Research Journal of Pharmacology and Toxicology 01[05] 2015
www.asdpub.com/index.php/rjpt

Original Article

The effect of erythropoietin on creatine kinase MB levels during ischemia reperfusion injury in rats

C. Tsompos*[1], C. Panoulis[2], K Toutouzas[3], A. Triantafyllou[4], G. Zografos[5], A. Papalois[6]

[1]Consultant A, Department of Obstetrics & Gynecology, Mesologi County Hospital, Etoloakarnania, Greece
[2]Assistant Professor, Department of Obstetrics & Gynecology, Aretaieion Hospital, Athens University, Attiki, Greece
[3]Assistant Professor, Department of Surgery, Ippokrateion General Hospital, Athens University, Attiki, Greece
[4]Associate Professor, Department of Biologic Chemistry, Athens University, Attiki, Greece
[5]Professor, Department of Surgery, Ippokrateion General Hospital, Athens University, Attiki, Greece
[6]Director, Experimental Research Centre ELPEN Pharmaceuticals, S.A. Inc., Co., Attiki, Greece

***Corresponding Author**
Tsompos Constantinos
Department of Obstetrics & Gynecology,
Mesologi County Hospital, Nafpaktou street
Mesologi 30200, Etoloakarnania, Greece
Tel: 00302631360237 & 00306946674264
Fax: 00302106811215
E-mail: Tsomposconstantinos@gmail.com

Keywords:
Ischemia,
Erythropoietin,
Creatine kinase MB,
Reperfusion

Abstract

Objective: This experimental study examined the effect of erythropoietin (Epo) in rat model and particularly in an ischemia-reperfusion (IR) protocol. The effect of that molecule was studied biochemically using blood mean creatine kinase MB (CK-MB) levels.
Materials and methods: 40 rats of mean weight 247.7 g were used in the study. CK-MB levels were measured at 60 min (groups A and C) and at 120 min (groups B and D) of reperfusion. Erythropoietin was administered only in groups C and D.
Results: Erythropoietin administration kept non significantly increased the predicted CK-MB values by 4.28%±5.11% (P= 0.3721). Reperfusion time non significantly decreased the predicted CK-MB values by the same quantities 4.28%±5.11% (P= 0.3721). Interaction of erythropoietin administration and reperfusion time kept non significantly increased the predicted CK-MB values by 2.32%±3.09% (P= 0.4430).
Conclusions: Erythropoietin administration whether it interacted or not with reperfusion time kept short-term non significantly increased the predicted CK-MB values.

1. Introduction

Erythropoietin (Epo) is generally one of the more well studied growth factors. Epo implicates over 28,595 known biomedical studies at present. 3.39% at least of these studies concern tissue ischemia and reperfusion (IR) experiments. Certainly, important progress has been made concerning the Epo usage in reversing the IR 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, not covering completely more specific matters. A numeric evaluation of the Epo efficacy was yielded by a meta-analysis of 25 published seric variables, based on the same experimental setting, at the same endpoints (Table 1).

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

Variable	1h rep	p-value	1.5h rep	p-value	2h rep	p-value	interaction of Epo and rep	p-value
White BCC	+24.01%±13.38%	0.1012	+22.09%±9.11%	0.0163	+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	-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
Albumins[2]	-4.61%±4.21%	0.2530	-9.28%±3.20%	0.0054	-13.96%±5.03%	0.0095	-5.37%±2.73%	0.0072
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
Calcium[1]	0.28%±1.19%	0.8065	-0.56%±1.13%	0.5761	-1.41%±2.08%	0.4100	-0.34%±0.68%	0.6095
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	+1%±6.20%	0.8596	-1.09%±3.34%	0.7248	-3.19%±3.90%	0.3729	-0.19%±1.93%	0.9197
Amylase	+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	+1.59%±9.55%	0.5941	+1.86%±8.93%	0.3753	+2.14%±10.73%	0.3558	+1.72%±5.59%	0.4186

The special aim of this experimental work was to study the effect of Epo on a rat model and mainly in an IR protocol. The effect of Epo molecule was tested by measuring the blood mean creatine kinase MB (CK-MB) levels.

2. Materials and Methods

2.1. Animal preparation

Prefectural veterinary Address of East Attiki licensed the 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. Normal 7 days pre-experimental housing in laboratory included ad libitum diet. Prenarcosis of animals, preceded of continuous intra-experimental general anesthesia [1-2], electrocardiogram, acidometry and oxygen supply. Post-experimental preservation of the rodents was not permitted even if euthanasia was required.

