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The acute effect of erythropoietin on mean corpuscular volume levels during hypoxia-reoxygenation injury in rats

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Abstract.
OBJECTIVE: This experimental study examined the effect of erythropoietin (epo) in a rat model and particularly in a hypoxia-reoxygenation (HR) protocol. The effect of epo was studied hematologically using the blood mean corpuscular volume (MCV) levels.
MATERIALS AND METHODS: 40 rats of mean weight 247.7±34.991 g were used in the study. MCV levels were measured at 60 min (groups A and C) and at 120 min (groups B and D) of reoxygenation. Epo was administered only in groups C and D.
RESULTS: Epo administration increased non-significantly the predicted MCV levels by $0.56\% \pm 0.66\%$ ($p = 0.3549$). The reperfusion time decreased non-significantly the predicted MCV levels by $0.55\% \pm 0.65\%$ ($p = 0.3721$). Epo administration and reperfusion time together did also not induce an increase of the MCV levels ($0.30\% \pm 0.39\%$; $p = 0.4430$).
CONCLUSIONS: Epo administration, reperfusion time and their interaction had no short – term effect on MCV within the time of 2 hours.

Keywords: Hypoxia, mean corpuscular volume, erythropoietin, reoxygenation

1. Introduction

Erythropoietin (Epo) is generally one of the well-studied growth factors. Epo implicates 28,508 known biomedical studies at present. 8.69% at least of these studies concern tissue hypoxia and reoxygenation (HR) experiments. Certainly, important progress has been made concerning the Epo usage in reversing the HR kind of transient or permanent injuries including adjacent organs and certainly patients' health. Nevertheless, satisfactory answers have not been provided yet to basic questions, as, its action velocity, the administration timing and the dosage. The concept is to forward the knowledge away from the original action of Epo in stem blood cells recovery. However, just few related reports were found, covering completely more specific matters. A numeric evaluation of the Epo efficacy was yielded by a meta-analysis of 23 published seric variables, based on the same experimental setting, at the same endpoints (Table 1).

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Table 1
The erythropoietin (Epo) influence (±SD) on the levels of some seric[1] variables concerning reperfusion (rep) time

Variable	1 h rep	*p*-value	1.5 h rep	*p*-value	2 h rep	*p*-value	interaction of Epo and rep	*p*-value
White BCC	+24.01% ± 13.38%	0.1012	+22.09% ± 9.11%	0.0351	+20.17% ± 12.94%	0.0902	+14.63% ± 5.40%	0.0080
Red BCC	+1.45% ± 3.31%	0.6589	+0.37% ± 3.02%	0.9048	–0.70% ± 4.68%	0.8844	+0.81% ± 1.79%	0.6446
Hematocrit	+0.14% ± 2.89%	0.9626	–0.61% ± 2.37%	0.8072	–1.37% ± 4.05%	0.7485	+0.24% ± 1.38%	0.8586
MCH	+0.01% ± 1.29%	0.9904	+0.67% ± 0.80%	0.3549	+1.34% ± 1.08%	0.1509	–0.36% ± 0.47%	0.4430
RbcDW[2]	–1.85% ± 4.24%	0.6703	–1.64% ± 2.53%	0.5159	–1.43% ± 3.34%	0.6078	–1.06% ± 1.43%	0.4733
Platelet DW	+1.60% ± 0.80%	0.0765	+1.36% ± 0.58%	0.0205	+1.13% ± 0.74%	0.1152	+0.37% ± 0.37%	0.0615
Platelet-crit	–16.47% ± 10.40%	0.0921	–13.74% ± 7.01%	0.0158	–11.01% ± 7.34%	0.0882	–6.88% ± 3.69%	0.0615
Urea	+21.42% ± 7.84%	0.0115	+20.11% ± 7.25%	0.0059	+18.80% ± 9.44%	0.0709	+15.64% ± 4.04%	0.0003
Creatinine	–0.10% ± 9.78%	0.9904	–4.84% ± 5.78%	0.3721	–9.59% ± 7.74%	0.1509	–2.62% ± 3.49%	0.4430
Uric acid	+10.13% ± 15.10%	0.4917	+15.86% ± 10.21%	0.1408	+21.59% ± 15.45%	0.1940	+9.33% ± 6.16%	0.1264
Total protei	–0.02% ± 2.47%	0.9904	–1.27% ± 1.51%	0.3721	–2.52% ± 2.03%	0.1509	–0.68% ± 2.48%	0.4430
ALT	+18.89% ± 12.42%	0.1372	+7.63% ± 18.94%	0.6396	–3.63% ± 25.19%	0.8617	+8.03% ± 11.36%	0.4698
γGT	–19.35% ± 18.58%	0.2362	–12.70% ± 13.11%	0.3541	–6.06% ± 19.96%	0.7800	–4.62% ± 7.97%	0.5534
ALP	+0.20% ± 18.57%	0.9904	+10.70% ± 12.78%	0.3549	+21.20% ± 17.11%	0.1509	+5.79% ± 7.72%	0.4430
ACP	+0.06% ± 5.79%	0.9904	+3.11% ± 3.71%	0.3172	+6.16% ± 4.97%	0.1509	+1.68% ± 2.23%	0.4430
CPK	+0.15% ± 14.09%	0.9904	+7.91% ± 9.44%	0.3549	+15.67% ± 12.65%	0.1509	+4.28% ± 5.70%	0.4430
LDH	+0.08% ± 7.92%	0.9904	+4.48% ± 5.35%	0.3549	+8.89% ± 7.17%	0.1509	+2.42% ± 3.22%	0.4430
Sodium	+0.72% ± 0.74%	0.3054	+0.21% ± 0.63%	0.7136	–0.29% ± 1.09%	0.7670	–0.11% ± 0.38%	0.7531
Potassium	–6.17% ± 4.94%	0.1540	–2.21% ± 3.66%	0.5134	+1.74% ± 5.43%	0.7299	+0.18% ± 2.22%	0.9338
Phosphorus	+1.92% ± 5.25%	0.6982	+3.95% ± 3.35%	0.2100	+5.98% ± 4.81%	0.2930	+2.45% ± 2.01%	0.2168
Magnesium[3]	+1% ± 6.20%	0.8596	–1.09% ± 3.34%	0.7248	–3.19% ± 3.90%	0.3729	–0.19% ± 1.93%	0.9197
Amylase[4]	+6.50% ± 9.15%	0.4161	+5.04% ± 6.12%	0.3831	+3.59% ± 8.42%	0.6649	+4.36% ± 3.65%	0.2258
Progesteron	–0.20% ± 18.65%	0.9904	–8.86% ± 10.58%	0.3549	–17.53% ± 14.15%	0.1509	–4.79% ± 6.39%	0.4430
Mean	+1.91% ± 9.88%	0.5997	+2.45% ± 8.98%	0.3835	+2.99% ± 10.61%	0.3685	+2.12% ± 5.61%	0.4282

