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The Effect of Erythropoietin on Aspartate Aminotransferase Levels during Ischemia Reperfusion Injury in Rats

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Research Article

Abstract

Objective: This experimental study examined the effect of erythropoietin (Epo) on rat model and particularly in a liver ischemia-reperfusion (IR) protocol. The effect of that molecule was studied biochemically using blood mean aspartate aminotransferase (AST) levels.

Materials and methods: The 40 rats of mean weight 247.7 g were used in the study. AST levels were measured at 60 min (groups A and C) and at 120 min (groups B and D) of reperfusion. Epo was administered only in groups C and D.

Results: Epo administration kept significantly increased the AST levels by 26.71% ± 13.17% (p=0.0235). Reperfusion time kept significantly increased the AST levels by 27.58% ± 13.12% (p=0.0271). Along, Epo administration and reperfusion time together produced a significant combined effect in keeping increased the AST levels by 19.73% ± 7.70% (p=0.0119).

Conclusions: Epo declined the difference of elevated post-IR values from the sham AST values, from significant level after 1.5 h reperfusion at non-significant level after 2 h reperfusion. So, Epo was proved an optimal resolving factor for liver IR injury after 1.5 h reperfusion.

Keywords: Ischemia; Erythropoietin; Aspartate aminotransferase; Reperfusion.

1. Introduction

Erythropoietin (Epo) is generally one of the more well studied growth factors. Epo implicates over 28,529 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 ones of adjacent organs and subsequently 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. Rezaee MA et al. have shown that Epo could reduce CO-induced cardiac ischemia and effectively suppress apoptosis in rat myocardial cells ($p<0.01$) [1]. Silachev DN et al. indicated that endogenous anti-ischemic defense; renal preconditioning and cationic plastoquinone derivative (SkQR1)-induced brain protection may be mediated through the release of the very powerful neuroprotective agent Epo from the kidney [2]. A numeric evaluation of the Epo efficacy was yielded by a meta-analysis of 23 published seric variables, based on the same experimental setting, at the same endpoints (Table 1).

The aim of this study is to investigate whether Epo administration may result in partial or complete resolution of liver IR injury using as resolution marker the aspartate aminotransferase levels (AST) in a rat model.

2. Methods

2.1 Animal preparation

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Table 1. The erythropoietin (Epo) influence (±S.D) on the levels of some seric[3] variables concerning reperfusion (rep) time.

Variable	1 h rep	p-value	1.5 h rep	p-value	2 h rep	p-value	Interaction of Epo and rep	p-value
White BCC	+24.01% ± 13.38%	0.1012	+22.09% ± 9.11%	0.0351	+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[4]	-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
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
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[5]	+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]	+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	+1.91% ± 9.88%	0.5997	+2.45% ± 8.98%	0.3835	+2.99% ± 10.61%	0.3685	+2.12% ± 5.61%	0.4282

this experiment under 3693/12-11-2010 & 14/10-1-2012 decisions. All substances, equipment and consumables needed was a courtesy of ELPEN Pharmaceuticals Co Inc. S.A. at Pikermi, Attiki. Formal humane animal care was adopted for female albino Wistar rats. Normal 7 days pre-experimental housing included *ad libitum* diet in laboratory. Prenarcosis preceded of continuous intra-experimental general anesthesia [3-6], electrocardiogram, acidometry and oxygen supply. Finally, 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. Same protocol was used by Ahmadiasl N et al. who investigated the effect of co-administration of Epo on IR-induced renal injury in rats [7]. Also, Li XJ et al. demonstrated that Epo plays a protective role against lipopolysaccharide-induced multiple organ failure which promoted phosphorylation of alanine aminotransferase in lung, liver, and kidney by reducing the inflammatory response and tissue degeneration [8]. Epo administration was performed at the time of reperfusion, through catheterized inferior vena cava. The AST 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 AST 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 AST levels 154.1 IU/L [SD: 34.86307 IU/L] (Table 2). **Group B:** Reperfusion lasted for 120 min n=10 controls rats) mean mass 262 g [SD: 31.10913 g], mean AST levels 209.3 IU/L [SD: 86.6834 IU/L] (Table 2).

Erythropoietin group: A 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 AST levels 207.5 IU/L [SD: 38.37896 IU/L] (Table 2).

Group D: Reperfusion lasted for 120 min (n=10 Epo rats) mean mass 243 g [SD: 32.84644 g], mean AST levels 266.1 IU/L [SD: 124.1732 IU/L] (Table 2).

