The Effect of Erythropoietin On Lactate Dehydrogenase Levels During Ischemia-Reperfusion Injury In Rats

Ratlarda İskemi-Reperfüzyon Sırasında Eritropoietinin Laktat Dehidrogenaz Seviyleri Üzerine Etkisi

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ABSTRACT

Objective: Aim of this experimental study was to examine the effect of erythropoietin (Epo) on a rat model and particularly in tissues by an ischemia-reperfusion (IR) protocol. The beneficial effect or non-effectiveness of that molecule were studied biochemically using mean blood lactate dehydrogenase (LDH) as a parameter of tissues' injury.
Methods: Forty rats with a mean weight of 247.7 g were used in the study. LDH levels were measured 60 min (groups A and C) and 120 min (groups B and D) after reperfusion. Placebo drug was administered in sham operated group A and B, while Epo was administered in groups C and D. The predicted LDH values, adjusted for rats' weight were calculated since there was a significant relation between rats' weight and LDH levels (p= 0.0469).
Results: Epo administration significantly increased the LDH levels by 778.3 IU/L [200.6627 IU/L - 1355.937 IU/L] (p= 0.0096). This finding was in accordance with the results of a paired t-test (p= 0.0004). Reperfusion time non-significantly decreased the LDH levels by 124.9 IU/L [-755.2258 IU/L - 505.4258 IU/L] (p= 0.6906), also in accordance with a paired t-test (p= 0.6354). Epo administration and reperfusion time together produced a significant combined effect of increasing LDH levels by 376.8 IU/L [16.55497 IU/L - 737.045 IU/L] (p= 0.0408).
Conclusion: Results of this study indicate that Epo administration alone or in interaction with reperfusion time, has increasing short – term effects on LDH levels. These increases are significant for the original LDH values and non-significant for predicted values. Considering the predicted values as more reliable, it is concluded that Epo administration declines the postischemically raised LDH levels effectively, suggesting a greater-than-expected restorating role of Epo in tissues.

Key Words: Ischemia, erythropoietin, lactate dehydrogenase levels, reperfusion, restoration, tissue damage

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ÖZET

Giriş: Bu deneysel çalışmanın amacı, bir rat modelinde ve özellikle dokularda iskemi-reperfüzyon (IR) protokolü ile eritropoietinin (Epo) etkisnin araştırılmasıdır.Molekülün yararlı etkisi ya da etkisizliğini belirlemek ve dokuların hasar parametresi olarak ortalama kan laktat dehidrogenazı (LDH) çalışılmıştır.
Yöntemler: Bu çalışmada ortalama ağırlıkları 247.7 g olan 40 rat çalışıldı. LDH düzeyleri reperfüzyon sonrası 60 dk (A ve C grupları) ve 120 dk (B ve D grupları) sonra çalışılmıştır.Plasebo ilaç, A ve B sham gruplarına verilirken Epo C ve D gruplarına uygulanmıştır.Tahmini LDH değerleri ratların ağırlıklarına göre hesaplanmıştır. Ratların ağırlkları ile LDH düzeyleri arasında belirgin bir ilişki bulunmuştur(p= 0.0469).
Bulgular: Epo uygulaması LDH düzeylerini 778.3 IU/L [200.6627 IU/L - 1355.937 IU/L] olacak şekilde belirgin olarak artırmıştır (p= 0.0096). Bu bulgu, paired t-testi sonuçları ile uyumlu bulunmuştur (p= 0.0004). Reperfüzyon süresi ise LDH düzeylerini 124.9 IU/L [-755.2258 IU/L - 505.4258 IU/L] olacak şekilde belirgin olarak azaltmamıştır (p= 0.6906), ayrıca bu sonuçlar paired t-testi ile de uyumludur (p= 0.6354). Epo uygulaması ve reperfüzyon süresi birlikte belirgin kombine etki göstererek LDH düzeylerini 376.8 IU/L [16.55497 IU/L - 737.045 IU/L] olacak şekilde yükseltmiştir (p= 0.0408).
Sonuç: Bu çalışmanın sonuçları, tek başına veya reperfüzyon süresi ile birlikte Epo uygulamasının LDH düzeyleri üzerine kısa sürede artırıcı etkisinin olduğunu gösermiştir.Bu artışlar, orijinal LDH değerleri için belirgin iken tahmin edilen değerlere göre kayda değer bulunmamıştır. Sonuç olarak; tahmini değerleri daha güvenilir olarak kabul edersek Epo uygulaması post iskemik olarak artmış olan LDH düzeylerini etkin şekilde azaltmaktadır. Bu da Epo'nun dokularda beklenendne daha büyük bir rol oynadığını önermektedir.