The rodents were randomly delivered to four experimental groups, each one consisted by 10 animals. The 4 groups had common the stage of preceded ischemia of 45 min induced by laparotomic forceps clamping inferior aorta over renal arteries. Afterwards, reperfusion was restored by removing the clamp and reestablishment of inferior aorta patency. Reperfusion of 60 min was followed for group A. Reperfusion of 120 min was followed for group B. Immediate Epo intravenous (IV) administration and reperfusion of 60 min was followed for group C. Immediate Epo IV administration and reperfusion 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 reperfusion, through catheterized inferior vena cava. The CK-MB levels evaluations were performed at 60 min of reperfusion for A and C groups and at 120 min of reperfusion for B and D groups. The mean mass of the forty (40) female Wistar albino rats used was 247.7 g [Standard Deviation (SD): 34.99172 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 CK-MB levels. This assumption was also investigated.

2.2 Model of ischemia-reperfusion injury

Control groups: 20 control rats (mean mass 252.5 g [SD: 39.31988 g]) experienced ischemia for 45 min followed by reperfusion.

Group A: Reperfusion lasted for 60 min (n=10 controls rats) mean mass 243 g [SD: 45.77724 g], mean CK-MB levels 299.4 IU/L [SD: 176.6592 IU/L] (Table 2).

Group B: Reperfusion lasted for 120 min (n=10 controls rats) mean mass 262 g [SD: 31.10913 g], mean CK-MB levels 315 IU/L [SD: 189.6535 IU/L] (Table 2).

Erythropoietin group: 20 Epo rats (mean mass 242.9 g [SD: 30.3105 g] experienced ischemia for 45 min followed by reperfusion in the beginning of which 10 mg Epo /kg body weight were IV administered.

Group C: Reperfusion lasted for 60 min (n=10 Epo rats) mean mass 242.8 g [SD: 29.33636 g], mean CK-MB levels 454.9 IU/L [SD: 126.1326 IU/L] (Table 2).

Group D: Reperfusion lasted for 120 min (n=10 Epo rats) mean mass 243 g [SD: 32.84644 g], mean CK-MB levels 429.7 IU/L [SD: 161.9678 IU/L] (Table 2).

Table 2: Weight and CK-MB mean levels and Std. Dev. of groups

Groups	Variable	Mean	Std. Dev
A	Weight	243 g	45.77724 g
	CK-MB	299.4 IU/L	176.6592 IU/L
B	Weight	262 g	31.10913 g
	CK-MB	315 IU/L	189.6535 IU/L
C	Weight	242.8 g	29.33636 g
	CK-MB	454.9 IU/L	126.1326 IU/L
D	Weight	243 g	32.84644 g
	CK-MB	429.7 IU/L	161.9678 IU/L

2.3 Statistical analysis

Every weight and CK-MB 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 CK-MB 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 CK-MB levels. The 3 independent variables were the Epo administration or no, the reperfusion time and their interaction. Inserting the rats' mass as independent variable at glm, a significant correlation appeared with CK-MB levels (p= 0.0368), so as to further investigation was needed. The predicted CK-MB values, adjusted for rats' weight were calculated and are depicted at table 5. Afterwards, every predicted CK-MB level 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 CK-MB levels. The 3 independent variables were again the Epo administration or no, the reperfusion time and their interaction. The statistical analysis was performed by Stata 6.0 software [Stata 6.0, StataCorp LP, Texas, USA].

3. Results

The first glm resulted in: Epo administration kept significantly increased the CK-MB levels by 135.1 IU/L [31.86157 IU/L - 238.3384 IU/L] (P= 0.0117). This finding was in accordance with the results of standard t-test (p=0.0078). Reperfusion time non-significantly decreased the CK-MB levels by 4.8 IU/L [-117.157 IU/L - 107.557 IU/L] (P= 0.9315), in accordance also with standard t-test (P= 0.8944). However, erythropoietin administration and reperfusion time together produced a significant combined effect in keeping increased the CK-MB levels by 69.10909 IU/L [5.262361 IU/L - 132.9558 IU/L] (P= 0.0346). Reviewing the above and table 3, the tables 4 and 5 sum up concerning the alteration influence of Epo in connection with reperfusion time. The second glm application resulted in: Erythropoietin administration kept non significantly increased the predicted CK-MB values by 15.74619 IU/L [-21.11541 IU/L - 52.60779 IU/L] (P= 0.3926), in accordance also with standard t-test (P= 0.3517). Reperfusion time non significantly decreased the predicted CK-MB values by 15.74622 IU/L [-52.60782 IU/L - 21.11539 IU/L] (P= 0.3926), in accordance also with standard t-test (P= 0.3517). Interaction of erythropoietin administration and reperfusion time kept non significantly increased the predicted CK-MB values by 8.529186 IU/L [-13.7414 IU/L - 30.79977 IU /L] (P= 0.4430). Reviewing the above and table 6, the tables 7 and 8 sum up concerning the alteration influence of Epo in connection with reperfusion time.