The aim of this experimental work was to study the effect of Epo on a rat model and mainly in a HR protocol. The effect of Epo molecule was tested by measuring the blood mean corpuscular volume (MCV) levels.

2. Materials and methods

2.1. Animal preparation

Prefectural veterinary Address of East Attiki licensed this experiment under 3693/12-11-2010 & 14/10-1-2012 decisions. All substances, equipment and consumable needed for the study was a courtesy of ELPEN Pharmaceuticals Co Inc. S.A. at Pikermi, Attiki. Formal human animal care was adopted for female albino Wistar rats. That care included normal 7 days pre-experimental housing in laboratory with *ad libitum* diet. Furthermore, it used preceded prenarcosis and non stop general anesthesia [1–4], electrocardiogram, acidometry and oxygen supply. Finally it did not permit post-experimental preservation of the rodents.

The rodents were randomly delivered to four experimental groups, each one consisted by 10 animals. The 4 groups had common the stage of preceded hypoxia of 45 min induced by laparotomic forceps clamping inferior aorta over renal arteries. Afterwards, reoxygenation was restored by removing the clamp and reestablishment of inferior aorta patency. Reoxygenation of 60 min was followed for group A. Reoxygenation of 120 min was followed for group B. Immediate Epo intravenous (IV) administration and reoxygenation of 60 min was followed for group C. Immediate Epo IV administration and reoxygenation of 120 min was followed for group D. The dosage for molecule Epo was 10 mg/kg body mass per animal. Epo administration was performed at the time of reoxygenation, through catheterized inferior vena cava. The MCV levels evaluations were performed at 60 min of reoxygenation for A and C groups and at 120 min of reoxygenation for B and D groups. The mean mass of the forty (40) female Wistar albino rats used was 247.7 g [Standard Deviation (SD): 34.991 g], min weight 165 g and max weight 320 g. Rats' mass could be probably a confusing factor, e.g. the more obese rats to have higher MCV levels. This assumption was also investigated.

2.2. Model of hypoxia-reoxygenation injury

2.2.1. Control groups

20 control rats of mean weight 252.5 g [SD: 39.319 g] experienced hypoxia for 45 min followed by reoxygenation.

Group A

Reoxygenation which lasted 60 min concerned 10 control rats of mean weight 243 g [SD: 45.777 g], mean MCV levels 59.17 fl [SD: 2.730 fl] (Table 2).

