10.5005/jp-journals-10028-1185

RESEARCH ARTICLE

The Effect of Erythropoietin on Amylase Levels during Ischemia-Reperfusion Injury in Rats

[1]Constantinos Tsompos, [2]Constantinos Panoulis, [3]Konstantinos Toutouzas, [4]George Zografos, [5]Apostolos Papalois

ABSTRACT

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

Materials and methods: Forty rats of mean weight 247.7 gm were used in the study. Amylase levels were measured at 60 minutes (groups A and C) and at 120 minutes (groups B and D) of reperfusion. Erythropoietin was administered only in groups C and D.

Results: Erythropoietin administration kept non-significantly increased the A levels by 5.04 ± 6.12% (p = 0.3831). Reperfusion time kept non-significantly increased the A levels by 10.08 ± 5.95% (p = 0.0615). However, EPO administration and reperfusion time together produced a non-significant combined effect in keeping increased the A levels by 4.36 ± 3.65% (p = 0.2258).

Conclusion: Erythropoietin administration, reperfusion time and their interaction kept non-significantly short-term increased the amylase levels. The restorating effect of EPO is satisfactory, since it reduced the discrepancy from baseline values at non-significant level.

Keywords: Amylase, Erythropoietin, Ischemia, Reperfusion.

How to cite this article: Tsompos C, Panoulis C, Toutouzas K, Zografos G, Papalois A. The Effect of Erythropoietin on Amylase Levels during Ischemia-Reperfusion Injury in Rats. J Postgrad Med Edu Res 2016;50(1):18-21.

Source of support: Nil

Conflict of interest: None

INTRODUCTION

Tissue ischemia and 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). 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 pancreas IR protocol. The effect of that molecule was studied by measuring the blood mean amylase (A) levels.

MATERIALS AND METHODS

Animal Preparation

This experimental study was licensed by Veterinary Address of East Attiki Prefecture under 3693/12-11-2010 and 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. SA at Pikerni, 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 minutes followed by reperfusion for 60 minutes (group A). Ischemia for 45 minutes followed by reperfusion for 120 minutes (group B). Ischemia for 45 minutes followed by immediate EPO intravenous (IV) administration and reperfusion for 60 minutes (group C). Ischemia for 45 minutes followed by immediate EPO IV administration and reperfusion for

[1]Consultant, [2,3]Assistant Professor, [4]Professor, [5]Director

[1]Department of Obstetrics and Gynecology, Mesologi County Hospital, Etoloakarnania, Greece

[2]Department of Obstetrics and Gynecology, Aretaieion Hospital Athens University, Attiki, Greece

[3,4]Department of Surgery, Ippokrateion Greece Hospital, Attiki Greece

[5]Experimental Research Center, Elpen Pharmaceuticals, Co. Inc., SA, Pikerni, Greece

Corresponding Author: Constantinos Tsompos, Consultant Department of Obstetrics and Gynecology, Mesologi County Hospital, Etoloakarnania, Greece, Phone: 00302631360237 e-mail: constantinostsompos@yahoo.com

120 minutes (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.[1,2] Oxygen supply, electrocardiogram and acidimetry 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 minutes after laparotomic access had been achieved. Reperfusion was induced by removing the clamp and re-establishment of inferior aorta patency. The molecules were administered at the time of reperfusion, through inferior vena cava after catheterization had been achieved. The amylase levels measurements were performed at 60 minutes of reperfusion (groups A and C) and at 120 minutes of reperfusion (groups B and D). The mean weight of the 40 female Wistar albino rats used was 231.875 gm (SD: 36.59703 gm), with minimum weight ≥165 gm and maximum weight ≤320 gm. Rats' weight could be potentially a confusing factor, e.g. fatter rats to have greater blood amylase levels. This suspicion was investigated.

MODEL OF ISCHEMIA-REPERFUSION INJURY

Control Group

Twenty control rats of mean weight 252.5 gm (SD: 39.31988 gm) suffered by ischemia for 45 minutes followed by reperfusion.

Group A: Reperfusion which lasted 60 minutes concerned 10 control rats of mean weight 243 gm (SD: 45.77724 gm) and mean A levels 1674 IU/l (SD: 282.0162 IU/l) (Table 2).

