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Original Article

The effect of erythropoietin on albumins levels during hypoxia reoxygenation injury in rats

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Abstract
Aim and objective: The aim of this experimental study was to examine the effect of erythropoietin on rat model and particularly in a hypoxia reoxygenation (HR) protocol. The effect of that molecule was studied biochemically using blood mean albumins levels.
Materials and methods: 40 rats of mean weight 247.7 g were used in the study. Albumins 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.
Result: Results were that Epo administration significantly decreased the albumins levels by 9.28%+3.20% (p=0.0054). Reperfusion time non-significantly increased the albumins levels by 3.09%+3.52% (p= 0.3405). However, erythropoietin administration and reperfusion time together produced a significant combined effect in keeping decreased the albumins levels by 5.37%+2.73% (p= 0.0072).
Conclusions: Conclusions are that erythropoietin administration whether it interacted or not with reoxygenation time, has significant decreasing effects on albumins levels in a short-term context of 2 hours.

1. Introduction

Tissue hypoxia and reoxygenation (HR) remain of the main causes of permanent or transient damage with serious implications on adjacent organs and certainly on patients' health. Although important progress has been made regarding the usage of erythropoietin (Epo) in managing this kind of damages, satisfactory answers have not been given yet to fundamental questions, as, by what velocity this factor acts, when it should be administered, and in which dosage. The particularly satisfactory action of Epo in stem blood cells recovery has been noted in several performed experiments. However, just few relative reports were found concerning Epo trial in HR experiments, not covering completely this particular matter. A meta-analysis of 14 published seric variables, coming from the same experimental setting, tried to provide a numeric evaluation of the Epo efficacy 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 blood cells	+24.01%±13.38%	0.1012	+22.09%±9.11%	0.0351	+20.17%±12.94%	0.0902	+14.63%±5.40%	0.0080
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
mean corpuscular hemoglobin	+0.01%+1.29%	0.9904	+0.67%±0.80%	0.3549	+1.34%±1.08%	0.1509	-0.36%±0.47%	0.4430
platelet distribution width	+1.60%±0.80%	0.0765	+1.36%±0.58%	0.0205	+1.13%±0.74%	0.1152	+0.37%±0.37%	0.0615
plateletcrit	-16.47%±10.40%	0.0921	-13.74%±7.01%	0.0158	-11.01%±7.34%	0.0882	-6.88%±3.69%	0.0615
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 protein	-0.02%±2.47%	0.9904	-1.27%±1.51%	0.3721	-2.52%±2.03%	0.1509	-0.68%±2.48%	0.4430
alkaline phosphatase	+0.20%±18.57%	0.9904	+10.70%±12.78%	0.3549	+21.20%±17.11%	0.1509	+5.79%±7.72%	0.4430
acid phosphatase	+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
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
progesterone	-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%±8.41%	0.6900	+3.27%±9.12%	0.3147	+4.95%±11.82%	0.2394	+2.02%±5.41%	0.3704

Furthermore, several publications addressed trials of other similar molecules of growth factors to which the studied molecule also belongs to.

The **aim** of this experimental study was to examine the effect of Epo on rat model and particularly in a liver ischemia reperfusion (IR) protocol. The effect of that molecule was studied by measuring the blood mean albumins levels.

2. Materials and methods

2.1 Animal preparation

This experimental study was lisenced 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 of Experimental Research Center of ELPEN Pharmaceuticals Co. Inc. S.A. at Pikermi, Attiki. Accepted standards of humane animal care were adopted for Albino female Wistar rats. Normal housing in laboratory 7 days before the experiment included continuous access to water and food. The experiment was acute, that means that awakening and preservation of the rodents was not following the experiment. They were randomly delivered to four experimental groups by 10 animals in each one. 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 Epo intravenous (IV) administration and reoxygenation for 60 min (group C). Hypoxia for 45 min followed by immediate Epo IV administration and reoxygenation for 120 min (group D). The molecule Epo dosage was 10 mg/Kg body weight of animals.