Group B

Reoxygenation which lasted 120 min concerned 10 control rats of mean weight 262 g [SD: 31.109 g], mean MCV levels 58.81 fl [SD: 1.397 fl] (Table 2).

2.2.2. Erythropoietin group

20 Epo rats of mean weight 242.9 g [SD: 30.310 g] experienced hypoxia for 45 min followed by reoxygenation in the beginning of which 10 mg Epo /kg body weight were IV administered.

Group C

Reoxygenation which lasted 60 min concerned 10 Epo rats of mean weight 242.8 g [SD: 29.336 g], mean MCV levels 58.28 fl [SD: 2.060] (Table 2).

Table 2
Weight and MCV levels and std. dev. of groups

Groups	Variable	Mean	Std. dev.
A	Weight [g]	243	45.777
	MCV [fl]	59.17	2.730
B	Weight [g]	262	31.109
	MCV [fl]	58.81	1.397
C	Weight [g]	242.8	29.336
	MCV [fl]	58.28	2.060
D	Weight [g]	243	32.846
	MCV [fl]	58.92	1.977

Table 3
Statistical significance of mean values difference for groups (DG) after statistical standard *t* test application

DG	Variable	Difference	*p*-value
A-B	Weight [g]	–19	0.242
	MCV [fl]	0.359	0.527
A-C	Weight [g]	0.2	0.990
	MCV [fl]	0.890	0.237
A-D	Weight [g]	0	1.000
	MCV [fl]	0.250	0.758
B-C	Weight [g]	19.2	0.259
	MCV [fl]	0.530	0.390
B-D	Weight [g]	19	0.101
	MCV [fl]	–0.109	0.880
C-D	Weight [g]	–0.2	0.988
	MCV [fl]	–0.639	0.442

Group D

Reoxygenaion which lasted 120 min concerned 10 Epo rats of mean weight 243 g [SD: 32.846 g], mean MCV levels 58.92 fl [SD: 1.977 fl] (Table 2).

3. Statistical analysis

Every weight and MCV level group was compared with each other from 3 remained groups applying respective statistical standard *t*-tests (Table 3). If any probable significant difference among MCV levels was raised, it would be investigated whether owed in any respective probable significant mass one (Table 3). Then, the application of generalized linear models (glm) was followed. It included as dependant variable the MCV levels. The 3 independent variables were the Epo administration or no, the reoxygenation time and their interaction. Inserting the rats' mass as independent variable at glm, a significant correlation appeared with MCV levels ($p = 0.0000$), so as to further investigation was required. The predicted MCV values were calculated for every rat, depicted at Table 5. Also, every predicted MCV group was compared with each other from 3 remained groups applying respective statistical standard *t*-tests (Table 6). Then, a second application of generalized linear models (glm) was followed. It included as dependant variable the predicted MCV levels. The 3 independent variables were the Epo administration or no, the reoxygenation time and their interaction.

Table 4
The alteration influence of erythropoietin in connection with reperfusion time

Change	95% c. in.	Reperfusion time	*p*-values	
			t-test	glm
–0.890 fl	–3.162 fl-1.382 fl	1 h	0.237	0.421
–0.390 fl	–1.706 fl 0.926 fl	1.5 h	0.444	0.552
0.109 fl	–1.498 fl-.718 fl	2 h	0.880	0.887
0.139 fl	–1.182 fl-1.462 fl	Reoxygenation time	0.831	0.775
–0.096 fl	–0.893 fl-0.700 fl	Interaction	0.808	

Table 5
Predicted MCV levels and std. dev. of groups

Group	Mean	Std. dev.
A	58.958 fl	1.595 fl
B	58.296 fl	1.083 fl
C	58.965 fl	1.022 fl
D	58.958 fl	1.144 fl

Table 6
Statistical significance of predicted MCV values differences for groups (DG) after statistical standard *t* test application

DG	Difference	*p*-value
A-B	0.662 fl	0.242
A-C	–0.006 fl	0.990
A-D	0 fl	1.000
B-C	–0.668 fl	0.259
B-D	–0.662 fl	0.101
C-D	0.006 fl	0.988