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	-19 g	0.2423
	CK-MB	-15.6 IU/L	0.7558
A-C	Weight	0.2 g	0.9900
	CK-MB	-155.5 IU/L	0.0344
A-D	Weight	0 g	1.0000
	CK-MB	-130.3 IU/L	0.0574
B-C	Weight	19.2 g	0.2598
	CK-MB	-139.9 IU/L	0.0813
B-D	Weight	19 g	0.1011
	CK-MB	-114.7 IU/L	0.1294
C-D	Weight	-0.2 g	0.9883
	CK-MB	25.2 IU/L	0.6519

Table 4: The restorative influence of erythropoietin in connection with reperfusion time

			p-values	
Increase	**95% c. in.**	**Reperfusion time**	**t-test**	**glm**
155.5 IU/L	11.28747 IU/L - 299.7125 IU/L	1h	0.0344	0.0361
135.1 IU/L	31.86157 IU/L - 238.3384 IU/L	1.5h	0.0078	0.0117
114.7 IU/L	-50.99605 IU/L - 280.396 IU/L	2h	0.1294	0.1631
-4.8 IU/L	117.157 IU/L - 107.557 IU/L	-reperfusion time	0.8944	0.9315
69.10909 IU/L	5.262361 IU/L - 132.9558 IU/L	interaction		0.0346

Table 5: Mean predicted CK-MB values adjusted for weight and Std. Dev. of groups

Groups	Mean	Std. Dev
A	382.4591 IU/L	75.08527 IU/L
B	351.2947 IU/L	51.02618 IU/L
C	382.7871 IU/L	48.11842 IU/L
D	382.4591 IU/L	53.87576 IU/L

Table 6: Statistical significance of mean values difference for groups (DG) after statistical standard t test application

DG	Difference	p-value
A-B	31.16439 IU/L	0.2423
A-C	-0.3280151 IU/L	0.9900
A-D	0.0000275 IU/L	1.0000
B-C	-31.4924 IU/L	0.2598
B-D	-31.16436 IU/L	0.1011
C-D	0.3280426 IU/L	0.9883

Table 7: The restorative influence of erythropoietin in connection with reperfusion time

				p-values
Increase	**95% c. in**	**Reperfusion time**	**t-test**	**glm**
0.3280151 IU/L	-58.9209 IU/L - 59.57693 IU/L	1h	0.9900	0.9908
15.74618755 IU/L	-21.11541 IU/L - 52.60779 IU/L	1.5h	0.3517	0.3926
31.16436 IU/L	-18.1347 IU/L - 80.46342 IU/L	2h	0.1011	0.2007
-15.74622 IU/L	-52.60782 IU/L - 21.11539 IU/L	reperfusion time	0.3517	0.3926
8.529186 IU/L	-13.7414 IU/L - 30.79977 IU /L	interaction		0.4430

Table 8: The (%) restorative influence of erythropoietin in connection with reperfusion time

Increase	±SD	Reperfusion time	p-values
0.08%	±7.90%	1h	0.9904
4.28%	±5.11%	1.5h	0.3721
8.49%	±6.85%	2h	0.1509
-4.28%	±5.11%	reperfusion time	0.3721
2.32%	±3.09%	interaction	0.4430

4. Discussion

Many clinical situations can prove how CK-MB levels are influenced by ischemic cases. Gonçalves *et al* noted [3] a significant increase in plasma CK-MB levels after 30 minutes IR in control rats than treated ones by ornithine α-ketoglutarate. Jebeli *et al* [4] found significantly higher CK-MB serum levels in myocardial ischemia or infarction patients with impaired left ventricular function [(LVEF) < 35%] undergoing on-pump coronary artery bypass graft (CABG) treated by placebo than treated by milrinone (50.5 μg/kg/min) ($p < 0.05$). Sala *et al* [5] found more frequent by 3.95-fold the CK-MB peak levels > 300 IU/L in patients with acute phase of myocardial infarction (AMI), suffered from ventricular tachycardia (VT) in coronary care unit. Serruys *et al* [6] foundno instances of CK-MB levels elevations 10 or more times the upper limit of normal in patients after successful completion of their first percutaneous coronary intervention PCI randomly receiving treatment with fluvastatin 80 mg/d, or matching placebo at hospital discharge for 3 to 4 years. Savchuk *et al* [7] increased the total coronary blood CK-MB activity by 57.3 ± 11.7 mE/ml ($p < 0.001$) after induced short-term myocardial ischemia in dogs.