Anahtar Sözcükler: İskemi, eritropoietin, laktat dehidrogenaz düzeyleri, reperfüzyon, restorasyon, doku hasarı

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INTRODUCTION

Tissue ischemia and reperfusion (IR) remain one of the main causes of permanent or transient damage with serious implications on adjacent organs and certainly to patients' health. The use of erythropoietin (Epo) has been a research subject for a lot of years. However, although important progress has been made, satisfactory answers have not been given yet to some fundamental questions such as how much powerful should an antioxidant be, when should it be administered, and in what dosage. The particularly satisfactory action of Epo in tissue protection has been noted in several experiments. After a careful literature search (PubMed - Medline) it was found that this factor has been tested in IR experiments. However, relatively few reports were found, not completely covering this particular issue (1,2,3,4,5). Also, a lot of publications addressed trials of other similar molecules of growth factors to which the studied molecule belongs to.

Lactate dehydrogenase (LDH) is found extensively in body tissues, such as blood cells, heart muscle and liver. Because it is released during tissue damage, it is a marker of common injuries like muscular failure and fatigue, tissue breakdown or turnover and hemolysis (6). It concerns all the ischemic tissues under the clapping level in the present experiment. The aim of this experimental study was to examine the effect of Epo on a rat model and particularly in half lower body tissues' IR protocol including hemolysis. Whether or not that molecule would be effective was studied by measuring blood LDH levels.

METHODS

Animal preparation

This experimental study was carried out at the Experimental Research Center of ELPEN Pharmaceuticals Co. Inc. S.A. at Pikermi, Attiki. All materials needed for the study including consumables, equipment and substances used, were a courtesy of the S.A. Measurements were taken by Nihon Kohden celltac, a MEK-6450 K automatic biochemistry analyzer, with preset rat type and cyanide-free reagents with high sensitivity. Wistar albino rats were used in accordance with accepted standards of humane animal care. They spent in laboratory 7 days before the experiment with easy access to water and food. The experiment was acute, that is, the animal usage was completed following experimental set of times without awakening and preservation of the rodents. They were randomly assigned to four experimental groups (10 animals in each group): 1) Ischemia for 45 min and afterwards reperfusion for 60 min (group A); 2) Ischemia for 45 min and afterwards reperfusion for 120 min (group B); 3) Ischemia for 45 min and afterwards immediate Epo intravenous (IV) administration and reperfusion for 60 min (group C); 4) Ischemia for 45 min and afterwards immediate Epo administration and reperfusion for 120 min (group D).

The molecule Epo dose was 10 mg/Kg of the body weight of animals. Commercial vial preparations of epoietin-α were used. They were diluted by distilled water. The experiment started with prenarcosis and general anesthesia administration in animals. Their electrocardiogram and acidometry were continuously monitored. The vessels concerning blood supply, were prepared so as their flow to be excluded by forceps. After exclusion, the protocol of IR was applied. The molecules were administered at the time of reperfusion, through inferior vena cava (catheterization preceded the beginning of the experiment and was after general anesthesia procedures). LDH is of medical significance because it is found extensively in body tissues mainly in the liver. Hemolysis contributes to LDH level rises but its exact contribution cannot be calculated. This contribution is even notable with general tissue injury and is not eliminated. Because it is released during tissue damage, it is a marker of common injuries and disease (6). The LDH level measurement was performed at the 60th min of reperfusion (groups A and C) and the 120th min of reperfusion (groups B and D).

