THE ACUTE EFFECT OF ERYTHROPOIETIN ON GLUCOSE LEVELS DURING ISCHEMIA REPERFUSION INJURY IN RATS

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ABSTRAK

Tujuan dari studi eksperimental ini adalah untuk menguji pengaruh erythropoietin pada model tikus dan khususnya dalam iskemia reperfusi (HR) protokol. Pengaruh molekul yang dipelajari biokimia menggunakan darah berarti glukosa (Gl) tingkat. Bahan dan metode: 40 tikus dari rata-rata berat 247,7 g yang digunakan dalam penelitian ini. tingkat gl diukur pada 60 menit (kelompok A dan C) dan pada 120 menit (kelompok B dan D) dari reperfusi. Erythropoietin diberikan hanya pada kelompok C dan D. Hasil itu bahwa pemberian Epo non-signifikan meningkatkan tingkat gl oleh 5,59% + 6.46% (p = 0,3208). Waktu reperfusi non-signifikan meningkatkan tingkat gl sebesar 5,63% + 6.45% (p = 0,4098). Namun, administrasi erythropoietin dan waktu reperfusi bersama-sama menghasilkan efek gabungan non signifikan dalam meningkatkan tingkat gl sebesar 4,94% + 3,81% (p = 0,1892). Kesimpulan: Hasil penelitian ini menunjukkan bahwa pemberian erythropoietin, waktu reperfusi, atau interaksi mereka non-signifikan meningkatkan kadar glukosa darah dalam jangka pendek. Data bibliografi berlawanan dianggap lebih dapat diandalkan, sampai sampel yang lebih besar memberikan hasil yang lebih jelas. ***(FMI 2016;52:14-18)***

Kata kunci: *iskemia,eritropoietin, kedar gula, reperfusi*

ABSTRACT

The aim of this experimental study was to examine the effect of erythropoietin on rat model and particularly in an ischemia reperfusion (HR) protocol. The effect of that molecule was studied biochemically using blood mean glucose (Gl) levels. Materials and methods: 40 rats of mean weight 247.7 g were used in the study. Gl 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 were that Epo administration non-significantly increased the gl levels by 5.59% +6.46% (p=0.3208). Reperfusion time non-significantly increased the gl levels by 5.63%+6.45% (p=0.4098). However, erythropoietin administration and reperfusion time together produced a non significant combined effect in increasing the gl levels by 4.94%+3.81% (p= 0.1892). Conclusions: Results of this study indicate that erythropoietin administration, reperfusion time, or their interaction non-significantly increase the blood glucose levels in short-term. Opposite bibliographic data are considered more reliable, until a greater sample provide clearer results. ***(FMI 2016;52:14-18)***

Keywords: *ischemia, erythropoietin, glucose levels, reperfusion*

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Abbreviations:
Gl: glucose; Epo: erythropoietin; MBG: mean blood glucose; AG: admission glucose

INTRODUCTION

Tissue ischemia reperfusion (IR) 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 should it 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 IR experiments, not covering completely this particular matter. A meta-analysis of 13 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) (Tsompos et al

2015a, Tsompos et al 2015b). Furthermore, several publications addressed trials of other similar molecules of growth factors to which the studied molecule also belongs to (Bader et al 2011, Wang et al 2011). The aim of this experimental study was to examine the effect of Epo on rat model and particularly in a pancreas IR protocol. The effect of that molecule was studied by measuring the blood mean glucose (Gl) levels.

MATERIALS AND METHODS

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 Exprerimental 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. Ischemia for 45 min followed by reperfusion for 60 min (group A). Ischemia for 45 min followed by reperfusion for 120 min (group B). Ischemia for 45 min followed by immediate Epo intravenous (IV) administration and reperfusion for 60 min (group C). Ischemia for 45 min followed by immediate Epo IV administration and reperfusion 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 (Tsompos et al 2015a, Tsompos et al 2015b). Oxygen supply, electrocardiogram and acidometry were continuously provided during whole experiment performance.

