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Research Article

The effect of erythropoietin on γ-glutamyltransferase during ischemia reperfusion injury in rats

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Abstract

The aim of this experimental study was to examine the effect of erythropoietin on rat model and particularly in an ischemia reperfusion (IR) protocol. The effect of that molecule was studied biochemically using blood mean γ-glutamyl transferase (γGT) levels.

40 rats of mean weight 247.7 g were used in the study. γGT 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 decreased the γGT levels by 12.70%±13.11% results of paired t-test (p= 0.3541). Reperfusion time kept non-significantly increased the γGT levels by 6.35%±13.24% (P=0.6264). However, erythropoietin administration and reperfusion time together produced a non-significant combined effect in keeping decreased the γGT levels by 4.62%±7.97% (P= 0.5534).

Conclusions are that erythropoietin administration whether it interactedor not with reperfusion time have non significant short – term decreasing effects on γGT levels.

1. Introduction

Tissue ischemia reperfusion (IR) remains 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 IR 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). 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 IR protocol. The effect of that molecule was studied by measuring the blood mean γ-glutamyltransferase (γGT) levels.

2. Materials and methods

2.1 Experimental animals

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 [1]. 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 γGT 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 231.875 g [Std. Dev: 36.59703 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 γGT levels. This suspicion was investigated.

2.2 Model of ischemia-reperfusion injury

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 γGT levels 1.7 IU/L [Std. Dev: 0.6749486 IU/L] (Table 2).

Group B: Reperfusion lasted for 120 min (n=10 controls rats) mean mass 262 g [Std. Dev: 31.10913 g], mean γGT levels 1.7 IU/L [Std. Dev: 0.6749486 IU/L] (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 γGT levels 1.4 IU/L [Std. Dev: 0.5163978 IU/L] (Table 2).

Group D: Reperfusion lasted for 120 min (n=10 Epo rats) mean mass 243 g [Std. Dev: 32.84644 g], mean γGT levels 1.6 IU/L [Std. Dev: 0.6992059 IU/L] (Table 2).

3. Results

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 γGT levels, was investigated whether owed in the above mentioned significant weight correlations. γGT 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). Applying generalized linear models (GLM) with dependant variable the γGT levels and independent variables the Epo administration or no, the reperfusion time and their interaction, resulted in: Epo administration non-significantly decreased the γGT levels by 0.2 IU/L [-0.6048788 IU - 0.2048788 IU/L] (P= 0.3236). This finding was in accordance with the results of paired t-test (p=0.3847). Reperfusion time kept non-significantly increased the γGT levels by 0.1 IU/L [-0.3088548 IU - 0.5088548 IU/L] (P= 0.6234), in accordance also with paired t-test (P=0.6295). However, erythropoietin administration and reperfusion time together produced a non-significant combined effect in keeping decreased the γGT levels by 0.0727273 IU/L [-0.3189142 IU - 0.1734597 IU/L] (P= 0.5534). 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 γGT levels (p= 0.7483), so as to further investigation is not needed.

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

Table 2: Weight and γGT levels and Std. Dev. of groups

Groups	Variable	Mean	Std. Dev
A	Weight	243 gr	45.77724 gr
	γGT	1.7 IU/L	0.6749486 IU/L
B	Weight	262 gr	31.10913 gr
	γGT	1.7 IU/L	0.6749486 IU/L
C	Weight	242.8 gr	29.33636 gr
	γGT	1.4 IU/L	0.5163978 IU/L
D	Weight	243 gr	32.84644 gr
	γGT	1.6 IU/L	0.6992059 IU/L

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 gr	0.2423
	γGT	0 IU/L	1.0000
A-C	Weight	0,2 gr	0.9900
	γGT	0.3 IU/L	0.1934
A-D	Weight	0 gr	1.0000
	γGT	0.1 IU/L	0.6783
B-C	Weight	19,2 gr	0.2598
	γGT	0.3 IU/L	0.2789
B-D	Weight	19 gr	0.1011
	γGT	0.1 IU/L	0.8114
C-D	Weight	-0,2 gr	0.9883
	γGT	-0.2 IU/L	0.4433

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

Decrease	95% c. in.	Reperfusion time	p-values	
			t-test	glm
0.3 IU/L	-0.8646058 IU - 0.2646058 IU/L	1h	0.1934	0.2790
0.2 IU/L	-0.6048788 IU - 0.2048788 IU/L	1.5h	0.3847	0.3236
0.1 IU/L	-0.7456515 IU - 0.5456515 IU/L	2h	0.8114	0.7486
-0.1 IU/L	-0.3088548 IU - 0.5088548 IU/L	reperfusion time	0.6295	0.6234
0.0727273 IU/L	-0.3189142 IU - 0.1734597 IU/L	interaction		0.5534

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

Decrease	±SD	Reperfusion time	p-values
-19.35%	±18.58%	1h	0.2362
-12.70%	±13.11%	1.5h	0.3541
-6.06%	±19.96%	2h	0.7800
+6.35%	±13.24%	reperfusion time	0.6264
-4.62%	±7.97%	interaction	0.5534

4. Discussion

γGT is being considered a reliable index, not only for liver function but also for general metabolism. Its production is being influenced by ischemia and particulary by certain mode, as the next references show. Seifert *et al*[2] did not notice significant γGT levels changes over the 14-day assessment period occurring in subjects which ingested 20 g/day of ribose. Cutrín *et al*[3] obtained γGT samples from both ischemic and not kidneys after a standard brief period of unilateral kidney IR in rats. γGT activity was found to be increased in both cortical and medullar zones of the ischemic kidneys obtaining a net pro-oxidant effect of up-regulated γGT during short-term ischemia. Bounous *et al*[4] estimated the total activities of γGT in the isolated brush border of the entire small bowel reduced by 21% than control values ($p < 0.001$) after IR of the superior mesenteric artery. Enzymatic activity expressed as U/mg protein, is significantly reduced in the case of γGT level by 48% than control values.