Rats underwent general anesthesia by initial intramuscular (IM) administration of 0.5 cc compound, which consisted of 0.25 cc xylazine, [25 cc, 20 mg/cc] and 0,25 cc ketamine hydrochloride [1000, 100 mg/cc, 10cc]. Before initiation of laparotomy, 0.03 cc butorphanol [10mg/cc, 10cc] anesthesia was administered subcutaneously (SC). Continuous oxygen supply was administered during the whole experimental procedures. Ischemia was caused by clapping inferior aorta for 45 min after laparotomic access was achieved. Reperfusion was induced by removing the clapping and the reestablishment of inferior aorta patency. Forty (40) female Wistar albino rats with a mean weight of 247.7 gr [Std. Dev: 34.99172 gr] were used with min weight ≥ 165 gr and max weight < 320 gr. Rats' weight could potentially be a confounding factor, such as fatter rats having greater blood LDH levels. This suspicion will be investigated.

Model of ischemia-reperfusion injury

Control groups

Twenty control rats with a mean weight of 252.5 gr [Std. Dev: 39.31988 gr] were subjected to ischemia for 45 min and then reperfusion was induced.

Group A

Reperfusion which lasted 60 min concerned 10 control rats with a mean weight of 243 gr [Std. Dev: 45.77724 gr] andmean LDH levels of 1609.9 IU/L [Std. Dev: 834.4269 IU/L] (Table 1).

Group B

Reperfusion which lasted 120 min concerned 10 control rats with a mean weight of 262 gr [Std. Dev: 31.10913 gr] and mean LDH levels of 1622.6 IU/L [Std. Dev: 793.438 IU/L] (Table 1).

Erythropoietin group

Twenty rats with a mean weight of 242.9 gr [Std. Dev: 30.3105 gr] suffered ischemia for 45 min and then reperfusion, in the beginning of which 10 mg Epo/kg of body weight were IV administered.

Group C

Reperfusion which lasted 60 min concerned 10 Epo rats with a mean weight of 242.8 gr [Std. Dev: 29.33636 gr] and mean LDH levels of 2525.8 IU/L [Std. Dev: 622.2959 IU/L] (Table 1).

Group D

Reperfusion which lasted 120 min concerned 10 Epo rats with a mean weight of 243 gr [Std. Dev: 32.84644 gr] and mean LDH levels of 2263.3 IU/L [Std. Dev: 1298.542 IU/L] (Table 1).

Table 1: Weight and LDH mean levels and Std. Dev. of groups.

Groups	Variable	Mean	Std. Dev[a]
A	Weight	243 g	45.77724 g
		LDH	
	1609.9 IU/L	834.4269 IU/L	
B	Weight	262 g	31.10913 g
		LDH	
	1622.6 IU/L	793.438 IU/L	
C	Weight	242.8 g	29.33636 g
	LDH	2525.8 IU/L	622.2959 IU/L
D	Weight	243 g	32.84644 g

Statistical analysis

STATA 6.0 software was used for statistical analysis. Groups A and B consisted of sham operated rats. The comparison was performed by both paired t-tests and generalized linear models (GLM) producing the exactly same results. Rats from every weight group was initially compared with the others from the three remaining groups through a paired t-test. (Table 2). Any emerging significant difference among LDH levels, was investigated to see whether weight plays a role. Rats from every LDH group was also compared with the others from the other three remaining groups through a paired t-test. (Table 2). A GLM model with the dependent variable LDH levels and the independent variables whether the Epo was administered or not, the reperfusion time and their interaction was tested both for original and predicted values adjusted for rats' weights.