Group B: Reperfusion which lasted 120 minutes concerned 10 control rats of mean weight 262 gm (SD: 31.10913 gm) and mean A levels 1877 IU/l (SD: 354.5749 IU/l) (Table 2).

Erythropoietin Group

Twenty EPO rats of mean weight 242.9 gm (SD: 30.3105 gm) suffered by ischemia for 45 minutes followed by reperfusion in the beginning of which 10 mg EPO/kg body weight were IV administered.

Group C: Reperfusion which lasted 60 minutes concerned 10 EPO rats of mean weight 242.8 gm (SD: 29.33636 gm) and mean A levels 1786.5 IU/l (SD: 372.3598 IU/l) (Table 2).

Group D: Reperfusion which lasted 120 minutes concerned 10 EPO rats of mean weight 243 gm (SD: 32.84644 gm) and mean A levels 1945.8 IU/l (SD: 315.7716 IU/l) (Table 2).

RESULTS

Initially, every one from 4 rats weight groups was compared with each other from 3 remained groups applying statistical paired t-test (Table 3). Any emerging significant difference among A levels, was investigated whether owed in the above mentioned significant weight correlations. Also, every one from 4 rats A levels groups was compared with each other from 3 remained groups applying statistical paired t-test. (Table 3). Applying generalized linear models (GLM) with dependant variable the A levels

Table 1: Meta-analysis of the EPO influence (±SD) on the levels of some seric variables concerning reperfusion (rep) time coming[1] from the same experimental setting

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 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
Creatine phosphokinase	0.15 ± 14.09%	0.9904	7.91 ± 9.44%	0.3549	15.67 ± 12.65%	0.1509	4.28 ± 5.70%	0.4430
Lactic dehydrogenase	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

and independent variables the EPO administration or no, the reperfusion time and their interaction, resulted in: EPO administration kept non-significantly increased the A levels by 90.65 IU/l (– 125.3341 IU/l-306.6341 IU/l) (p = 0.4008). This finding was in accordance with the results of paired t-test (p = 0.3654). Reperfusion time kept non-significantly increased the A levels by 181.15 IU/l (– 28.60304 IU/l-390.903 IU/l) (p = 0.0885), in accordance also with paired t-test (p = 0.0346). However, erythropoietin administration and reperfusion time together produced a non significant combined effect in keeping increased the A levels by 78.40909 IU/l (– 50.51936 IU/l-207.3375 IU/l) (p = 0.2258). Reviewing the Tables 3 to 5 sum up concerning the decreasing influence of EPO along with reoxygenation time. Inserting the rats weight as independent variable at GLM, a non-significant relation turns on A levels (p = 0.5804), so as to further investigation is not needed.

Table 2: Weight and amylase mean levels and standard deviation of groups

Groups	*Variable*	*Mean*	*SD*
A	Weight	243 gm	45.77724 gm
	Amylase	1674 IU/l	282.0162 IU/l
B	Weight	262 gm	31.10913 gm
	Amylase	1877 IU/l	354.5749 IU/l
C	Weight	242.8 gm	29.33636 gm
	Amylase	1786.5 IU/l	372.3598 IU/l
D	Weight	243 gm	32.84644 gm
	Amylase	1945.8 IU/l	315.7716 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 gm	0.2423
	Amylase	– 203 IU/l	0.1832
A–C	Weight	0.2 gm	0.9900
	Amylase	– 112.5 IU/l	0.3761
A–D	Weight	0 gm	1.0000
	Amylase	– 271.8 IU/l	0.0054
B–C	Weight	19.2 gm	0.2598
	Amylase	90.5 IU/l	0.6528
B–D	Weight	19 gm	0.1011
	Amylase	– 68.8 IU/l	0.6776
C–D	Weight	– 0.2 gm	0.9883
	Amylase	– 159.3 IU/l	0.0858

Table 4: Increasing influence of EPO in connection with reperfusion time

Increase	*95% CI*	*Reperfusion time*	*p-values t-test*	*GLM*
112.5 IU/l	– 197.829 IU/l–422.829 IU/l	1 hour	0.3761	0.4561
90.65 IU/l	– 125.3341 IU/l–306.6341 IU/l	1.5 hours	0.3654	0.4008
68.8 IU/l	– 246.6428 IU/l–384.2428 IU/l	2 hours	0.6776	0.6523
181.15 IU/l	– 28.60304 IU/l–390.903 IU/l	Reperfusion time	0.0346	0.0885
78.40909 IU/l	– 50.51936 IU/l–207.3375 IU/l	Interaction	–	0.2258