At first, the animals were submitted into prenarcosis followed by general anesthesia. The detailed anesthesiologic technique is described in related references[1]. Oxygen supply, electrocardiogram and acidometry were continuously provided during whole experiment performance.

The protocol of HR was followed. Hypoxia was caused by forceps clamping inferior aorta over renal arteries for 45 min after laparotomic access had been achieved. Reoxygenation was induced by removing the clamp and reestablishment of inferior aorta patency. The molecules were administered at the time of reoxygenation, through inferior vena cava after catheterization had been achieved. The albumins levels measurements were performed at 60 min of reoxygenation (for groups A and C) and at 120 min of reoxygenation (for groups B and D). The mean weight of the forty (40) female Wistar albino rats used was 247.7 g [Std. Dev: 34.99172 g], with min weight ≥ 165 g and max weight ≤ 320 g. Rats' weight could be potentially a confusing factor, e.g. the more obese rats to have greater albumins levels. This suspicion was investigated.

2.2 Model of hypoxia-reoxygenation injury

Control groups: 20 control rats (mean mass 252.5 g [Std. Dev: 39.31988 g] suffered by hypoxia for 45 min followed by reoxygenation.

Group A: Reoxygenation lasted for 60 min (n=10 controls rats) mean mass 243 g [Std. Dev: 45.77724 g], mean albumins levels 3.55 g/dL [Std. Dev: 0.3308239 g/dL] (Table 2).

Group B: Reoxygenation lasted for 120 min (n=10 controls rats) mean mass 262 g [Std. Dev: 31.10913 g], mean albumins levels 3.83 g/dL [Std. Dev: 0.3917198 g/dL] (Table 2).

Erythropoietin group: 20 Epo rats (mean mass 242.9 g [Std. Dev: 30.3105 g] suffered by 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 for 60 min (n=10 Epo rats) mean mass 242.8 g [Std. Dev: 29.33636 g], mean albumins levels 3.39 g/dL [Std. Dev: 0.2766867 g/dL] (Table 2).

Group D: Reoxygenation lasted for 120 min (n=10 Epo rats) mean mass 243 g [Std. Dev: 32.84644 g], mean albumins levels 3.33 g/dL [Std. Dev: 0.3591657 g/dL] (Table 2).

2.3 Statistical analysis

Weight comparison of everyone from 4 rats groups initially was performed with each other from 3 remained groups applying statistical paired t-test (Table 3). Any emerging significant difference among albumins levels, was investigated whether owed in the above mentioned significant weight correlations. Albumins levels comparison of everyone from 4 rats groups initially was performed with each other from 3 remained groups applying statistical paired t-test (Table 3). The application of generalized linear models (glm) with dependant variable the albumins levels and independent variables the Epo administration or no, the reoxygenation time and their interaction was followed. Inserting the rats' weights as independent variable at glm, a non-significant relation turned on albumins levels (p= 0.0874), so as to further investigation was not needed.

3. Results

Epo administration significantly decreased the albumins levels by 0.33 g/dL [-0.5533603 g/dL - -0.1066396 g/dL] (P= 0.0049). This finding was in accordance with the results of paired t-test (p=0.0059). Reoxygenation time non-significantly increased the albumins levels by 0.11 g/dL [-0.1356204 g/dL - 0.3556204 g/dL] (P= 0.3703), in accordance also with paired t-test (P= 0.3107). However, erythropoietin administration and reoxygenation time together produced a significant combined effect in keeping decreased the albumins levels by 0.1909091 g/dL [-0.3268572 g/dL -0.054961 g/dL] (P= 0.0072). Reviewing the above and table 3, the tables 4 and 5 sum up concerning the alteration influence of Epo in connection with reoxygenation time.

