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# The Effect Of Erythropoietin On Testosterone Levels During Ischemia Reperfusion Injury In Rats

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ABSTRACT
Objective: This experimental study examined the effect of erythropoietin (Epo) in a rat model and particularly in an adrenal ischemia-reperfusion (IR) protocol. The effect of that molecule was studied biochemically using blood mean testosterone levels (T).
Materials and methods: 40 rats of mean weight 247.7 g were used in the study. T 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: Erythropoietin administration non significantly increased the testosterone levels by 71.21%+44.19% (p=0.1080). Reperfusion time non-significantly decreased the testosterone levels by 65.17%+44.45% (p=0.0792). However, erythropoietin administration and reperfusion time together produced a non-significant combined effect in increasing the testosterone levels by 27.65%+27.21% (p= 0.3006).
Conclusions: Erythropoietin administration whether it interacted or not with reperfusion time has increasing non significant short-term effects on testosterone levels. Perhaps, a longer study time or a higher Epo dose, may reveal clearer and more significant effects.

Keywords: ischemia, erythropoietin, testosterone levels, reperfusion

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## Introduction

Erythropoietin (Epo) is generally one of the more well studied growth factors. Epo implicates over 28,623 known biomedical studies at present. 3.4% at least of these studies concern tissue ischemia-reperfusion (IR) experiments. Certainly, important progress has been made concerning the Epo usage in reversing the IR kind of transient or permanent injuries including adjacent organs and certainly 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. A numeric evaluation of the Epo efficacy was yielded by a meta-analysis of 28 published seric variables, based on the same experimental setting, at the same endpoints (Table I).

The special aim of this experimental work was to study the effect of Epo on a rat model and mainly in an adrenal IR protocol. The effect of Epo molecule was tested by measuring the blood mean testosterone levels (T).

*Table I: The erythropoietin (Epo) influence (+SD) on the levels of some seric1 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 BCC | +24.01%+13.38% | 0.1012 | +22.09%+9.11% | 0.0163 | +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 |
| MCHC5 | +1.82%+0.56% | 0.0076 | +1.73%+0.50% | 0.0016 | +1.65%+0.92% | 0.0721 | +0.89%+0.31% | 0.0061 |
| RbcDW | -1.85%+4.24% | 0.6703 | -1.64%+2.53% | 0.5159 | -1.43%+3.34% | 0.6078 | -1.06%+1.43% | 0.4733 |
| Plt4 | -7.32%+13.11% | 0.5219 | -2.14%+8.04% | 0.7581 | +3.04%+10.78% | 0.7204 | -0.16%+4.76% | 0.9725 |
| 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 |
| Albumins2 | -4.61%+4.21% | 0.2530 | -9.28%+3.20% | 0.0054 | -13.96%+5.03% | 0.0095 | -5.37%+2.73% | 0.0072 |
| 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 |
| AST3 | +29.53%+9.72% | 0.0096 | +26.71%+13.17% | 0.0235 | +23.89%+21.59% | 0.1709 | +19.73%+7.70% | 0.0119 |
| 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 |
| Calcium1 | 0.28%+1.19% | 0.8065 | -0.56%+1.13% | 0.5761 | -1.41%+2.08% | 0.4100 | -0.34%+0.68% | 0.6095 |
| 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 | +1%+6.20% | 0.8596 | -1.09%+3.34% | 0.7248 | -3.19%+3.90% | 0.3729 | -0.19%+1.93% | 0.9197 |
| Amylase | +6.50%+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 | +2.27%+10.60% | 0.5497 | +2.60%+9.68% | 0.3630 | +2.93%+10.92% | 0.3520 | +2.27%+6.29% | 0.4092 |

## Material and method

### Animal preparation

Prefectural veterinary Address of East Attiki licensed the experiment under 3693/12-11-2010 & 14/10-1-2012 decisions. All substances, equipment and consumable needed for the study was a courtesy of ELPEN Pharmaceuticals Co Inc. S.A. at Pikermi, Attiki. Formal human animal care was adopted for female albino Wistar rats. Normal 7 days pre-experimental housing in laboratory included *ad libitum* diet. Prenarcosis of animals, preceded of continuous intra-experimental general anesthesia [1-5], electrocardiogram, acidometry and oxygen supply. Post-experimental euthanasia of the rodents did not permit their preservation.

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. Epo administration was performed at the time of reperfusion, through catheterized inferior vena cava. The T 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 T levels. This assumption was also investigated.

**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 T levels 0.091 ng/ml [SD: 0.0455705 ng/ml] (Table II).

Group B

Reperfusion lasted for 120 min (n=10 controls rats) mean mass 262 g [SD: 31.10913 g], mean T levels 0.034 ng/ml [SD: 0.0084327 ng/ml] (Table II).

Erythropoietin group

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 T levels 0.147 ng/ml [SD: 0.1997804 ng/ml] (Table II).

Group D

Reperfusion lasted for 120 min (n=10 Epo rats) mean mass 243 g [SD: 32.84644 g], mean T levels 0.096 ng/ml [SD: 0.0880909 ng/ml] (Table II).

*Table II: Weight and testosterone mean levels and Std. Dev. of groups*

| Groups | Variable | Mean | Std. Dev |
|---|---|---|---|
| A | Weight | 243 g | 45.77724 g |
| | Testosterone | 0.091 ng/ml | 0.0455705 ng/ml |
| B | Weight | 262 g | 31.10913 g |
| | Testosterone | 0.034 ng/ml | 0.0084327 ng/ml |
| C | Weight | 242.8 g | 29.33636 g |
| | Testosterone | 0.147 ng/ml | 0.1997804 ng/ml |
| D | Weight | 243 g | 32.84644 g |
| | Testosterone | 0.096 ng/ml | 0.0880909 ng/ml |

**Statistical analysis**

Every weight and T level group was compared with each other from 3 remained groups applying respective statistical standard t-tests (Table III). If any probable significant difference among T levels was raised, it would be investigated whether owed in any respective probable significant mass one (Table III). Then, the application of generalized linear models (glm) was followed. It included as dependant variable the T levels. The 3 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 T levels (p = 0.1800), suggesting that further investigation was not needed.