Table 2: Statistical significance of mean values difference for groups after statistical paired t test application.

DG[a]	Variable	Difference	p-value
A-B	Weight	-19 g	0.2423
	LDH	-12.7 IU/L	0.9738
A-C	Weight	0.2 g	0.9900
	LDH	-915.9 IU/L	0.0006
A-D	Weight	0 g	1.0000
	LDH	-653.4 IU/L	0.1147
B-C	Weight	19.2 g	0.2598
	LDH	-903.2 IU/L	0.0143
B-D	Weight	19 g	0.1011
	LDH	-640.7 IU/L	0.0737
C-D	Weight	-0.2 g	0.9883
	LDH	262.5 IU/L	0.4974

[a]difference for groups

RESULTS

Epo administration significantly increased the LDH levels by 778.3 IU/L [200.6627 IU/L - 1355.937 IU/L] (P= 0.0096). This finding was in accordance with the results of a paired t-test (P= 0.0004). Reperfusion time non-significantly decreased the LDH levels by 124.9 IU/L [-755.2258 IU/L - 505.4258 IU/L] (P= 0.6906). This finding was also in accordance with paired t-test results (P= 0.6354). Epo administration and reperfusion time together produced a significant combined effect in increasing the LDH levels by 376.8 IU/L [16.55497 IU/L - 737.045 IU/L] (P= 0.0408). Reviewing the above and table 2, the table 3 sums up the alteration influence of Epo as a function of reperfusion time. By including the rats' weight as the independent variable in the GLM model, a very significant effect on LDH levels was obtained (p= 0.0469), necessitating a further investigation. The predicted LDH values, adjusted for rats' weight were calculated and are depicted at table 4. Afterwards, the predicted LDH values for each of the 4 rat groups were compared with those of the other 3 remaining groups through a paired t-test. (Table 5). Applying again the glm procedure to the model with the dependant variable predicted LDH values and the independent variables Epo administration, the reperfusion time and their interaction produced the following results: Epo administration non-significantly increased the predicted LDH by 84.48171 IU/L [-113.2886 IU/L - 282.252 IU/L] (P= 0.3926). This finding was also confirmed in a paired t-test (P= 0.3172). Reperfusion time non-significantly decreased the predicted LDH by 84.48179 IU/L [-282.2521 IU/L - 113.2885 IU/L] (P= 0.3926) which was also confirmed by a paired t-test (P= 0.3926). Epo administration and reperfusion time together produced a non-significant combined effect of increasing the predicted LDH by 45.76091 IU/L [-73.72549 IU/L - 165.2473 IU /L] (P= 0.4430) (Table 6).

Table 3: The increasing influence of erythropoietin in connection with reperfusion time

			p-values	
Increase	**95% c. in.[a]**	**Reperfusion time**	**t-test**	**glm**
915.9 IU/L	224.3422 IU/L - 1607.458 IU/L	1h	0.0006	0.0123
778.3 IU/L	200.6627 IU/L - 1355.937 IU/L	1.5h	0.0004	0.0096
640.7 IU/L	-370.3121 IU/L - 1651.712 IU/L	2h	0.0737	0.1997

[a]confidence interval

Table 4: Mean predicted LDH values adjusted for weight and Std. Dev. of groups

Groups	**Mean**	**Std. Dev[a]**
A	2046.761 IU/L	402.8484 IU/L
B	1879.558 IU/L	273.7663 IU/L
C	2048.521 IU/L	258.1654 IU/L
D	2046.761 IU/L	289.0548 IU/L

[a]standard deviation

Table 5: Statistical significance of mean values difference for groups after statistical paired t test application.

