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The effect of erythropoietin on chloride 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 that molecule was studied biochemically using blood mean chloride (Cl) levels.
Materials and methods. 40 rats of mean weight 247.7 g were used in the study. Cl levels were measured at 60 min (groups A and C) and at 120 min (groups B and D) of reoxygenation. Erythropoietin was administered only in groups C and D.
Results. Epo administration non-significantly decreased Cl levels by 1.07%+0.91% (p=0.2635). Reoxygenation time non-significantly decreased Cl levels by 0.68%+0.92% (P= 0.4457). However, erythropoietin administration and reoxygenation time together produced a non-significant combined effect in decreasing Cl levels by 0.74%+0.54% (P= 0.1701).
Conclusions. Epo administration, reoxygenation time and their interaction have non-significant, short-term, decreasing effects on Cl levels.

Key words: chloride, hypoxia, erythropoietin, reoxygenation

INTRODUCTION

Erythropoietin (Epo) is generally one of the more well studied growth factors. Epo is implicated in over 29,194 known biomedical studies at present. At least 10.39% of these studies concern tissue hypoxia and reoxygenation (HR) experiments. Important progress has been made concerning Epo usage in reversing HR injuries of adjacent organs and certainly patients' health. Satisfactory answers to basic questions have not yet been provided, such as its action velocity, the administration timing and the dosage. The concept is the knowledge promotion away from the original action of Epo as a glycoprotein cytokine secreted by the kidney in response to cellular hypoxia; which stimulates red blood cell production (erythropoiesis) in the bone marrow. However, just a few related reports were found, not completely covering more specific matters. A numeric evaluation of Epo efficacy was yielded by a meta-analysis of 34 published studies, based on the same experimental setting, using the same endpoints (table 1).
The aim of this experimental study was to examine the effect of Epo on a rat model and particularly in an HR protocol. The effects of that model were studied by measuring the blood mean chloride (Cl) levels.

MATERIALS AND METHODS

Animal preparation

The prefectural vet of East Attiki licensed this experiment under 3693/12-11-2010 & 14/10-1-2012 decisions. All substances, equipment and consumables needed for the study were donated as a courtesy of ELPEN Pharmaceuticals Co Inc. S.A. at Pikermi, Attiki. Appropriate care was adopted for female albino Wistar rats. Seven days pre-experimental normal housing in laboratory included ad libitum diet. Prenarcosis preceded of non-stop intra-experimental general anesthesia, (1) electrocardiogram, acidometry and oxygen supply. Post-experimental euthanasia did not permit preservation of the rodents.
The rodents were randomly delivered to four experimental groups, each one consisted of 10 animals. The 4 groups had common the stage of preceded hypoxia of 45 min induced by laparotomic clamping inferior aorta over renal arteries by forceps. Afterwards, the clamp removal restored reoxygenation by inferior aorta patency reestablishment. 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 of molecule Epo was 10 mg/kg body mass per animal. Epo administration was performed at the time of reoxygenation, through inferior vena cava catheter. The Cl 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): 34.99172 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 Cl levels. This assumption was also investigated.

Model of hypoxia-reoxygenation injury

Control groups
20 control rats of mean weight 252.5 g [SD: 39.31988 g] experienced hypoxia for 45 min followed by reoxygenation.

Group A
Reoxygenation lasted 60 min concerning 10 control rats of mean weight 243 g [SD: 45.77724 g] and mean Cl levels 103.4 mmol/l [SD: 2.1187 mmol/l] (table 2).

Group B
Reoxygenation lasted 120 min concerning 10 control rats of mean weight 262 g [SD: 31.10913 g] and mean Cl levels 102.9 mmol/l [SD: 1.911951 mmol/l] (table 2).

Erythropoietin group
20 Epo rats of mean weight 242.9 g [SD: 30.3105 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 lasted 60 min concerning 10 Epo rats of mean weight 242.8 g [SD: 29.33636 g] and mean Cl levels 102.5 mmol/l [SD: 2.068279 mmol/l] (table 2).

Group D
Reoxygenation lasted 120 min concerning 10 Epo rats of mean weight 243 g [SD: 32.84644 g] and mean Cl levels 101.6 mmol/l [SD: 4.695151 mmol/l] (table 2).

Statistical analysis

Every weight and Cl 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 Cl 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 Cl 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 non significant correlation appeared with Cl levels (p=0.0577), so as to further investigation was not required. The statistical analysis was performed by Stata 6.0 software [Stata 6.0, StataCorp LP, Texas, USA].