The protocol of IR was followed. Ischemia was caused by forceps clamping inferior aorta over renal arteries for 45 min after laparotomic access had been achieved. Reperfusion was induced by removing the clamp and reestablishment of inferior aorta patency. The molecules were administered at the time of reperfusion, through inferior vena cava after catheterization had been achieved. The Gl levels measurements were performed at 60 min of reperfusion (for groups A and C) and at 120 min of reperfusion (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 $\geq$ 165 g and max weight $\leq$ 320 g. Rats' weight could be potentially a confusing factor, e.g. the more obese rats to have greater gl levels. This suspicion was investigated.

Model of ischemia-reperfusion injury in each groups was as follows: Control groups: 20 control rats (mean mass 252.5 g [Std. Dev: 39.31988 g] suffered by ischemia for 45 min followed by reperfusion. Group A: Reperfusion lasted for 60 min (n=10 controls rats) mean mass 243 g [Std. Dev: 45.77724 g], mean gl levels 420.6 mg/dl [Std. Dev: 69.20373 mg/dl] (Table 2). Group B: Reperfusion lasted for 120 min (n=10 controls rats) mean mass 262 g [Std. Dev: 31.10913 g], mean gl levels 423.6 mg/dl [Std. Dev: 102.9997 mg/dl] (Table 2). Erythropoietin group: 20 Epo rats (mean mass 242.9 g [Std. Dev: 30.3105 g] suffered by 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 [Std. Dev: 29.33636 g], mean gl levels 423.8 mg/dl [Std. Dev: 73.60676 mg/dl] (Table 2). Group D: Reperfusion lasted for 120 min (n=10 Epo rats) mean mass 243 g [Std. Dev: 32.84644 g], mean gl levels 470.3 mg/dl [Std. Dev: 101.9979 mg/dl] (Table 2).

RESULTS

Weight comparison of every one 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 gl levels, was investigated whether owed in the above mentioned significant weight correlations. Gl levels comparison of every one from 4 rats groups initially was performed with each other from 3 remained groups applying statistical paired t-test (Table 3). Applying generalized linear models (glm) with dependant variable the gl levels and independent variables the Epo administration or no, the reperfusion time and their interaction, resulted in: Epo administration non-significantly increased the gl levels by 24.75 mg/dl [-31.36051 mg/dl - 80.86051 mg/dl] (P= 0.3775). This finding was in accordance with the results of paired t-test (p=0.2642). Reperfusion time non-significantly increased the gl levels by 24.95 mg/dl [-31.15096 mg/dl - 81.05096 mg/dl] (P= 0.3736), also in accordance with paired t-test (p=0.4461). However, erythropoietin administration and reperfusion time together produced a non significant combined effect in increasing the gl levels by 22.06364 mg/dl [-11.34881 mg/dl - 55.47608 mg/dl] (P= 0.1892). Reviewing the above and table 3, the tables 4 and 5 sum

up concerning the alteration influence of Epo in connection with reperfusion time. Inserting the rats weight as independent variable at glm, a non significant relation turns on gl levels (p= 0.2104), so as to further investigation is not needed.

Table 1. The erythropoietin (Epo) influence (+SD) on the levels of some seric1 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[2]	+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
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	+1.57%±8.76%	0.6894	+3.22%±9.49%	0.3228	+4.87%±12.29%	0.2353	+1.99%±5.63%	0.3823

Table 2. Weight and mean glucose levels and Std. Dev. of groups

Groups	Variable	Mean	Std. Dev
A	Weight	243 g	45.77724 g
	Glucose	420.6 mg/dl	69.20373 mg/dl
B	Weight	262 g	31.10913 g
	Glucose	423.6 mg/dl	102.9997 mg/dl
C	Weight	242.8 g	29.33636 g
	Glucose	423.8 mg/dl	73.60676 mg/dl
D	Weight	243 g	32.84644 g
	Glucose	470.3 mg/dl	101.9979 mg/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
	Glucose	-3 mg/dl	0.9165
A-C	Weight	0.2 g	0.9900
	Glucose	-3.2 mg/dl	0.9401
A-D	Weight	0 g	1.0000
	Glucose	- 49.7 mg/dl	0.3125
B-C	Weight	19.2 g	0.2598
	Glucose	-0.2 mg/dl	0.9966
B-D	Weight	19 g	0.1011
	Glucose	- 46.7 mg/dl	0.3765
C-D	Weight	-0.2 g	0.9883
	Glucose	- 46.5 mg/dl	0.1897