DG[a]	**Difference**	**p-value**
A-B	167.2035 IU/L	0.2423
A-C	-1.76001 IU/L	0.9900
A-D	0.0000854 IU/L	1.0000
B-C	-168.9635 IU/L	0.2598
B-D	-167.2034 IU/L	0.1011
C-D	1.760095 IU/L	0.9883

[a]difference for groups

Table 6: The increasing influence of erythropoietin in connection with reperfusion time.

			p-values	
Increase	**95% c. in[a]**	**Reperfusion time**	**t-test**	**glm**
1.76001 IU/L	-316.1229 IU/L - 319.643 IU/L	1 h	0.9900	0.9908
84.481705 IU/L	-113.2886 IU/L - 282.252 IU/L	1.5 h	0.3172	0.3926
167.2034 IU/L	-97.29645 IU/L - 431.7033 IU/L	2 h	0.1011	0.2007

[a]confidence interval

DISCUSSION

A lot of clinical observations show how LDH levels are influenced in ischemic cases. Pisarenko et al. studied (7) isolated working rat hearts subjected to global IR. Enhanced functional recovery was combined with an increase in LDH levels and LDH/pyruvate levels ratio leakage in perfusate at early reperfusion. Wang et al. generated (8) a mouse model with cardiac-specific overexpression of functional consequence of microRNAs miR-494. Transgenic and wild-type hearts were subjected to global I/R. Transgenic hearts exhibited remarkable decreases in both LDH levels release and myocardial infarction size, than wild-type ones. Yamagishi et al. clarified (9) the characteristics of improved ischemic tolerance induced by severe, short-term food restriction in isolated, perfused rat hearts. Wistar rats were given a food intake equivalent to a 70% reduction on the food intake of ad-libitum fed rats (FR group and AL group, respectively). After this period, hearts were subjected to global IR. During preischemic perfusion, the levels of LDH released into the coronary effluent in the FR group were inversely lower than in the AL group. These results suggested that severe, short-term food restriction improves ischemic tolerance in rat hearts. Xu et al. first determined (10) the effects of IR on cardiomyocyte shortening in female rats and then the roles of β2-adrenoceptor in the levels of LDH released in culture medium, during hypoxia in isolated myocytes. Ciminelli et al. submitted (11) hearts from control and trained (treadmill dunning) rats to IR. Training also reduced the entrapment of mitochondrial DOG ratio units, $p < 0.01$. However, released LDH levels in the coronary effluent were similar in both experimental groups. Van der Hoeven et al. used (12) sham-operated Wistar rats as controls (1 hour and 6 hours). Organ function was studied by monitoring LDH levels. Progressive organ and particularly kidney dysfunction and also, inflammatory responses reflecting tissue injury, were most pronounced in hemodynamically unstable brain-dead donors. Wang et al. examined (13) the role of Na+-H+ exchanger isoform 1 (NHE1) in IR injury using gene-targeted NHE1-null mutant (Nhe1-/-) mice. Nhe1-/- and wild-type hearts were perfused in both absence and presence of the NHE1 inhibitor under IR. In the absence of inhibitor, the released LDH levels were also significantly less impaired in Nhe1-/- hearts relative to wild-type hearts. Wang et al. studied (14) the protective effect of ischemic preconditioning (IP) on IR injury of limb. As compared with the controls, the release of skeletal muscle intracellular enzyme LDH decreased in experiment groups. Baron et al. assessed (15) the effects of mycophenolic acid (MPA 100 microg/ml) in rat liver IR. There was no significant influence of MPA on postischemic efflux rates of LDH. Ko et al. subjected (16) isolated-perfused hearts prepared from control and diabetic rats to myocardial IR injury. A brief period (20 min) of post-ischemic reperfusion caused a LDH leakage to a smaller extent. Kume et al. reported (17) that ischemic preconditioning (IP) of the heart or brain has a possible relevance to heat shock protein (HSP). This IP also attenuated the liver damage in the subsequent IR injury, improving the restoration of hepatic function during reperfusion as explained by LDH release. Jaeschke et al. tested (18) the hypothesis that intracellular generation of reactive oxygen species in hepatocytes may cause IR injury in isolated,perfused livers of Fischer rats as explained by LDH release.