Yang *et al* [8] decreased blood CK-MB levels and the markers of kidney injury by treatment with rhEpo (300 U/kg) 48 h after glycerol-induced rhabdomyolysis - one of the causes of acute renal failure - in rats. Popov *et al* [9] suggested that the risk allele (T) about 36% of homozygous genotype distribution in single-nucleotide polymorphism (SNP) rs1617640 at the promoter of the Epo gene plays a role in the development of renal dysfunction after cardiac surgery with cardiopulmonary bypass (CPB). Patients with the TT risk allele also associated with serum CK-MB levels increase (P=0.03), required more frequent acute renal replacement therapy. Wu *et al* [10] further increased the already significantly increased blood CK-MB levels by Epo 300U/kg, IV administration after Escherichia coli lipopolysaccharide (20mg/kg) induced endotoxin shock in conscious rats. Cho *et al* [11] found the most frequent adverse events (AEs) by 25% occurrences of elevated serum CK-MB levels in the rhEpo with albumin formulation administration over 32 hours than combined albumin-free rhEpo and existing rhEpo with albumin formulations in healthy male volunteers. Groopman JE [12] included the usage of hematopoietic growth factors, such as erythropoietin in alleviation of the zidovudine hematologic toxicity. Anemia, leukopenia and myopathy, also appears to be time and dose dependent. Patients often exhibit an associated elevation in CK-MB level.

5. Conclusion

Erythropoietin administration whether it interacted or not with reperfusion time kept short-term non significantly increased the predicted CK-MB values. Epo got on decline the CK-MB values from significant to non-significant levels proving an encouraging restorative short-term capacity, with interesting impacts in Cardiology.

Acknowledgment

This study was funded by Scholarship by the Experimental Research Center ELPEN Pharmaceuticals (E.R.C.E), Athens, Greece. The research facilities for this project were provided by the aforementioned institution

References

[1] Tsompos C, Panoulis C, Toutouzas K, Triantafyllou A, Zografos G, Papalois A. The effect of erythropoietin on calcium levels during hypoxia reoxygenation injury in rats. *Geriatric care* 2016; 2:5722:12-16.

[2] Tsompos C, Panoulis C, Toutouzas K, Zografos G, Papalois A. The effect of erythropoietin on albumins levels during hypoxia reoxygenation injury in rats. *Research Journal of Pharmacology and Toxicology* 2015; 1(3):42-45.

[3] Gonçalves ES, Rabelo CM, Prado Neto AX, *et al*: Effect of short-term ornithine alpha-ketoglutarate pretreatment on intestinal ischemia-reperfusion in rats. *Acta Cir Bras*. 2011; 26 Suppl 1:2-7.

[4] Jebeli M, Ghazinoor M, Mandegar MH, *et al*: Effect of milrinone on short-term outcome of patients with myocardial dysfunction undergoing coronary artery bypass graft: A randomized controlled trial. *Cardiol J.* 2010; 17(1):73-8.

[5] Sala MF, Bárcena JP, Rota JI, *et al*: Sustained ventricular tachycardia as a marker of inadequate myocardial perfusion during the acute phase of myocardial infarction. *Clin Cardiol*. 2002 Jul; 25(7):328-34.

[6] Serruys PW, de Feyter P, Macaya C, *et al*: Fluvastatin for prevention of cardiac events following successful first percutaneous coronary intervention: a randomized controlled trial. *JAMA.* 2002 Jun 26; 287(24):3215-22.

[7] Savchuk VI, Vinogradov AV, Pozin VM, *et al*: Activity of total serum creatine phosphokinase and its MB fraction in reversible coronary blood flow disorders in dogs in a chronic experiment. *Kardiologiia.* 1980 Jan; 20(1):58-60.

[8] Yang FL, Subeq YM, Chiu YH, *et al*: Recombinant human erythropoietin reduces rhabdomyolysis-induced acute renal failure in rats. *Injury.* 2012 Mar; 43(3):367-73.

[9] Popov AF, Schulz EG, Schmitto JD, *et al*: Relation between renal dysfunction requiring renal replacement therapy and promoter polymorphism of the erythropoietin gene in cardiac surgery. *Artif Organs.* 2010 Nov; 34(11):961-8.

[10] Wu WT, Hu TM, Lin NT, *et al*: Low-dose erythropoietin aggravates endotoxin-induced organ damage in conscious rats. *Cytokine.* 2010 Feb; 49(2):155-62.

[11] Cho SH, Lim HS, Ghim JL, *et al*: Pharmacokinetic, tolerability, and bioequivalence comparison of three different intravenous formulations of recombinant human erythropoietin in healthy Korean adult male volunteers: an open-label, randomized-sequence, three-treatment, three-way crossover study. *Clin Ther*. 2009 May; 31(5):1046-53.

[12] Groopman JE. Zidovudine intolerance. *Rev Infect Dis*. 1990 Jul-Aug; 12 Suppl 5:S500-6.