Table 5: Percentage increasing influence of EPO in connection with reperfusion time

Increase	*±SD*	*Reperfusion time*	*p-values*
6.50%	±9.15%	1 hour	0.4161
5.04%	±6.12%	1.5 hours	0.3831
3.59%	±8.42%	2 hours	0.6649
10.08%	±5.95%	Reperfusion time	0.0615
4.36%	±3.65%	Interaction	0.2258

DISCUSSION

Examples are described herein concerning whether pancreatic ischemia can influence the amylase levels. Fisher et al[3] predicted greater increases in heart rate (HR), decreases in respiratory sinus arrhythmia (RSA) and decreased alteration of salivary alpha-amylase (SAA) levels by higher baseline ones in induced-stressed worriers than healthy controls. Schneeberger et al did not find any difference[4] of serum amylase values in 6 months observation period pancreas transplant graft function between mean cold ischemia time of 10 hours histidine-tryptophan-ketoglutarate and University of Wisconsin solutions. Woessner et al[5] noted a well-known increase in SAA levels through the infusion of 6% hydroxyethyl starch (HES) 130/0.4 than crystalloid solution as continuous infusion over 4 days in patients suffering from an acute ischemic stroke. Viola et al[6] demonstrated pancreatic IR damage by increased SAA levels than sham operated rats. Freiburghaus et al[7] observed no difference for SAA levels between ischemic pancreatic branches of the splenic artery than sham operated ones with saline until 9 weeks in rats. Chronic pancreatitis follows acute necrotizing pancreatitis. Fiolet et al[8] measured the extracellular volume decreased by about 20% by the non-cardiac enzyme alpha-amylase in suspensions of isolated rat ventricular myocytes after 30 minutes total IR. Költringer et al[9] found significantly increased the fasting levels of SAA in patients with II b stage peripheral arterial occlusive disease after 500 ml Elohaest administration during the first 4 treatment days. Spormann et al[10] analyzed alpha-amylase in acute pancreatitis of ischemic rats until 24 hours postoperatively. Letko et al[11] found SAA significantly increased 24 hours after acute ischemic pancreatitis than control cases. Sokolowski et al[12] found the alpha-amylase release dependent on the duration of ischemia within 24 hours postoperatively in acute pancreatitis rats. Sokolowski et al[13] caused a drastical

2.5-fold increase in initial phase alpha-amylase levels, remaining 24 hours postoperatively at distinct pathological level in ischemic acute pancreatitis rats. Fleischer et al[14] did not alter alpha-amylase levels after pancreatic 20 minutes intervals IR in mongrel dogs serum. Scheele et al remarked an extreme initial rise in the levels of alpha-amylase gradually normalized in postoperative course of a[15] complete transected hepatoduodenal ligament occurred in a 59-year-old female patient.

Also the following examples concern the influence of EPO fluctuation on the amylase levels. Li et al[16] showed significantly decreased serum amylase activity until 12 hours ($p < 0.05$) after severe acute pancreatitis (SAP) in EPO rats group than normal saline SAP group. Erythropoietin can effectively alleviate SAP in rats. Eliopoulos et al[17] showed also significantly decreased amylase blood concentrations in recipient mice with mesenchymal stromal cells (MSCs) gene-enhanced to secrete EPO et al[18] observed no significant change in amylase level after hrEpo administration in weekly increasing doses.

CONCLUSION

Erythropoietin administration, reperfusion time and their interaction kept non-significantly short-term increased the amylase levels. The restorating effect of EPO is satisfactory since it reduced the discrepancy from baseline values at non-significant level. Perhaps, a longer study time than 2 hours or a greater EPO dose might reveal full restoration.

ACKNOWLEDGMENT

This study was funded by scholarship by the Experimental Research Center ELPEN Pharmaceuticals (ERCE), Athens, Greece. The research facilities for this project were provided by the aforementioned institution.