Table 2: Weight and albumin mean levels and Std. Dev. of groups

Groups	Variable	Mean	Std. Dev
A	Weight	243 g	45.77724 g
	Albumin	3.55 g/dL	0.3308239 g/dL
B	Weight	262 g	31.10913 g
	Albumin	3.83 g/dL	0.3917198 g/dL
C	Weight	242.8 g	29.33636 g
	Albumin	3.39 g/dL	0.2766867 g/dL
D	Weight	243 g	32.84644 g
	Albumin	3.33 g/dL	0.3591657 g/dL

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

DG	Variable	Difference	p-value
A-B	Weight	-19 g	0.2423
	Albumin	-0.28 g/dL	0.1189
A-C	Weight	0.2 g	0.9900
	Albumin	0.16 g/dL	0.2500
A-D	Weight	0 g	1.0000
	Albumin	0.22 g/dL	0.0338
B-C	Weight	19.2 g	0.2598
	Albumin	0.44 g/dL	0.0170
B-D	Weight	19 g	0.1011
	Albumin	0.5 g/dL	0.0109
C-D	Weight	-0,2 g	0.9883
	Albumin	0.06 g/dL	0.6275

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

Decrease	95% c. in	p-values		
		Reperfusion time	t-test	Glm
0.16 g/dL	-0.4465275 g/dL - 0.1265276 g/dL	1h	0.2500	0.2560
0.33 g/dL	-0.5533603 g/dL - -0.1066396 g/dL	1.5h	0.0059	0.0049
0.5 g/dL	-0.8530827 g/dL - -0.1469173 g/dL	2h	0.0109	0.0081
-0.11 g/dL	-0.1356204 g/dL - 0.3556204 g/dL	reperfusion time	0.3107	0.3703
0.1909091 g/dL	-0.3268572 g/dL - 0.054961 g/dL	interaction		0.0072

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

Decrease	±SD	Reperfusion time	p-values
-4.61%	±4.21%	1h	0.2530
-9.28%	±3.20%	1.5h	0.0054
-13.96%	±5.03%	2h	0.0095
+3.09%	±3.52%	reperfusion time	0.3405
-5.37%	±2.73%	interaction	0.0072

4. Discussion

A lot of clinical situations can show how ischemia influences the albumins levels. Liepinsh *et al* [2] demonstrated that ischemic damage is significantly lower in the fed state compared with fasted state in Wistar and diabetic Goto-Kakizaki rat's hearts. Even overnight fasting could provoke and aggravate cardiovascular events and high-risk cardiovascular patients should avoid prolonged fasting periods. Abubakar *et al* [3] determined the mean serum albumin significantly higher by 45.67% in short-term first-ever acute stroke favourable outcome patients than those with unfavourable outcome (p=0.0001). Patients that died had significantly lower serum albumin (1.66 g/dl) than survivors (p=0.0001). Serum albumin of 1.55 g /dL has sensitivity of 100% and specificity of 61.5%. Low admission serum albumin was an independent determinant of poor outcome. Herisson *et al* [4] found neither difference nor correlation in admission baseline ischemia-modified albumin (IMA) levels within 4.5 hours of acute ischemic and hemorrhagic stroke onset patients. Consuegra-Sanchez *et al*[5] found the 30-day combined end point 1.48-fold (P = 0.017) and the 1-year mortality rate 1.78-fold (P = 0.028) significantly[5] higher in patients with IMA levels > 93.3 U/ml obtained on admission compared with lower IMA or IMA as a general independent predictor patients presenting to the emergency department with typical acute chest pain. Polk *et al*[6] measured significantly higher cobalt-albumin binding assay (CABA) test values by 1.67-fold in clinically diagnosed intestinal ischemia patients, than control patients without intestinal ischemia (p = 0.00023). This resulted in a sensitivity of 100% and a specificity of 85.7% for the CABA test, making it a useful tool for risk stratification of intestinal ischemia. Dusek *et al* [7] used a negative IMA marker as an aid to rule out acute coronary syndrome (ACS) in low risk symptomatic patients with non-diagnostic ECG and normal troponin. Worster *et al*[8] found likelihood ratios 1.35 and 0.98 for IMA < and > 80 U/mL respectively within 6 hours after chest pain in order to predict a serious cardiac outcome within the following 72 hours, suggest IMA as a poor short term predictor of serious cardiac outcomes. Rafael Sadaba *et al* [9] reduced the mean tissue perfusion of the upper limb by 15.38% (P = 0.000555) using Technetium-99m human serum albumin after removal of the radial artery, but did not affect short term hand function. Steinbauer *et al* [10] enhanced the half-life and antioxidant activity of nitroxides by their covalent binding to human serum albumin, resulting in polynitroxyl albumin (PNA) in an IR hamster dorsal skinfold chamber model. PNA in the dose 1% b.w. and--to a lesser extent albumin--effectively reduced postischemic microvascular perfusion failure, and tissue injury. Although free oxygen radical scavenging seems to be an underlying mechanism leading to the beneficial effects of PNA on IR injury, hemodilution and known radical scavenging properties of pure albumin contribute in part to the observed effects. Donaldson *et al* [11] explained why deaths from arterial disease are more prevalent in winters associating short-term falls in temperature with significant and prolonged haemoconcentration and hypertension which produce significant increases in serum albumin and in mortalities from ischemic heart disease and cerebrovascular disease. Arend *et al* [12] found similar significant decreases in serum albumin values within 24 h in heart patients both treated and control ones with nitroglycerin. Pollock *et al*[13] produced a significant increase by 2.05-fold nearly identical to those of atrial natriuretic factor (ANF) at 0.5 μg/kg/min in glomerular filtration rate (GFR) administering 10 μg/kg/min A68828 after acute renal IR failure compared with vehicle controls (P < 0.05). These results indicate that infusion of a reduced-size analog of ANF, A68828ANF improves renal function in the immediate postischemic period in Sprague-Dawley rats. Tilton *et al* [14] decreased the rate of intravascular clearance of radiolabelled albumin by 36% and increased the mean transit time of the coronary vasculature by albumin approximately 1.875-fold in ischemic hearts than control hearts from rabbits fed normal chow and 2-fold increase prior to ischemia in rabbits fed cholesterol for 2-3 weeks.