LDH levels may be influenced also by Epo administration. Zhou et al. evaluated (19) possible neuroprotection of rhEPO in a model of sepsis. They determined hippocampal neuronal apoptosis by LDH release, after treatment with lipopolysaccharide. They performed cecal ligation and perforation surgery in vivo. Treatment with rhEPO reduces apoptosis and increases cell viability in lipopolysaccharide-treated neuronal cultures. These results indicated that rhEPO improves brain dysfunction by reducing neuronal apoptosis. Application of rhEPO may serve as a potential therapy for the treatment of septic encephalopathy. Pottgiesser et al. investigated (20) 3 km altitude camping in cyclists for 26 nights. There was a substantial decrease in serum EPO (-34%) two days after return to the sea level. Dulaney et al. assessed the viability (21) of the fetal mouse liver cells by cellular retention of LDH affecting it very little at amines, diamines and polyamines concentrations that substantially discontinue heme synthesis inhibiting the Epo-stimulated incorporation of 59Fe into newly-synthesized heme.

CONCLUSION

Epo administration either alone, or in interaction with reperfusion time has increasing short – term effects on LDH levels. These increases, which stand for increasing distance from baseline levels due to ischemic damage, are significant for the original LDH values and non-significant for predicted values. Significance stands for the difference in values from baseline levels, which is, longer for original and smaller for predicted ones.

Considering the predicted values as more reliable, it is concluded that Epo administration decreases the gap between postischemic LDH levels more effectively than what was previously believed to be the case, suggesting a greater restorating role of Epo in general tissue injuries including blood cells. So, Epo can assist in quicker recession of situations such as fatigue, common injuries and diseases, acidosis, hemolysis, inflammations and infections.

Limitations of the study

Groups A and B were assigned as both control and sham ones at once, since every animal could provide only one value. So the unique value available was preferred to be either the sham or the case one.

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Conflict of Interest

No conflict of interest was declared by the authors.

REFERENCES

1. Bakan V, Ciralik H, Tolun FI, Atli Y, Mil A, Ozturk S. Protective effect of erythropoietin on torsion/detorsion injury in rat model. J Pediatr Surg 2009;44:1988-94.

2. Karaca M, Odabasoglu F, Kumtepe Y, Albayrak A, Cadirci E, Keles ON. Protective effects of erythropoietin on ischemia/reperfusion injury of rat ovary. Eur J Obstet Gynecol Reprod Biol. 2009;144:157-62.

3. Yano T Miki T, Tanno M, Kuno A, Itoh T, Takada A, Sato T, Kouzu H, Shimamoto K,Miura T. Hypertensive hypertrophied myocardium is vulnerable to infarction and refractory to erythropoietin-induced protection. Hypertension 2011;57:110-5.

4. Bader A, Pavlica S, Deiwick A, Lotkova H, Kucera O, Darsow K, Bartel S, Schulze M, Lange HA, Cervinkova Z. Proteomic analysis to display the effect of low doses of erythropoietin on rat liver regeneration. Life Sci 2011;89:827-33.

5. Wang Q, Gorbey S, Pfister F, Höger S, Dorn-Beineke A, Krügel K, Berrone E, Wu L, Korff T, Lin J, Busch S, Reichenbach A, Feng Y, Hammes HP. Long-term treatment with suberythropoietic Epo is vaso- and neuroprotective in experimental diabetic retinopathy. Cell Physiol Biochem 2011;27:769-82.

6. Spriet LL, Howlett RA, Heigenhauser GJ.An enzymatic approach to lactate production in human skeletal muscle during exercise.Med Sci Sports Exerc 2000;32:756-63.

