The aim of this experimental study was the trial of erythropoietin in rat animal model and certainly in liver ischemia/reperfusion protocol. The benefit or not of that particular molecule was studied measuring alanine aminotransferase (ALT). This experimental study was approved by Scientific committee of Ippokrateion General Hospital, Athens University, and by Veterinary Address of East Attiki Prefecture and institutional and national guide for the care and use of laboratory animals was followed.

**AIM**

Aim of present experimental study was the trial of erythropoietin in rat animal model and certainly in liver ischemia/reperfusion protocol. The benefit or not of that particular molecule was studied measuring alanine aminotransferase (ALT). This experimental study was approved by Scientific committee of Ippokrateion General Hospital, Athens University, and by Veterinary Address of East Attiki Prefecture and institutional and national guide for the care and use of laboratory animals was followed.

**EXPERIMENTAL GROUPS**

This experimental study was laid out by Experimental Research Center of ELPEN Pharmaceuticals Co. Inc. S.A. at Pikermi, Attiki, and all of the settings including of consumables, equipment and substances used, were a courtesy of that S. A. 40 female white
Wistar rats of mean weight 247.7 gr [Std. Dev: 34.99172 gr] were used, min weight $\geq$ 165 gr and max weight $< 320$ gr. They were naturalized in laboratory for 7 days before experimentation. They had free access to water and food. They were accidentally separated in the following experimental groups (10 animals in each group). The experiment was acute, that is, the animal use was completed by following experimentation times expiring as awakening and preservation did not exist.

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 erythropoietin IV administration and reperfusion for 60 min (group C).
4 - Ischemia for 45 min and afterwards immediate erythropoietin IV administration and reperfusion for 120 min (group D).

The molecule erythropoietin dose was 10 mg/Kg body weight of animals.

The experiment was beginning by prenarcosis and general anaesthesia 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 ischemia/reperfusion was applied, described more in experimental groups. The molecules were administered at the time of reperfusion, through inferior vena cava (catheterization had been preceded at experiment beginning, after general anaesthesia establishment).

The ALT measurement was performed on these time points:
1 - on 60 min of reperfusion (groups A and C),
2 - on 120 min of reperfusion (groups B and D).

**PROTOCOL**

ALT is considered a reliable index substance of liver metabolism being of great clinical diagnostic value concerning general metabolism and liver function. Also, rats weight could be potentially a confusing factor, e.g. fatter rats to have greater blood ALT levels. This suspicion will be investigated and will be rejected.

Introduction into general anaesthesia was becoming by initial IM administration of 0.5 cc compound, constituted by 0.25cc xylazine, [25cc, 20mg/cc] and 0.25cc ketamine hydrochloride [1000, 100mg/cc, 10cc], 0.03cc butorphanol [10mg/cc, 10cc] anaesthesia was administered s.c. before a laparotomy. Continuous oxygen supply was administered during the whole experiment performance. Ischemia was caused by clapping inferior aorta for 45 min after laparotomic access. Reperfusion was achieved by removing clapping and inferior aorta patency re-establishment.

### TABLE 1

<table>
<thead>
<tr>
<th>Groups</th>
<th>Variable</th>
<th>Mean</th>
<th>Std. dev</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Weight</td>
<td>243 gr</td>
<td>45.77724 gr</td>
</tr>
<tr>
<td></td>
<td>ALT</td>
<td>43.6 IU/L</td>
<td>5.853774 IU/L</td>
</tr>
<tr>
<td>B</td>
<td>Weight</td>
<td>262 gr</td>
<td>31.10913 gr</td>
</tr>
<tr>
<td></td>
<td>ALT</td>
<td>64.5 IU/L</td>
<td>39.42715 IU/L</td>
</tr>
<tr>
<td>C</td>
<td>Weight</td>
<td>242.8 gr</td>
<td>29.33636 gr</td>
</tr>
<tr>
<td></td>
<td>ALT</td>
<td>52.7 IU/L</td>
<td>16.64699 IU/L</td>
</tr>
<tr>
<td>D</td>
<td>Weight</td>
<td>243 gr</td>
<td>32.84644 gr</td>
</tr>
<tr>
<td></td>
<td>AST</td>
<td>62.2 IU/L</td>
<td>25.74145 IU/L</td>
</tr>
</tbody>
</table>