RESULTS

Epo administration non significantly decreased the Cl levels by 1.1 mmol/l [-2.944603 mmol/l - 0.7446032 mmol/l] (P=0.2348). This finding was in accordance with the results of standard t-test (p=0.2922). Reoxygenation time non-significantly decreased the Cl levels by 0.7 mmol/l [-2.565532 mmol/l - 1.165532 mmol/l] (P= 0.4522), in accordance also with standard t-test (P= 0.4392). However, erythropoietin administration and reoxygenation time together produced a non significant combined effect in decreasing the Cl levels by 0.7636364 mmol/l [-1.869013 mmol/l - 0.3417403 mmol/l] (P= 0.1701). Reviewing the above and table 3, the tables 4 and 5 sum up concerning the decreasing influence of Epo in connection with reoxygenation time.

Table 1. Erythropoietin (Epo) influence (+SD) on the levels of some serum (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
Hemoglobin	+4.09%+5.20%	0.3350	+2.15%+2.63	0.4527	+0.20%+5.08%	0.9584	+1.31%+1.59%	0.3984
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
MCV	+0.01%±1.08%	0.9904	+0.56%±0.66%	0.3549	+1.12%±0.91%	0.1509	+0.30%±0.39%	0.4430
MCHC	+1.82%+0.56%	0.0076	+1.73%+0.50%	0.0016	+1.65%+0.92%	0.0721	+0.89%+0.31%	0.0061
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
Plt C	-7.32%+13.11%	0.5219	-2.14%+8.04%	0.7581	+3.04%+10.78%	0.7204	-0.16%+4.76%	0.9725
MPV	+3.82%+4.10%	0.3105	-0.12%+2.13%	0.9513	-4.07%+3.75%	0.2608	-0.27%+0.92%	0.7585
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
Glucose (7)	+0.75%+8.11%	0.9307	+5.59%+6.46%	0.3208	+10.44%+10.99%	0.3491	+4.94%+3.81%	0.1892
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	-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
AST	+29.53%+9.72%	0.0096	+26.71%+13.17%	0.0235	+23.89%+21.59%	0.1709	+19.73%+7.70%	0.0119
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
CK-MB	+0.08%+7.90%	0.9904	+4.28%+5.11%	0.3721	+8.49%+6.85%	0.1509	+2.32%+3.09%	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	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
Testosteron (2)	+47.05%+58.96%	0.4120	+71.21%+44.19%	0.1080	+95.38%+46.14%	0.0470	+27.65%+27.21%	0.3006
Mean	+3.52%+12.31%	0.5694	+4.60%+14.69%	0.3743	+5.69%+18.79%	0.3463	+2.93%+7.21%	0.4114

Table 2. Weight and chloride (Cl) mean levels and Std. dev. of groups

Groups	Variable	Mean	Std. Dev
A	Weight	243 g	45.77724 g
A	Cl	103.4 mmol/l	2.1187 mmol/l
B	Weight	262 g	31.10913 g
B	Cl	102.9 mmol/l	1.911951 mmol/l
C	Weight	242.8 g	29.33636 g
C	Cl	102.5 mmol/l	2.068279 mmol/l
D	Weight	243 g	32.84644 g
D	Cl	101.6 mmol/l	4.695151 mmol/l

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
A-B	Cl	0.5 mmol/l	0.6262
A-C	Weight	0.2 g	0.9900
A-C	Cl	0.9 mmol/l	0.3783
A-D	Weight	0 g	1.0000
A-D	Cl	1.8 mmol/l	0.3203
B-C	Weight	19.2 g	0.2598
B-C	Cl	0.4 mmol/l	0.7109
B-D	Weight	19 g	0.1011
B-D	Cl	1.3 mmol/l	0.4987
C-D	Weight	-0.2 g	0.9883
C-D	Cl	0.9 mmol/l	0.5692

Table 4. The decreasing influence of erythropoietin in connection with reperfusion time.

			p-values	
Decrease	95% c. in.	Reperfusion time	t-test	Glm
0.9 mmol/l	-2.867103 mmol/l - 1.067103 mmol/l	1h	0.3783	0.3492
1.1 mmol/l	-2.944603 mmol/l - 0.7446032 mmol/l	1.5h	0.2922	0.2348
1.3 mmol/l	-4.668034 mmol/l - 2.068034 mmol/l	2h	0.4987	0.4280

0.7 mmol/l	-2.565532 mmol/l - 1.165532 mmol/l	reperfusion time	0.4392	0.4522
0.7636364 mmol/l	-1.869013 mmol/l - 0.3417403 mmol/l	interaction	-	0.1701

Table 5. The (%) decreasing influence of erythropoietin in connection with reperfusion time.