7. Pisarenko OI, Shulzhenko VS, Pelogeĭkina IuA, Studneva IM, Kkhatri DN, Bespalova ZhD, et al. Effects of exogenous apelin-12 on functional and metabolic recovery of isolated rat heart after ischemia. Kardiologiia 2010; 50: 44-9.

8. Wang X, Zhang X, Ren XP, Chen J, Liu H, Yang J, et al. MicroRNA-494 targeting both proapoptotic and antiapoptotic proteins protects against ischemia/reperfusion-induced cardiac injury. Circulation 2010; 28;122: 1308-18.

9. Yamagishi T, Bessho M, Yanagida S, Nishizawa K, Kusuhara M, Ohsuzu F, et al. Severe, short-term food restriction improves cardiac function following ischemia/reperfusion in perfused rat hearts. Heart Vessels 2010; 25: 417-25.

10. Xu C, Liu A, Sun H, Sun Y, Wang G, Gao L, et al. beta2-Adrenoceptor confers cardioprotection against hypoxia in isolated ventricular myocytes and the effects depend on estrogenic environment. J Recept Signal Transduct Res 2010; 30: 255-61.

11. Ciminelli M, Ascah A, Bourduas K, Burelle Y. Short term training attenuates opening of the mitochondrial permeability transition pore without affecting myocardial function following ischemia-reperfusion. Mol Cell Biochem 2006; 291: 39-47.

12. van der Hoeven JA, Molema G, Ter Horst GJ, Freund RL, Wiersema J, van Schilfgaarde R. Relationship between duration of brain death and hemodynamic (in)stability on progressive dysfunction and increased immunologic activation of donor kidneys. Kidney Int 2003; 64: 1874-82.

13. Wang Y, Meyer JW, Ashraf M, Shull GE. Mice with a null mutation in the NHE1 Na+-H+ exchanger are resistant to cardiac ischemia-reperfusion injury. Circ Res 2003; 17;93: 776-82.

14. Wang WG, Deng ZS, Gong JL, Zhu Y. Protective effect of ischemic preconditioning on ischemia-reperfusion injury. Hunan Yi Ke Da Xue Xue Bao 2000; 28;25: 351-3.

15. Baron A, Bilzer M, Gerbes AL. Short-term treatment with mycophenolic acid increases bile flow in continuously perfused and cold-preserved rat livers and does not affect hepatic ischemia-reperfusion injury. Transpl Int 2002; 15: 265-71.

16. Ko KM, Mak DH, Poon MK, Yiu HY. Altered susceptibility to ischemia-reperfusion injury in isolated-perfused hearts of short-term diabetic rats associated with changes in non-enzymatic antioxidants. Jpn J Pharmacol 2001; 85: 435-42.

17. Kume M, Yamamoto Y, Saad S, Gomi T, Kimoto S, Shimabukuro T, et al. Ischemic preconditioning of the liver in rats: implications of heat shock protein induction to increase tolerance of ischemia-reperfusion injury. J Lab Clin Med 1996; 128: 251-8.

18. Jaeschke H, Smith CV, Mitchell JR. Reactive oxygen species during ischemia-reflow injury in isolated perfused rat liver. J Clin Invest 1988; 81: 1240-6.

19. Ischemia, erythropoietin, lactate dehydrogenase levels, reperfusion, restoration, tissue damage Zhou TF, Yu JG. Recombinant human erythropoietin attenuates neuronal apoptosis and cognitive defects via JAK2/STAT3 signaling in experimental endotoxemia. J Surg Res 2013 ; 183: 304-12.

20. Pottgiesser T, Garvican LA, Martin DT, Featonby JM, Gore CJ, Schumacher YO. Short-term hematological effects upon completion of a four-week simulated altitude camp. Int J Sports Physiol Perform 2012; 7: 79-83.

21. Dulaney JT, Hatch FE Jr, Young J. Effect of amines on erythropoietin-stimulated heme synthesis in fetal mouse liver cells. Life Sci 1985; 29;36: 1633-42.