### TABLE 2

<table>
<thead>
<tr>
<th>DG</th>
<th>Variable</th>
<th>Difference</th>
<th>p-value</th>
</tr>
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<tbody>
<tr>
<td>AB</td>
<td>Weight</td>
<td>-19 gr</td>
<td>0.2423  gr</td>
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<tr>
<td></td>
<td>ALT</td>
<td>-20.9 IU/L</td>
<td>0.01022 IU/L</td>
</tr>
<tr>
<td>AC</td>
<td>Weight</td>
<td>0.2 gr</td>
<td>0.9900  gr</td>
</tr>
<tr>
<td></td>
<td>ALT</td>
<td>-9.1 IU/L</td>
<td>0.1541 IU/L</td>
</tr>
<tr>
<td>AD</td>
<td>Weight</td>
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<td>1.0000  gr</td>
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<tr>
<td></td>
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<td>-18.6 IU/L</td>
<td>0.0344 IU/L</td>
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<tr>
<td>BC</td>
<td>Weight</td>
<td>19.2 gr</td>
<td>0.2598  gr</td>
</tr>
<tr>
<td></td>
<td>ALT</td>
<td>11.8 IU/L</td>
<td>0.3545 IU/L</td>
</tr>
<tr>
<td>BD</td>
<td>Weight</td>
<td>19 gr</td>
<td>0.1011  gr</td>
</tr>
<tr>
<td></td>
<td>ALT</td>
<td>2.3 IU/L</td>
<td>0.8444 IU/L</td>
</tr>
<tr>
<td>CD</td>
<td>Weight</td>
<td>-0.2 gr</td>
<td>0.9883  gr</td>
</tr>
<tr>
<td></td>
<td>ALT</td>
<td>-9.5 IU/L</td>
<td>0.3138 IU/L</td>
</tr>
</tbody>
</table>
Control groups

20 control rats (controls: 1 - 20) mean weight 252.5 gr [Std. Dev: 39.31988 gr] suffered by ischemia for 45 min and then a reperfusion.

Group A

Reperfusion which lasted 60 min concerned 10 controls rats of mean weight 243 gr [Std. Dev: 45.77724 gr], mean ALT 43.6 IU/L [Std. Dev: 5.853774 IU/L] (Table 1).

Group B

Reperfusion which lasted 120 min concerned 10 controls rats of mean weight 262 gr [Std. Dev: 31.10913 gr], mean ALT 64.5 IU/L [Std. Dev: 39.42715 IU/L] (Table 1).

Erythropoietin group

20 rats (L: 1 - 20) of mean weight 242,9 gr [Std. Dev: 30.3105 gr] suffered by ischemia for 45 min and then the reperfusion at the beginning of which 10 mg erythropoietin /kg body weight were IV administered.

Group C

Reperfusion which lasted 60 min concerned 10 L rats of mean weight 242.8 gr [Std. Dev: 29.33636 gr], mean ALT 52.7 IU/L IU/L [Std. Dev: 16.64699 IU/L] (Table 1).

Group D

Reperfusion which lasted 120 min concerned 10 L rats of mean weight 243 gr [Std. Dev: 32.84644 gr], mean ALT 62.2 IU/L IU/L [Std. Dev: 25.74145 IU/L] (Table 1).

Weight comparison

Weight comparison of each one from 4 rats groups initially was performed with other one from 3 remainder groups applying statistical paired t-test. (Table 2).

Some weight correlations resulted statistically important. Any emerging important difference among ALT, will be investigated whether owed in the above mentioned important weight correlations.

<table>
<thead>
<tr>
<th>Alteration</th>
<th>95% c.in.</th>
<th>Reperfusion time</th>
<th>p-values; t-test</th>
<th>glm</th>
</tr>
</thead>
<tbody>
<tr>
<td>-9.1</td>
<td>-2.623613 IU/L-20.82361 IU/L</td>
<td>1 h</td>
<td>0.1541</td>
<td>0.1203</td>
</tr>
<tr>
<td>-3.4</td>
<td>-13.1482 IU/L-19.9482 IU/L</td>
<td>1.5 h</td>
<td>0.5994</td>
<td>0.6798</td>
</tr>
<tr>
<td>2.3</td>
<td>-33.58274 IU/L-28.98274 IU/L</td>
<td>2 h</td>
<td>0.8444</td>
<td>0.8790</td>
</tr>
</tbody>
</table>

ALT levels comparison

ALT comparison of each one from 4 rats groups initially was performed with other one from 3 remainder groups applying statistical paired t-test. (Table 2).