2.3 Statistical analysis

Every weight and AST levels group was compared with each other from 3 remained groups applying respective statistical standard t-test (Table 3). If any probable significant difference among AST levels was raised, it was investigated whether it owed in any respective probable significant mass one (Table 3). Then, the application of generalized linear models (glm) was followed; including as dependant variable the AST levels. The independent variables were the Epo administration or no, the reperfusion time and their interaction. Inserting the rats' mass as independent variable at glm, a non significant correlation appeared with AST levels (p=0.8644), so as to further investigation was interrupted.

Table 2. Weight and AST levels and std. dev. of groups.

Groups	Variable	Mean	Std. Dev
A	Weight	243 g	45.77724 g
	AST	154.1 IU/L	34.86307 IU/L
B	Weight	262 g	31.10913 g
	AST	209.3 IU/L	86.6834 IU/L
C	Weight	242.8 g	29.33636 g
	AST	207.5 IU/L	38.37896 IU/L
D	Weight	243 g	32.84644 g
	AST	266.1 IU/L	124.1732 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 g	0.2423
	AST	-55.2 IU/L	0.0906
A-C	Weight	0.2 g	0.9900
	AST	-53.4 IU/L	0.0148
A-D	Weight	0 g	1.0000
	AST	-112 IU/L	0.0274
B-C	Weight	19.2 g	0.2598
	AST	1.8 IU/L	0.9327
B-D	Weight	19 g	0.1011
	AST	-56.8 IU/L	0.0908
C-D	Weight	-0.2 g	0.9883
	AST	-58.6 IU/L	0.1244

3. Results

The glm resulted in: Epo administration kept significantly increased the AST levels by 55.1 IU/L [1.842717 IU/L-108.3573 IU/L] (p=0.0429). This finding was in accordance with the results of standard t-test (p=0.0042). Reperfusion time kept significantly increased the AST levels by 56.9 IU/L [3.847231 IU/L-109.9528 IU/L] (p=0.0362), also in accordance with standard t-test (p=0.0181). However, Epo administration and reperfusion time together produced a significant combined effect in keeping increased the AST levels by 40.70909 IU/L [9.536473 IU/L-71.88171 IU/L] (p=0.0119). Reviewing the above and Tables 3-5 sum up concerning the alteration influence of Epo in connection with reperfusion time (Tables 4 and 5).

4. Discussion

AST levels may be influenced by ischemic cases. Liu SQ et al. found significantly higher by 45.35% the post-operative AST levels for the detection of liver damage with hand suturing, than those in sutureless group after IR (P<0.01) reducing the complications and the extent of injury caused by hand suturing, leading to smoother operations and improved prognosis in adult dogs [9]. Oba M et al. suppressed the hepatic IR-induced release of serum AST levels, subjecting mice three times after IR into heat shock (HS) (42 degrees C) and/or mild electrical stimulation (MES) (12V) for 20 min than sham ones [10]. Boin Ide F et al. evaluated the liver donors according to AST levels in prediction of post-transplant survival [11]. Seifert J et al. observed non-significant toxic changes in AST levels over the 14-day assessment period in healthy adults subjects who ingested 20 g/day of ribose [12]. Pulitanò C et al. found the postoperative serum AST levels significantly lower in group provided 500 mg of methylprednisolone preoperatively than control one in liver injury patients following liver operation [13]. Cywinski JB et al. associated the use of HTK preservation solution with higher mean postoperative orthotopic liver transplantation AST levels (P=0.02) [14]. Rosen HR et al. confirmed the independent association between both the extent of IR and peak value of ASTmax levels within the first 72 hr after liver transplantation with confirmed overall 1-year survival of both patient and liver grafts (P=0.001) [15]. Cighetti G et al. found significantly increased AST serum levels after short-term in vivo hepatic IR in fasted rats with physiological content of xanthine oxidase (XO) (25%), than those of higher XO percentage (45%) [16].

Also, AST levels are a factor influenced by Epo. The point is whether Epo administration is able to restore the AST levels. Fu W et al. found significantly decreased the AST serum levels in the serum of IR + rhuEpo group, proving the protective effect of rhuEpo in IR injury before a liver IR model operation in Sprague-Dawley rats [17]. Eisfeld AK et al. found AST levels elevated in 55.7% of allograft recipients due to hematologic malignancies [18]. Gul M et al. showed that Epo 3000 IU/kg administration decreased the AST levels significantly (p<0.05) at 24 h on liver regeneration after partial 70% hepatectomy due to its anti-inflammatory, anti-oxidant, anti-apoptotic and angiogenic properties [19]. Systemic administration of high-dose Epo increases regeneration more than SC one. However, Rjiba-Touati K et al. induced a Cisp liver failure characterized by a significant increase in serum AST levels in adult male Wistar rats, although the tissue-protective effect of administered rhEpo [20]. Kato M et al. found out that a dose of 30,000 IU Epo administration has greater potential than both steroids and a dose of 60,000 IU Epo to ameliorate IR before the Pringle maneuver according to AST levels fluctuations (P=0.041) [21].