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

Increase	95% c. in.	Reperfusion time	p values	
			t-test	glm
3.2 mg/dl	-63.92145 mg/dl - 70.32145 mg/dl	1h	0.9401	0.9213
24.75 mg/dl	-31.36051 mg/dl - 80.86051mg/dl	1.5h	0.2642	0.3775
46.7 mg/dl	- 49.60499 mg/dl - 143.005 mg/dl	2h	0.3765	0.3218
24.95 mg/dl	-31.15096 mg/dl - 81.05096 mg/dl	reperfusion time	0.4461	0.3736
22.06364 mg/dl	-11.34881 mg/dl - 55.47608 mg/dl	Interaction		0.1892

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

Increase	+SD	Reperfusion time	p-values
0.75%	+8.11%	1h	0.9307
5.59%	+6.46%	1.5h	0.3208
10.44%	+10.99%	2h	0.3491
5.63%	+6.45%	reperfusion time	0.4098
4.94%	+3.81%	Interaction	0.1892

DISCUSSION

The following clinical situations show how hyper-glycemia favors ischemia. Gąsecki et al (2012) significantly associated favorable early outcome with adjustment for blood Gl level on admission in stroke patients (P = 0.001). Nardi et al (2012) found that admission hyperglycemia (≥143mg/dL) is a strong and independent predictor for 72-hour fatality, especially in patients with no prior history of diabetes mellitus (overall: OR=4.0, p=0.003; non-diabetics: OR=4.9, p=0.004). Admission hyperglycemia increases the risk of death in first-ever acute ischemic stroke patients (Nardi et al 2012). Yang et al (2010) found that elevated mean blood Gl (equal or greater than 7.1 - 8.5 mmol/L) levels is an independent predictor and superior to admission Gl (AG) (P < 0.001) on predicting 7-and 30-day mortality and combined end point events on predicting short-term prognosis in acute myocardial infarction patients.

Also, the following situations reflect the effect Epo has on Gl levels. Lagarto et al (2012) observed a slight increase in Gl level within the normal range after 28 days of intra-nasal dosing of 6900 UI/kg/day Epo in healthy Wistar rats. Lee et al (2006) found that Gl and insulin concentrations were significantly lowered by the 25-day mountaineering activity where Epo increases, in male subjects group. Spaia et al (2000) proved the beneficial effect of Epo treatment on insulin resistance in non-obese, non-diabetic, stable dialysis patients, could be attributed to the Epo itself. Berridge & Tan (1995) showed that growth factors can still promote short term cell survival responses, increasing affinity for Gl and thus the intracellular Gl concent-ration. Kokot et al (1994) noticed an increase of fasting insulinemia and a decrease of basal plasma level of glucagon and PP after 6 months of rHuEpo treatment. At that time point rHuEpo therapy also increased the response of insulin, glucagon, and gastrin to the test meal (Kokot et al 1994). Salvesen et al (1993) found the mean umbilical venous blood Epo significantly higher in diabetic pregnancies than the appropriate normal mean for gestation. There were significant associations between fetal hemoglobin and maternal glycosylated hemo-globin. Obviously, maternal hyperglycemia causes fetal hyperglycemia. The increase in fetal hemoglobin may be mediated by either Epo or hyperinsulinemia (Salvesen et al 1993). Mulay & Congote (1985) found that Epo had a specific stimulatory effect on embryonic-type globins in fetal liver cells in rat fetuses of diabetic mothers, which was significantly higher than control ones until 14 days of gestation. After this endpoint, these differences are abolished, but until then, a complicity between hyper-glycemia and Epo is denoted (Mulay & Congote 1985). Katz et al (2010) demons-trated Epo-mediated decrease in blood Gl levels, attenuation of body weight gain and reduction of hemoglobin A1c in all mice models tested. Nakao et al (1998) correlated inversely the changes in HbA1c with both the changes in Hct and the reticulocyte counts by Epo treatment, though there were no significant changes in blood Gl levels during the 2-weeks study period in 15 non-diabetic hemodialysis patients.

CONCLUSION

Epo administration, reperfusion time, or their interaction non-significantly increase the blood glucose levels in short-term. The opposite bibliographic data are con-sidered more reliable. A greater sample would provide

more clearer results. Seven from nine studies show clearly that Epo has a definite declining effect on Gl levels.

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.

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