Applying generalised linear models (glm) with dependant variable the ALT levels and independent variables the erythropoietin administration or no, the reperfusion time and their interaction, results in: 1) erythropoietin administration increased significantly the ALT by 15.2 IU/L [-0.6168546 IU - 31.01685 IU/L] (P= 0.0591), in accordance also with paired t-test (P= 0.0480), 2) reperfusion time increased non significantly the ALT by 3.4 IU/L [-13.1482 IU - 19.9482 IU/L] (P= 0.6798), in accordance also with paired t-test (P=0.5994), and 3) interaction with erythropoietin administration and reperfusion time increased non significantly the ALT levels by 3.581818 IU/L [-6.350404 IU - 13.51404 IU/L] (P= 0.4698).

Reviewing the above and table 2, the table 3 sums up concerning the alteration influence of erythropoietin in connection with reperfusion time.

Inserting the rats weight as an independent variable at glm, a non significant relation turns on ALT levels (p=0.2628), so as to further investigation does not need.

DISCUSSION

A lot of clinical situations can prove how ALT levels are influenced by ischemic cases. Liu SQ et al detected \(^1\) that ALT levels for the detection of liver damage after operation in adult dogs with hand suturing were significantly higher than those in the sutureless group after reperfusion (P < 0.01) reducing the complications caused by hand suturing, and the extent of IR injury, leading to smoother operations and improved prognosis. Sosnowski P et al observed\(^2\) that induced ischemia of hind limb caused an increase in ALT activity and an increase in the level of free radicals in analyzed liver tissues of rats. Teoh NC et al fed foz/foz mice with a high-fat diet (HFD) to cause nonalcoholic steatohepatitis NASH or chow simple steatosis (SS) and then subjected\(^3\) them into hepatic IR which was exacerbated compared with HFD-fed or chow-fed wild-type littermates by ALT release assessed at 24 hours. Ikeda A et
al preserved rat liver grafts in University of Wisconsin (UW) solution containing 5% CO (CO-UW solution) for 21 hours and transplanted them into syngeneic Lewis rats. After LTx serum alanine aminotransferase levels were significantly less in the CO-UW group than in the control UW group. In conclusion, CO delivery to excised liver grafts efficiently ameliorates hepatic I/R injury. Oba M et al subjected mice to hepatic IR after treatment three times with heat shock HS (42 degrees C) and/or mild electrical stimulation MES (12V) for 20 min than sham ones. Liver injury was assessed by evaluating the levels of serum ALT. HS+MES pretreatment suppressed the hepatic I/R-induced release of serum ALT. Kim KH et al explored the role of the transcription factor interferon regulatory factor-1 (IRF-1) in a model of orthotopic rat liver transplantation pretreated 4 days before graft harvesting and in uninfected control grafts. Rats pretreated with control gene vector AdIRF-1 before transplantation had elevated ALT levels and in the short-term period (3 hours) when compared to donor’s livers pretreated with Adnull. IRF-1 is an important regulator of IR injury after OLT in rats and may be a potential strategy to ameliorate ischemic hepatic liver injury after transplantation. Pulitanti C et al provided in liver injury patients following liver operation 500 mg of methylprednisolone preoperatively. Postoperative serum levels of ALT were significantly lower in the steroid group than in controls. Fiorini RN et al investigated the effects of epigallocatechin gallate EGCG on hepatic steatosis and markers of cellular damage at baseline and after I/R injury in ob/ob mice as being a potent antioxidant that inhibits fatty acid synthase (FAS) in vitro. Animals were pretreated with 85 mg/kg EGCG via intraperitoneal (ip) injection for 2 days or oral consumption for 5 days before I/R. ALT levels decreased in all EGCG-treated animals compared with control animals after I/R. Also, ALT levels are a factor influenced by erythropoietin. Is suitable erythropoietin administration able to influence indirectly ALT levels? Coilly A et al have significantly improved sustained virological response SVR rates in HCV patients by protease inhibitors (PI) with peg-interferon/ribavirin. Liver transplant recipients were treated with boceprevir or telaprevir. The indication for therapy was HCV recurrence (fibrosis stage II or fibrosing cholestatic hepatitis). After 12 weeks of PI therapy, a complete virological response was obtained in 89% of patients treated with boceprevir, 58% with telaprevir (P=0.06) and 92% with anemia treated by erythropoietin. Ta’al F et al described primary hyperoxaluria as a rare autosomal recessive disorder. Type 1 PH is the most common form and develops due to a defect in a liver specific enzyme the ALT enzyme with erythropoietin-resistant anemia. Carrió JA et al distributed patients receiving antiviral treatment into 3 groups: control group, multidisciplinary support programme MSP group, and Epo-MSCs. Also, higher SVR rates were observed. However, the on-treatment virological response was high. Fu W et al injected rHuEPO and/or LY294002 before a liver I/R model operation in Sprague-Dawley rats. The serum levels of ALT were significantly decreased in the serum of I/R + rHuEPO group, proving the protective effect of rHuEPO in I/R injury. Eisfeld AK et al found ALT elevated in 55.7% of allograft recipients due to hematologic malignancies. Rjibatouati K et al induced a Cisp liver failure characterized by a significant increase in ALT levels in serum of adult male Wistar rats although tissue-protective effect of administered rHuEPO. Eliopoulos N et al implanted mouse. Epo-secreting mesenchymal stromal cells MSCs by intraperitoneal injection in allogeneic mice previously administered cisplatin to induce acute kidney injury AKI. Epo-MSCs recipient mice also showed significantly decreased ALT blood concentrations. Ishikawa Y et al studied the benefits of perioperative oral nutrition (ON) with branched chain amino acids BCAA in hepatectomy patients and in the control group. Serum levels of ALT, did not differ significantly between the BCAA group and control group; however, peak values were lower in the BCAA group. Short-term BCAA support was associated with higher serum EPO levels than a