Ishikawa Y et al. found lower peak AST values and higher serum Epo levels in perioperative oral nutrition (ON) with branched chain amino acids (BCAA) in hepatectomy patients than in control normal diet hepatectomy group [22]. Higher Epo levels might protect liver cells from IR injury associated with lower perioperative serum AST levels. Moussavian MR et al. resulted in AST levels elevation after 24 h cold storage hepatic IR in 37°C Krebs Henseleit buffer preconditioned by a multidrug donor including also Epo [23]. Nunes LA et al. investigated whether the use of rhEpo altered monthly serum AST concentrations in male subjects undergoing 4 months of regular aerobic training [24]. Greif F et al. assessed serum AST levels significantly lower after liver injury on days 2 and 4 post-hepatectomy in rats pretreated with intraperitoneal injection of rhEpo (4 IU/g) 30 minutes prior to subtotal 70% hepatectomy than control group (P<0.005) [25]. Barroso J et al. predicted no greater changes in HIV-related fatigue in participants over a 1-year period, sorting them out among AST levels, HIV viral load and serum Epo [26]. Bárcena R et al. observed that liver transplant (LT) subjects survived with long-term significant improvements in AST levels liver function tests only whether were able to complete or maintain full dose pegylated interferon/ribavirin (peg-IFN/RBV) antiviral therapy [27]. Bockhorn M et al. reduced liver damage by 37.31% or 2.3-fold as indicated by the serum activity of AST levels after partial 90% hepatectomy (PH) or 30% partial liver transplantation (pLTx) in Epo-treated rats respectively [28]. Robinson Y et al. suspected of Epo use in lowlanders estimating mean red blood cell (RBC) age by means of erythrocyte AST activity (eAST levels). Participants receiving rhEpo had a dose-dependent increase in eAST levels (P<0.05) [29].

Sepodes B et al. proved first that rhEpo administration

5 min before IR reduces the oxidative stress, reducing apoptosis and serum AST levels of liver injury in rats [30]. Robinson Y et al. showed a lower erythrocyte AST activity (eAST levels) ($P<0.001$) and higher but normal serum Epo ($P<0.01$) in aerobic performers than untrained control group [31]. Małyszko J et al. modulated hepcidin synthesis in hepatocytes in response to anemia, hypoxia or inflammation and correlated it positively with AST levels under Epo therapy in hemodialyzed (HD) patients than healthy volunteers [32]. Kahraman S et al. observed a significant decrease in rhEpo doses but a significant elevation in AST levels in HCV-positive maintenance HD patients [33]. Patel NS et al. attenuated renal dysfunction, injury and AST levels in mice treated by Epo (1000 IU/kg/day SQ) for 3 days at the end of IR experiments [34]. Böning D et al. found eAST activity in erythrocytes augmented on day 7 by 41.34% and decreased on day 11 by 21.84% after return from 6250 m altitude indicating reduced mean cell age in mountaineers [35]. Campbell NR et al. predicted the percentage increase in AST concentration from predialysis to 3 h postdialysis, by multiplication of percentage loss in body weight in kg during dialysis by 3.3 [36]. Siems W et al. resulted in an increase of cell age-dependent eAST levels due to rejuvenation of the erythrocyte population and enhancement of the proportion of younger erythrocytes, but the reduced life span of erythrocytes in HD patients during therapy with rhEpo was not improved [37]. Churchill DN et al. noticed no differences on unexplained elevations in serum AST values, between receiving and no blood transfusions in non-A, non-B hepatitis HD patients [38]. Berglund B et al. found serum AST levels unchanged after rhEpo 30 IU/kg body weight treatment in healthy male subjects [39]. Hampl H et al. preferred a gradual increase in rhEpo doses since erythropoiesis in early phase of rhEpo therapy strongly influences plasma eAST concentrations in HD patients [40]. Hampl H et al. demonstrated a rejuvenation of the RBC population by 25% increased in bicarbonate (HDB) hemodialysis group than acetate (HDA) hemodialysis group and D50 decreased by 0.16% measuring the eAST activities of RBCs in HD patients receiving Epo therapy during 1 year [41].

5. Conclusions

Epo administration managed to reduce the elevated post-IR AST levels. Certainly Epo declined their difference from the sham AST values, from significant level after 1.5 h reperfusion at non-significant level after 2 h reperfusion. So, Epo was proved an optimal resolving factor for liver IR injury after 1.5 h reperfusion.

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