## Results

The glm application resulted in: Epo administration non-significantly increased the testosterone levels by 0.059 ng/ml [-0.012771 ng/ml - 0.130771 ng/ml] (p= 0.1043). This finding was in accordance with the results of standard t-test (p= 0.1118). Reperfusion time non-significantly decreased the testosterone levels by 0.054 ng/ml [-0.1261943 ng/ml - 0.0181943 ng/ml] (P= 0.1382), also in accordance with standard t-test (p= 0.0203). However, Epo administration and reperfusion time together non-significantly increased the testosterone levels by 0.0229091 ng/ml [-0.021284 ng/ml -

0.0671022 ng/ml] (p= 0.3006). Reviewing the above and table III, the tables IV and V sum up concerning the increasing influence of Epo in connection with reperfusion time.

*Table III: Statistical significance of mean values difference for groups (DG) after statistical standard t test application.*

| DG | Variable | Difference | p-value |
|---|---|---|---|
| A-B | Weight | -19 g | 0.3555 |
| | Testosterone | 0.057 ng/ml | 0.0031 |
| A-C | Weight | 0.2 g | 0.9900 |
| | Testosterone | -0.056 ng/ml | 0.4253 |
| A-D | Weight | 0 g | 1.0000 |
| | Testosterone | -0.005 ng/ml | 0.8801 |
| B-C | Weight | 19.2 g | 0.0478 |
| | Testosterone | -0.113 ng/ml | 0.1123 |
| B-D | Weight | 19 g | 0.2113 |
| | Testosterone | -0.062 ng/ml | 0.0541 |
| C-D | Weight | -0.2 g | 0.9883 |
| | Testosterone | 0.051 ng/ml | 0.2493 |

*Table V: The % alteration influence of erythropoietin in connection with reperfusion time.*

| Alteration | +SD | Reperfusion time | p-values |
|---|---|---|---|
| +47.05% | +58.96% | 1h | 0.4120 |
| +71.21% | +44.19% | 1.5h | 0.1080 |
| +95.38% | +46.14% | 2h | 0.0470 |
| -65.17% | +44.45% | Reperfusion | 0.0792 |
| +27.65% | +27.21% | Interaction | 0.3006 |

## Discussion

T is considered a reliable index substance of adrenals metabolism being of great clinical significance. Examples are described herein concerning whether adrenal ischemia can influence the T levels. Cakir E et al found [6] total T levels significantly higher in PCOS women also having higher risk for cardiovascular disease (CVD) and myocardial ischemia than control subjects. Guven S et al found[7] significantly higher concentrations of serum total T (P = 0.031), elevated serum ischemia-modified albumin (IMA) concentrations - a clinical marker of ongoing myocardial ischemia - well correlated with total T levels (P = 0.022) in women with PCOS than control group. Shaw LJ et al found8 more frequent and heavy angiographic coronary artery disease (CAD) (P = 0.04) and 9.8% less cumulative 5-yr cardiovascular (CV) event-free survival (P = 0.006) in women with clinical features of PCOS as defined by top T quartile (> 30.9 ng/dl) than normal control women. PCOS remained a significant predictor (P < 0.01) in prognostic models for suspected ischemia and CV disease. Kovalenko AN et al determined[9] peripheral blood T concentrations with consequences on metabolic background conducting to cerebral atherosclerosis and ischemic stroke development in clinically normal elderly than younger subjects.

Also the following examples concern the influence of Epo fluctuation on T levels. Klotz RK et al revealed [10] a Leydig cell tumor operationally removed in the hilus and stroma in left ovary of a

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

| | | | p-values | |
|---|---|---|---|---|
| **Increase** | **95% c. in.** | **Reperfusion time** | **t-test** | **glm** |
| 0.056 ng/ml | -0.0801373 ng/ml - 0.1921373 ng/ml | 1h | 0.4253 | 0.3988 |
| 0.059 ng/ml | -0.012771 ng/ml - 0.130771 ng/ml | 1.5h | 0.1118 | 0.1043 |
| 0.062 ng/ml | 0.0032075 ng/ml - 0.1207925 ng/ml | 2h | 0.0541 | 0.0399 |
| -0.054 ng/ml | -0.1261943 ng/ml - 0.0181943 ng/ml | reperfusion time | 0.0203 | 0.1382 |
| 0.0229091 ng/ml | -0.021284 ng/ml - 0.0671022 ng/ml | interaction | - | 0.3006 |

58-year-old woman with a profound elevation of T levels (7.5 μg/l), polyglobulia, remarkably elevated red blood count, hemoglobin and hematocrit values, while Epo was within normal limits. Stephen MR et al revealed [11] an unusual steroid cell tumour after hysterectomy in left ovary of a 67-year-old woman that caused T concentration 44 nmol/l (normal < 5) and virilisation accompanied with symptoms of secondary polycythemia presumably as a result of Epo production.

## Conclusion

Epo administration whether it interacted or not with reperfusion time, has increasing non significant short-term effects on T levels. Perhaps, a longer study time than 2 hours or a higher Epo dose than 10 mg/Kg, may reveal more clear and significant effects.

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