normal diet in hepatectomy patients. Higher EPO levels might protect liver cells from ischemic injury associated with lower perioperative levels of ALT in serum. Moussavain MR et al resulted in an ALT elevation after 24 h cold storage hepatic IR in 37°C Krebs Henseleit buffer preconditioned by a multidrug donor included also the erythropoietin. Greif F et al assessed serum ALT levels after liver injury on days 2 and 4 post-hepatectomy significantly lower in rats pretreated with intraperitoneal injection of rhEPO (4U/g) 30 minutes prior to subtotal 70% hepatectomy in comparison with the control group (P < 0.005). Barroso J et al did not predict greater changes in HIV-related fatigue in participants over a 1-year period, in an effort to sort out among ALT, HIV viral load and serum erythropoietin. Schmeding M et al induced fatty liver (≥50% steatosis) by a special diet in donor Lewis rats and treated them by 1000 IU rhEpo or saline injection (controls). ALT values were significantly reduced for 1000 IU rHuEPO epo-treated recipients rats than an equal amount of saline (control) 48 hr after reperfusion. Bárzana R et al observed that only liver transplant (LT) subjects able to complete or maintain
full dose pegylated interferon/ribavirin (peg-IFN/RBV) antiviral therapy survived, with long-term significant improvements in ALT liver function tests. Schmeding M et al treated donor liver Wistar rats with either 1000 IU rHuEpo or saline injection (controls). ALT values were significantly reduced among the 1000 IU rHuEpo-treated recipients animals than an equal amount of saline in control ones at reperfusion 24 and 48h after liver transplantation (LT). Erythropoietin reduces ischemia-reperfusion injury after orthotopic liver transplantation in rats. Bockhorn M et al reduced liver damage by 37.31% or 2.3-fold as indicated by the serum activity of ALT after partial 90% hepatectomy (PH) or 30% partial liver transplantation (pLTx) in EPO-treated rats respectively. Yilmaz S et al divided adult male Sprague-Dawley rats into three groups: group I, hepatic I/R; group II, hepatic I/R+ EPO of 1000 U/kg 120 minutes before IR; and group III, sham. In rats with hepatic ischemia, serum levels of ALT, after 45 minutes of reperfusion were reduced by the administration of erythropoietin. This study demonstrates that pre-ischemic administration of EPO has protective effects on hepatic I/R injury. Lin YL et al found ALP (P < 0.0001) significantly more elevated in chronic hepatitis group demanding less erythropoietin dose than in hepatitis-free group in haemodialysis patients. Carrió JA et al treated patients with severe recurrence cholestatic hepatitis C by peginterferon-α2b/ribavirin. Among treated patients, ALT became normal (OR 5.3 P < .01) demonstrating that, treatment for 48 weeks slows mild hepatitis C recurrence (fibrosis stage) progression (particularly in sustained virological responders) in liver transplantation recipients. Sepodes B et al provided the first evidence that rhEPO administration 5 min before ischemia reduces the oxidative stress suggesting the subsequent reduction of apoptosis and causes a substantial reduction of the serum levels of ALT as biochemical evidence of liver injury induced by I/R in rats. Tipoe GL et al shown the effect of chronic hypoxia on the expression of hypoxia-inducible factor-1 HIF-1α and respective-target genes in liver. Intriguingly, serum ALT levels are within normal range in chronic hypoxia, suggesting the absence of significant oxidative stress. Callahan SM et al analyzed ALT levels until 14th day in Sprague-Dawley rats given viral particles (vp)/kg of either: AdlacZ, Ad expressing murine erythropoietin (Epo), Ad without a transgene (Null), or phosphate-buffered saline (Vehicle). Di Fazio I et al diagnosed chronic hepatitis C by at least 2-fold higher ALT serum levels than normal values for at least 12 months and the presence of anti-HCV antibodies). At the end of the follow-up period, significant differences were seen in ALT levels. All differences favored patients who received IFN-α2a and rHuEPO. Boysen T et al found no relations between TNTC infection (68% of population of Danish hemodialysis patients) and elevated levels of ALT under treatment with erythropoietin (EPO), although 50% of TNTV-positive patients had a high TNTV viral load. Saab S. et al found a significant decrease of mean ALT value by 2.24-fold (p = 0.002) in orthotopic liver transplantation OLT recipients patients treated with ribavirin monotherapy for recurrent HCV. Their results suggest that HCV disease can progress despite a significant decrease in ALT values rendering ALT values an inadequate marker. Borawska J et al found thrombo- modulin TM and tissue factor TF levels higher and directly associated with serum enzymes and use of erythropoietin therapy in HD patients than healthy controls. ALT and use of erythropoietin independently predicted both TF and TM levels. HD patients with ALT levels lower than a middle, and not treated with erythropoietin had normal TF but increased TM concentrations compared with levels in healthy controls. Shakil AO et al caused a further decrease in serum ALT levels treated recurrent hepatitis C patients at least 6 months posttransplantation with IFN-α2a 3*106 IU 3 times a week subcutaneously and ribavirin monotherapy maintenance 800 mg daily p.o. for 48 weeks. Several patients required treatment with erythropoietin for anemia. Borawska J et al associated directly pre-dialysis serum hepatocyte growth factor HGF with the presence of ALT, with thrombomodulin TM levels, with duration of HD and usage of recombinant erythropoietin (rHuEpo) treatment in hepatitis B and C markers in HD patients. Radovic M et al found Hb levels maximal at the time of serum ALT normalization in anemic hepatitis B (HBV) and hepatitis C virus (HCV) infected patients on regular hemodialysis under serum erythropoietin (Epo) treatment. Chan TM et al normalized deranged ALT levels, within 2-8 weeks of interferon-α2b IFN therapy in the treatment of chronic hepatitis C virus (HCV) infection in patients on maintenance haemodialysis deserving special attention in erythropoietin resistance. Berglund B et al found serum ALT unchanged after rHuEpo 30 IU/kg body weight treatment in healthy male subjects.