Also, albumines is a factor influenced by Epo administration. Kojima *et al* [15] switched patients from conventional in-center hemodialysis to home hemodialysis (HHD) improving serum albumin levels and simultaneously reducing erythropoietin-stimulating agent levels. Capelli *et al* [16] found a local 69.31-fold and a national 4.22-fold mortality increase for albumin > 4.0 than albumin <3.5 as statistically significant predictors of survival. These findings indicate that individually higher albumin levels improve the survival rates and hospitalizations for end-stage renal disease (ESRD) patients under erythropoietic stimulating agents (epoetin-α) use. Costa *et al*[17] found statistically significant differences between responders and nonresponders (resistance) to rhEPO therapy hemodialysis chronic kidney disease (CKD) patients which may be due to the erythropoiesis-suppressing effect of pro-inflammatory cytokines, for albumin (lower in nonresponders) levels both compared with healthy control ones. Lin *et al* [18] found significantly increased TS at following 4 months in chronic HD patients with type II diabetes. Aguilera *et al*[19] induced a temporary, non inflammatory immune hyperactivity mediated by TNFα increasing progressively albumin 7% and short term normalized protein catabolic rate (nPCR) 12% daily and then decreased progressively albumin 5% and nPCR 5% daily too, in peritoneal dialysis (PD) rHuEPO (40-70 subcutaneous units/kg weekly) treated patients than control group. rHuEPO could increase food intake and improve the nutritional status of PD patients. Ortega *et al* [20] showed lower (P<0.05) albumin concentration and predicted poorer response to weekly Epo dose in pre-dialysis patients with higher prevalence of inflammation and CRP levels (>6 mg/l) at baseline. Rudduck *et al* [21] analyzed the stimulating effect of bovine serum albumin (BSA) and Epo on the erythroid mitoses in short-term marrow cultures. Krystal *et al* [22] reduced markedly the specific activities of 100-300 units of partially purified Epo urine preparations stored in solution in the presence of 1% bovine serum albumin.

5. Conclusion

Erythropoietin administration whether it interacted or not with reoxygenation time, has significant decreasing effects on albumins levels in a short-term context of 2 hours. It seems that starvation, or launched anabolic pathways consume albumins. Further human clinical or molecular studies are required to make this effect clearer.

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