REFERENCES

1. Tsompos C, Panoulis C, Toutouzas K, Zografos G, Papalois A. The effect of erythropoietin on total protein levels during ischemia reperfusion injury in rats. Int J Neurorehabil 2015; 2(1):146.
2. Tsompos C, Panoulis C, Toutouzas K, Zografos G, Papalois A. The acute effect of erythropoietin on hematocrit levels during hypoxia re-oxygenation injury in rats. Pren Méd Argent Marzo 2015;101(1):21-32.
3. Fisher AJ, Newman MG. Heart rate and autonomic response to stress after experimental induction of worry versus relaxation in healthy, high-worry, and generalized anxiety disorder individuals. Biol Psychol 2013 Apr;93(1):65-74.
4. Schneeberger S, Biebl M, Steurer W, et al. A prospective randomized multicenter trial comparing histidine-tryptophane-ketoglutarate versus University of Wisconsin perfusion solution in clinical pancreas transplantation. Transpl Int 2009 Feb;22(2):217-224.
5. Woessner R, Grauer MT, Dieterich HJ, et al. Influence of a long-term, high-dose volume therapy with 6% hydroxyethyl starch 130/0.4 or crystalloid solution on hemodynamics, rheology and hemostasis in patients with acute ischemic stroke. Results of a randomized, placebo-controlled, double-blind study. Pathophysiol Haemost Thromb 2003 May-Jun; 33(3):121-126.
6. Viola G, Al-Mufti RA, Sohail M, et al. Nitric oxide induction in a rat model of selective pancreatic ischemia and reperfusion. Hepatogastroenterology 2000 Sep-Oct;47(35):1250-1255.
7. Freiburghaus AU, Redha F, Ammann RW. Does acute pancreatitis progress to chronic pancreatitis? A microvascular pancreatitis model in the rat. Pancreas 1995 Nov;11(4):374-381.
8. Fiolet JW, Schumacher CA, Baartscheer A, et al. Osmotic changes and transsarcolemmal ion transport during total ischaemia of isolated rat ventricular myocytes. Basic Res Cardiol 1993 Sep-Oct;88(5):396-410.
9. Költringer P, Langsteger W, Lind P, et al. Kinetic effects of 6% hydroxyethyl starch 200.000/0.6-0.66 in hypervolemic hemodilution. Acta Med Austriaca 1991;18(Suppl 1):20-22.
10. Spormann H, Sokolowski A, Letko G. Experimental acute pancreatitis: a quantification of dynamics at enzymic and histomorphologic levels. Pathol Res Pract 1989 Sep;185(3):358-362.
11. Letko G, Spormann H, Sokolowski A, et al. Influence of antisera to pancreatic acinar cells on rat pancreas in situ. Dtsch Z Verdau Stoffwechselkr 1987;47(1):26-32.
12. Sokolowski A, Spormann H, Letko G. Influence of pancreatic edema and short-term ischemia of rat pancreas on lipase-and alpha-amylase-activities in the serum and in the pancreas. Dtsch Z Verdau Stoffwechselkr 1987;47(3):119-127.
13. Sokolowski A, Spormann H, Urbahn H, et al. Contribution of pancreatic edema and short-term ischemia to experimental acute pancreatitis in the rat II: behaviour of serum parameters. Z Exp Chir Transplant Kunstliche Organe 1986;19(6):331-339.
14. Fleischer GM, Herden P, Spormann H. Animal experiment studies on the role of ischemia in the pathogenesis of acute pancreatitis. Z Exp Chir Transplant Kunstliche Organe 1984; 17(3):179-187.
15. Scheele J, Schepke P, Gall FP. Successful reconstruction of the completely transected hepatoduodenal ligament: case report on ischemia tolerance of the human liver. Chirurg 1982 Oct; 53(10):622-627.
16. Li J, Luo Y, Li Z, et al. Effects of erythropoietin pretreatment on pro-and anti-inflammatory balance in rats with severe acute pancreatitis. Nan Fang Yi Ke Da Xue Xue Bao 2012 Jan; 32(1):93-96.
17. Eliopoulos N, Zhao J, Forner K, et al. Erythropoietin gene-enhanced marrow mesenchymal stromal cells decrease cisplatin-induced kidney injury and improve survival of allogeneic mice. Mol Ther 2011 Nov;19(11):2072-2083.
18. Kakuk G, Kárpáti I, Mátyus J. Recombinant human erythropoietin in the therapy of anemia in hemodialyzed patients. Orv Hetil 1990 Oct 28;131(43):2365-2366, 2369-2371.