CONCLUSION

Erythropoietin administration, reperfusion time and their interaction have non significant short – term increasing effect on ALT levels. It seems that erythropoietin itself exerts a catabolic influence on ALT on time, although huge nearly launching trends appearing during liver IR injury. The sure is that ALT levels would be found out still greater if the erythropoietin was not administered. Further human clinical or molecular studies are required to make this effect clearer.

SUMMARY

Cilj ove eksperimentalne studije je ispitivanje efekta eritropoetina na animalnom modelu (pacov) u uslovima ischemije-reperfuzije. Postojanje ili izostanak efekta ispitivan je biohemijskim merenjem alanin-aminotransferaze u serumu.
The effect of erythropoietin on alanine aminotransferase during ischemia-reperfusion injury in rats

Materijal i metode: Studija je radjena na 40 pacova prošće težine 247,7 gr. Alanin aminotransferaza je merena u sledećim vremenskim intervalima: 60. minut posle reperfuzije (grupe A i C), i 120. minut posle reperfuzije (grupe B i D). Grupa A i B je bila bez datog eritropoetina dok je u grupi C i D administriran eritropoetin.

Rezultati: 1) davanje eritropoetina je značajno povećalo nivo ALT za 15.2 IU/L [-0.616 8546 IU - 31.016 85 IU/L] (P= 0.0591), u skladu sa rezultatom t-testa (P= 0.0480), 2) reperfuziono vreme nije značajno povećalo nivo ALT - 3.4 IU/L [-13.1482 IU - 19.9482 IU/L] (P= 0.6798), a prema t-testu (P= 0.5994), i 3) interakcija eritropoetina i intervala reperfuzije nije značajno povećala nivo ALT - 3.58 1818 IU/L [-6.350404 IU - 13.51404 IU/L] (P= 0.4698).

Zaključak: Davanje eritropoetina, interval reperfuzije i njihova interakcija nemaju značajan kratkoročni efekat povećanja nivoa alanin aminotransferaze u serumu.

Ključne reči: eritropoetin, alanin aminotransferaza, reperfuzija

REFERENCES


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