in protecting Wistar rats heart muscle from IR damage. The typical morphological aspects of lipoperoxidative injury were shown in hearts transplanted without treatment: swollen mitochondria with disrupted cristae, damaged endothelial cells with the nucleous bulging into the lumen and a discontinued endothelial lining with diffuse edema among the fibers. Lazaroid treatment attenuated most of these damages in hearts. As for the biochemical findings, serum creatine kinase activity was lower in treated by U-74389G than in those untreated ($p < 0.05$). Taken together, all these results indicate that U-74389G treatment is effective in protecting cardiac muscle from structural and functional IR injuries.

Conclusion

U-74389G administration either alone or interacted with reoxygenation time has significant increasing short – term effects on the pathophysiology recovery of MCH values. It is concluded that the specific antioxidant action of U-74389G releases some anabolic action on MCH levels. U-74389G remains to be tested within human clinical trials with greater samples. Also a molecular investigation is required connecting both the antioxidant and anabolic capacities of the drug.

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References

1. https://www.caymanchem.com/app/template/Product.vm/catalog/75860.
2. Fenglin Shi, Jennifer Cavitt, Kenneth L Audus. 21-aminosteroid and 2-(aminomethyl)chromans inhibition of arachidonic acid-induced lipid peroxidation and permeability enhancement in bovine brain microvessel endothelial cell monolayers. Free Radical Biology and Medicine, 1995;19:349-357.
3. http://www.nlm.nih.gov/medlineplus/ency/article/003648.htm.
4. Aikimbaev K, Guvenc B, Canataroglu A, et al. Value of duplex and color doppler ultrasonography in the evaluation of orbital vascular flow and resistance in sickle cell disease. Am J Hematol. 2001;67:163-167.
5. Wilke A, Kruse T, Maisch B. Angina pectoris in leiomyoma. Herz. 1998;23:299-302.
6. Wolf PL, Nitti GJ, Bookstein R. An analysis of serum enzyme changes and clinical biochemical abnormalities of the anemia in Olympic runners. Am J Cardiovasc Pathol. 1988;2:231-240.
7. Gorokhova SG, Atamanova MA. Characteristics of erythrocytes and hemoglobin in patients with ischemic heart disease and type 2 diabetes mellitus. Ter Arkh. 2008;80:16-21.
8. Waheed N, Butt MA. Iron status: is there a role in febrile seizures? J Ayub Med Coll Abbottabad. 2012;24:128-30.
9. Liu B, Wang ZL, Lu JQ, Yang YY. Comparison of hemogram changes under chronic intermittent hypoxia in Lasiopodomys mandarinus and Kunming Mus musculus. Sheng Li Xue Bao. 2010;62:137-42.
10. Liu B, Wang Z, Lu J. Response to chronic intermittent hypoxia in blood system of Mandarin vole (Lasiopodomys mandarinus). Comp Biochem Physiol A Mol Integr Physiol. 2010 Aug;156(4):469-74.
11. Shah SL. Hematological parameters in tench Tinca tinca after short term exposure to lead. J Appl Toxicol. 2006;26:223-8.
12. Achermann R, Ohlerth S, Fidel J, Gardelle O, Gassmann M, Roos M, Saunders HM, Scheid A, Wergin M, Kaser-Hotz B. Ultrasound guided, pre-radiation oxygen measurements using polarographic oxygen needle electrodes in spontaneous canine soft tissue sarcomas. In Vivo. 2002;16:431-7.
13. Garruto RM, Dutt JS. Lack of prominent compensatory polycythemia in traditional native Andeans living at 4,200 meters. J Phys Anthropol. 1983;61:355-66.
14. Armitage KB. Hematological values for free-ranging yellow-bellied marmots. Comp Biochem Physiol A Comp Physiol. 1983;74:89-93.
15. Pearson TC, Grimes AJ, Slater NG, Wetherley-Mein G. Viscosity and iron deficiency in treated polycythaemia. Br J Haematol. 198149:123-7.
16. Hutton RD. The effect of iron deficiency on whole blood viscosity in polycythaemic patients. Br J Haematol. 1979;43:191-9.
17. Horáková L, Ondrejicková O, Bachratá K, et al. Preventive effect of several antioxidants after oxidative stress on rat brain homogenates. Gen Physiol Biophys. 2000;19:195-205.
18. Nediani C, Perna AM, Liguori P, et al. Beneficial effects of the 21-aminosteroid U 74389G on the ischemia-reperfusion damage in pig hearts. J Mol Cell Cardiol. 1997;29:2825-2835.

19. Perna AM, Liguori P, Bonacchi M, et al. Protection of rat heart from ischaemia-reperfusion injury by the 21-aminosteroid U-74389G. Pharmacol Res. 1996;34:25-31.
20. Tsompos C, Panoulis C, Toutouzas K, Zografos G, Papalois A. The effect of the antioxidant drug "U-74389G" on alkaline phosphatase levels during ischemia reperfusion injury in rats. J. Exp. Clin. Med., 2014;31:99-102.
21. Tsompos C, Panoulis C, Toutouzas K, Zografos G, Papalois A. The effect of the antioxidant drug "U-74389G" on sodium levels during ischemia reperfusion injury in rats. Crit Care & Shock 2014;2:31-36.
22. C Tsompos C, Panoulis C, Toutouzas K, Zografos G, Papalois A. The effect of the antioxidant drug "U-74389G" on chloride during ischemia reperfusion injury in rats. Medical review 2014;50:40-44.
23. Tsompos C, Panoulis C, Toutouzas K, Zografos G, Papalois A. The effect of the antioxidant drug "U-74389G" on phosphorus levels during ischemia reperfusion injury in rats. Psychogeriatria Polska 2013;10:103-108.