Also, γGT level is a factor influenced by Epo. Is suitable Epo dose administration able to restore the rised γGT levels? Endre *et al*[5] found no difference in the incidence of Epo in the primary outcome between placebo and Epo treated groups, after randomization triggered by the enzyme γGT concentration product to levels above 46.3 in ICU patients. Although the marker elevations were transient, selected patients were determined by more severe illness and at greater risk of acute kidney injury, dialysis, or death. Lau *et al*[6] treated Sprague-Dawley rats by PHE/HQ (1.1 mmol/kg/0.9 mmol/kg, ip) and benzene. 2,6-GS-HQ and 2,3,5-GS-HQ were also found in the bone marrow of them. Hydroquinone (HQ) readily oxidizes to 1,4-benzoquinone (1,4-BQ) followed by the reductive addition of glutathione (GSH). Hydroquinone (HQ) may activate oxygen via redox cycles in biological systems and may also deplete glutathione (GSH). Both these reactions are potentially harmful, and they may be involved in hydroquinone-induced development of γGT -positive enzyme-altered foci in rat liver. Moreover, 2,6-GS-HQ and 2,3,5-GS-HQ were hematotoxic when administered to rats. All of the HQ-GSH conjugates retain the ability to redox cycle and generate reactive oxygen species (ROS), and to arylate target proteins. The generation of ROS and alkylation of proteins may both contribute to benzene-mediated myelotoxicity, and the two processes may be inter-dependent. Within 18h of administration of PHE/HQ to rats a significant decrease in blood lymphocyte count was observed, but erythrocyte counts and hemoglobin concentrations remained within the normal range. Stenius *et al*[7] confirmed also that hydroquinone (HQ) may activate oxygen via redox cycles in biological systems, may also deplete glutathione (GSH) and both these reactions are potentially harmful. They studied their possible involvement in hydroquinone-induced development of γGT-positive enzyme-altered foci in rat liver. Gold *et al*[8] faced a young horse with erythrocytosis and increased serum γGT levels. Biopsy revealed hepatoblastoma and also bone marrow erythroid hyperplasia. Serum Epo concentration was 2.37-fold above upper reference value supporting production by the tumor and secondary inappropriate erythrocytosis. This report documents secondary erythrocytosis in a horse with hepatoblastoma. Chakraborty *et al*[9] saw fourfold activity of γGT, a neoplastic marker, in the host cells bearing in Dalton's lymphoma host mice. They confirmed the lymphoma progression via modulating erythropoiesis. De Paoli Vitali *et al*[10] determined serum Epo activity non significantly reduced in skiers than normal population ($P < 0.01$). In athletes Epo, urinary γGT levels, were determined before and after a ski race. A significant increase of these variables was found after the competition ($P < 0.001$).

It is concluded that they were not accompanied by a renal hypoxia sufficient to stimulate Epo overproduction during the strenuous race. Barroso *et al*[11] did not predict greater HIV participants-related fatigue by physiological changes measurements including γGT, HIV viral load and serum Epo levels, over a 1-year period.

Nevertheless, Takigawa *et al*[12] investigated the main factors correlated with the serum γGT activity in healthy Japanese people. The mean serum γGT activity was 29 IU/L. γGT activities of males and persons older than 45 years were significantly higher than each counterpart. γGT levels were increased significantly with the number of cigarettes smoked per day and the frequency of alcohol consumption except for the persons who did not take alcohol. Additionally, γGT was significantly correlated with urinary 8-hydroxydeoxyguanine, and with more blood factors including serum tocopherols, carotenoids, antioxidative enzymes, lipid peroxide, and free fatty acids than urinary 8-hydroxydeoxyguanine did. γGT had significant associations with retinol, 8-hydroxydeoxyguanine, docosahexaenoic acid, and cigarette smoking. They suppose the hypothesis that γGT can be used as a marker related with oxidative stress. Onur *et al*[13] found a strong positive association between coenzyme Q10 (CoQ10) status and serum γGT activity in healthy participants. A gender-specific examination revealed differences between male and female volunteers regarding the association between CoQ10 status and serum γGT activity. In the second step, ubiquinol (Q10H2) supplementation (150 mg/d) caused a significant decrease in serum γGT activity ($p < 0.001$). γGT1 mRNA levels declined 1.49-fold after Q10H2 supplementation opposed to other liver enzymes (i.e., aspartate aminotransferase, AST) which were not affected by Q10H2 supplementation. This effect might be caused by gene expression. Serum γGT activity is associated with cardiovascular diseases. In a physiological range, activity of γGT is a potential early and sensitive marker of inflammation and oxidative stress. Overall, they provided preliminary evidence that higher Q10H2 levels improve oxidative stress via reduction of serum γGT activity in humans.

The two last references show that the molecule of γGT may serve as an oxidative marker. This, not only cancels its study implication out as associated with an antioxidant drug, but also warrants it too. Successful drug is the one which involutes and improves the enzyme values until it coincides with the sham values or does not differ significantly from those. This is achieved in this experiment and there is the paradox: while the result is declared as non-significant, however, it is significant both statistically and clinically because a disturbed enzyme level was restored from significant to non-significant level.

Conclusions are that erythropoietin administration whether it interacted or not with reperfusion time has non significant short – term decreasing effects on γGT levels. It seems that either Epo itself exerts an over-restorating short-term influence on γGT levels, since these are found lower than sham operated ones, or γGT is enough valnerable in antioxidant capacity.

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