Decrease	+SD	Reperfusion time	p-values
0.87%	+0.97%	1h	0.3637
1.07%	+0.91%	1.5h	0.2635
1.27%	+1.68%	2h	0.4633
0.68%	+0.92%	reperfusion time	0.4457
0.74%	+0.54%	interaction	0.1701

DISCUSSION

Frequently, the change in Cl levels is associated with relevant changes in sodium levels. Sodium is the main electrolyte found in extracellular fluid and is involved in fluid balance and blood pressure control. All known higher life forms require a subtle and complex electrolyte balance between the intracellular and extracellular environment. In particular, the maintenance of precise osmotic gradients of electrolytes is of major importance. Such gradients affect and regulate the hydration of the body as well as blood pH. However, when sodium chloride (NaCl) is placed in serum, the salt dissolves into its component ions, due to thermodynamic interactions between serum and solute molecules according to dissociation reaction salvation: NaCl(s) → Na+(aq) + Cl−(aq)

Thus, the vast content of chloride in serum is as independent chloride ion Cl-(aq) and not as salt. For this reason, an individual and autonomous consideration of chloride regardless of sodium conjugate should be considered complete. Unpleasantly, there are not described situations concerning whether hypoxia can influence only the Cl levels in bibliography. On the contrary, there are a lot of cases reporting how the Cl levels fluctuations affect the function of various organs. Such examples are described herein. Nevertheless, isolated Cl administration is impossible. It is meant that, Cl is administered conjugated with another ion or factor possibly influencing the Cl levels. Le LL et al. achieved (3) brain ischemia-reperfusion (IR) injury three weeks after ischemic preconditioning (IPC) in rats. 2,3,5-triphenyltetrazolium Cl staining showed that IPC significantly reduced brain infarct area and improved neurological function of future cerebral injury. Rehni AK et al. measured cerebral infarct size by using triphenyltetrazolium Cl staining and found (4) beneficial effects of IPC on global cerebral IR-induced cerebral injury and behavioral deficits in mice. Hinkel R et al. determined segmental endocardial shortening in coronary IR infarct zone and infarct size by triphenyl tetrazolium Cl viability and found the 24 hours survival of neonatal pigs cardiomyocytes increased (5) by 2.8-fold than control ones after embryonic endothelial progenitor cells (5x106 cells) application in vivo. Murlasits Z et al. determined infarct area by a histological [triphenyltetrazolium Cl (TTC)] method, resulting in (6) cardioprotection as evidenced by reduced infarct size ($P<0.05$) in exercise-trained male Sprague-Dawley rats followed by bouts (70% of O2max). Mozaffari MS et al. found markedly worsened (7) contractile function following IR injury but infarct size reduced by 5 weeks salt (1% NaCl solution) regimen administration than control groups in male rats. Birnbaum Y et al. assessed (8) infarct size by triphenyltetrazolium Cl in male Sprague-Dawley both case and placebo rats groups ($p = 0.503$). Kadambi A et al. observed (9) elevated xanthine oxidase levels in rat muscles subjected to IR + 0.9% saline than non ischemic controls ones.

Also the majority of the following examples concern the influence of Cl levels fluctuation on Epo levels. The minority of examples concern the influence of Epo fluctuation on the Cl levels. Tringali G et al. found (10) that brain hypoxia-ischemia increases the Epo levels which inhibited the 56 mM KCl-induced CRH release than control ones. Freudenthaler S et al. found none concomitant alteration of Epo concentrations affected after high (HS), normal (NS) or low (LS) salt diet administration ($P = 0.54$) in (11) volunteer ones. Freudenthaler SM et al. demonstrated (12) no significant differences of AUC (Epo(0-48 h)) although Epo concentration in plasma was increased up to 290% of the baseline level, treating human volunteers by a short-term 0.9% NaCl administration during the period of hypoxia than control group.

CONCLUSION

Erythropoietin administration, reoxygenation time and their interaction have non significant short-term decreasing effects on Cl levels. Perhaps, a longer study time or a greater Epo dose may reveal clearer and significant effects.

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