4. Results

The first glm resulted in: Epo administration non-significantly decreased the MCV levels by 0.390 fl [–1.706 fl –0.926 fl] ($p = 0.552$). This finding was in accordance with the results of standard *t*-test ($p = 0.444$). Reperfusion time non-significantly increased the MCV levels by 0.139 fl [–1.182 fl–1.462 fl] ($p = 0.831$) also in accordance with standard *t*-test ($p = 0.775$). However, Epo administration and reperfusion time together produced a non-significant combined effect in decreasing the MCV levels by 0.096 fl [–0.893 fl–0.700 fl] ($P = 0.808$). Reviewing the above and Table 3, the Table 4 sums up concerning the alteration influence of Epo in connection with reperfusion time. The second glm resulted in: Epo administration non-significantly increased the predicted MCV levels by 0.334 fl [–0.448 fl–1.117 fl] ($p = 0.392$). This finding was in accordance with the results of standard *t*-test ($p = 0.317$). Reperfusion time non-significantly decreased the predicted MCV levels by 0.334 fl [–1.117 fl–0.448 fl] ($p = 0.392$), also in accordance with standard *t*-test ($p = 0.351$). However, Epo administration and reperfusion time together produced a non-significant combined effect in increasing the predicted

Table 7
The predicted increasing influence of erythropoietin in connection with reperfusion time

Change	95% c. in.	Reperfusion time	*p*-values	
			t-test	glm
0.006 fl	–1.251 fl–1.265 fl	1 h	0.990	0.990
0.334 fl	–0.448 fl–1.117 fl	1.5 h	0.317	0.392
0.662 fl	–0.385 fl–1.709 fl	2 h	0.101	0.200
–0.334 fl	–1.117 fl–0.448 fl	Reoxygenation time	0.392	0.351
0.181 fl	–0.291 fl–0.654 fl	Interaction	0.443	

Table 8
The (%) predicted increasing influence of erythropoietin in connection with reperfusion time

Change	±SD	Reperfusion time	*p*-values
+0.01%	±1.08%	1 h	0.990
+0.56%	±0.66%	1.5 h	0.354
+1.12%	±0.91%	2 h	0.150
–0.55%	±0.65%	Reoxygenation time	0.372
+0.30%	±0.39%	Interaction	0.443

MCV levels by 0.181 fl [–0.291 fl–0.654 fl] ($p = 0.443$). Reviewing the above and Table 6, the Tables 7 and 8 sum up concerning the alteration influence of Epo in connection with reperfusion time.

5. Discussion

Hypoxia may influence MCV levels. Yildiz H et al. described significant decreases of blood MCV levels ($p < 0.05$) after both testicular torsion by rotating the right testis 720° for 2 h and low dose sildenafil citrate administration in male Wistar rats [5]. Nemeth N et al. found higher MCV levels for one week measured daily in early postoperative period after unilateral 1 h hind-limb IR on femoral vessels (+allopurinol) than in sham operated and control groups in male rats [6]. Berra HH et al. reported enhanced MCV levels after 14 days in experimentally infected rats with Trypanosoma cruzi (Chagas' disease) in comparison with control animals [7]. Putintsev VI et al. revealed an increase on MCV levels in an exacerbated chronic obstructive bronchitis combined with coronary heart disease [8]. Mueller T et al. predicted MCV as an independent factor of severe atherosclerosis in the iliac arterial disease (OR = 2.72 for an increment of 5 fl) and the femoral-popliteal arterial disease segment (OR = 3.13 for an increment of 5 fl) [9]. Higher MCV values contributed to symptomatic peripheral arterial occlusive disease (PAOD) lumen reductions >75% of the proximal segments in patients compared with male age-matched control subjects. Elevated MCV values should be considered in PAOD patients. Wilke A et al. diagnosed an intestinal leiomyoma that led to chronic anemia and conspicuous MCV of 63 fl and further to angina pectoris [10]. Penix LP et al. showed an increased MCV normalized by vitamin B12 injections at two ischemic cerebral infarctions 16 years after ileal resection for Crohn's disease [11]. Donaldson GC et al. produced significant almost immediately decreases in MCV levels, persisted for longer intervals of up to 1-2 days after short-term falls in temperature [12]. Chen YM et al. provided regular transfusions and chelation therapy in 75.6% of Diamond Blackfan anemia patients having decreased MCV and 24.4% having macrocytic anemia unresponsive to corticosteroids [13].

However MCV can be influenced also by Epo. Schwartz AB et al. compared RBC size and MCV changes after rhEpo administration in chronic renal failure anemia patients with themselves as own controls. MCV levels at both short term (53 d) ($p = 0.02$) and intermediate term (136 d) ($p < 0.01$) treated groups were decreased; there was no change ($p = 0.71$) at the long term (221 d) ones. A significant ($p < 0.01$) trend toward decrease of MCV and microcytosis at short- and intermediate terms was shown consistent with iron deficiency secondary to the early, rapid increase in bone marrow iron utilization and early increased reticulocytosis [14].

6. Conclusion

Epo administration, reperfusion time and their interaction had no significant short – term effect on MCV within the observation time period of 2 hours. A longer study time or a higher epo dose are recommended for finding significant results.

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