

Cardioprotective Effect of Erythropoietin on Isoprenaline Induced Myocardial Infarction in Rats

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Abstract

Background: Erythropoietin (Epo) has been shown to have important cytoprotective properties against ischemic damage of the myocardium besides its important hematopoietic effect.

Aim: This study was designed to find out the possible effects of treatment with recombinant human erythropoietin in rat model of myocardial infarction induced by isoproterenol (Isoprenaline).

Materials and Methods: Adult male albino rats weighing 200 – 220 gm were divided into 4 groups: group I (Control group): Received no medications and given free access to food and water. Group II (ISO group): Injected intraperitoneal "i.p." with a single dose of isoprotrenol in a dose of 75 mg/kg body weight for induction of myocardial infarction. Group III (ISO + rhEPO): Injected i.p. by single dose recombinant human EPO (rhEPO) in a dose of 5000 I.U/kg body weight, 2 hours after induction of myocardial infarction with isoprotrenol. Group IV (rhEPO 10 days + ISO): Injected i.p. with rhEPO in a dose of 5000 I.U/kg body weight/day for 10 days before induction of myocardial infarction with isoproterenol.

Results: A significant reduction in the infarction size, serum cardiac enzymes (CPK and LDH), significant increases in plasma NO and non-significant change in both mean systemic arterial blood pressure (MSABP) and hematocrit (Hct) value were observed in rats injected with a single dose of rhEPO (5000 I.U/ kg body weight), 2 hours after induction of myocardial infarction when compared with rat model of myocardial infarction (ISO group). Pretreatment of rats with rhEPO (5000 I.U/ kg body weight/day) for 10 days before induction of myocardial infarction causes significant

decrease in cardiac enzymes and infarction size, non-significant change in plasma NO, significant increase in Hct value and MSABP when compared with rat model of myocardial infarction. While on comparing these results with those rats treated with single dose of rhEPO, 2 hours after induction of myocardial infarction, there was significant decrease in cardiac enzyme, plasma NO, Significant increase in MSABP, Hct value and non- significant change in the infarction size.

Conclusion: Cardioprotective effect conferred by rhEPO has many mechanisms. One of them is by modulating the hemodynamic functions through increasing the plasma NO concentration. Although, treatment with rhEPO for longer periods may result in increased hematocrit associated side effects such as hypertension or thromboembolism.

Introduction

Erythropoietin (EPO) is a 165 amino acid glycoprotein hormone and a member of the large and diverse cytokine superfamily produced by the fetal liver and adult kidney. It is mainly synthesized in the peritubular cells in the corticomedullary border of the kidney in response to hypoxia to increase erythropoiesis by stimulating erythroid progenitor (precursor) cells proliferation and differentiation thus increases the number of circulating mature red blood cells, thereby increasing O₂ carrying capacity and protecting red blood cells from apoptosis [1]. Thus, it also plays a major role in regulating plasma haemoglobin (Hb) concentration [2]. So it became widely used in treating anaemias resulting from chronic kidney disease and myelodysplasia occurring after chemotherapy or radiation [3].

The effects of EPO are mediated by a specific transmembrane EPO receptor (EPO-R) present in the kidney and erythroid precursors [4]. Expression of EPO-R was observed in various non haematopoietic cells such as neuronal cells [5], vascular smooth muscle cells, endothelial cells, cardiac myocytes, megakaryocytes, skeletal myoblasts [6] and nephrons [7]. EPO was observed to interact with 2 different receptors. The classic EPO receptor is responsible for the red blood cell response, whereas the interaction with the β - receptor is responsible for the cardiac tissue protective effects [8].

Recent studies have identified multiple paracrine and autocrine functions of EPO such as coordinating local responses to injury, maintaining vascular autoregulation, stimulating endothelial progenitor cells (EPCs) formation and mobilization, enhancing new vessel formation and

attenuating both primary (apoptotic) and secondary (inflammatory) causes of cell death [9] so multiple researches are now focusing on the non-hematopoietic effects of erythropoietin and its potential use against tissue ischemia and its role of in tissue repair and regeneration after brain and spinal cord injuries [10].

Cardiovascular disease is fast becoming the number one health concern worldwide. The mortality and morbidity from ischemic cardiovascular diseases remain the greatest unsolved public health challenge throughout the industrialized world due to their high mortality rate. Although the technical advances of thrombolysis and angioplasty have resulted in a remarkable increase over the last 20 years in both the short and long term survival of patients reaching advanced medical care but a significant percentage of surviving patients remain severely disabled. Novel approaches that protect against injured heart tissue would constitute important advances in the therapy of this disease [9, 11].

Materials and Methods:

I-Chemicals used:

1. Recombinant human erythropoietin: Provided in vials (Erypoietin). Each vial containing 4000 I.U/ml. (*Sigma CO., USA*).
2. DL-Isoproterenol hydrochloride: provided as white powder (*Sigma CO., USA*). It was dissolved in distilled water before use.
3. Urethane: Provided as white powder (*Sigma Co., U.S.A.*). It was dissolved in saline before use.
4. Triphenyl tetrazolium chloride: Provided as white powder (by MP biomedical, France).
5. Creatine phosphokinase (CPK) kits (*Spinreact Co., Spain*).
6. Lactate dehydrogenase (LDH) kits (*Spinreact Co., Spain*).
7. Plasma Nitric Oxide commercial kit (*Roche Co., Germany*).

II- Animals used:

Experimental protocol for the study was approved by the ethics committee on animal experiments in Benha University. Twenty eight adult male albino rats weighting 200 – 220 gm. averaging 18 weeks old were brought from Experimental Animal Breeding Farm, Helwan – Cairo to be

used in this study. They were housed in cages (7 rats/cage) under standard laboratory conditions (12h light/dark cycle, 20 – 25 °C, relative humidity 55%). The animals were given commercial standard caloric diet and tap water ad libitum. All animals received human care according to the criteria outlined in the “Guide for the Care and Use of Laboratory Animals” prepared by the National Academy of Sciences. After acclimatization for 1 week, the rats were randomly classified into 4 equal experimental groups: **Group I (Control group):** Rats of this group received no medication and given free access to food and water. **Group II (ISO group):** In which, rats were injected by a single dose of isoprotrenol (75 mg/kg body weight) i.p. for induction of myocardial infarction. **Group III (ISO + rhEPO):** In which, rats were injected by a single dose of rhEPO (5000 I.U/kg body weight) i.p., 2 hours after induction of myocardial infarction with isoproterenol. **Group IV (rhEPO for 10 days + ISO):** Rats of this group were injected by rhEPO i.p. in a dose of 5000 I.U/kg body weight/day for 10 days before induction of myocardial infarction with isoprotrenol.

IV-Procedure of the experiment:

No rats were died during the experimental periods. Three hours after induction of myocardial infarction by isoprotrenol, the rats were anaesthized by urethane and a longitudinal incision in the anterior aspect of the neck was done for measurement of arterial blood pressure then the chest was opened and intracardiac blood samples was collected and Hct value was determined. The plasma was taken for measurement of plasma nitric oxide (NO) and cardiac enzymes (CPK and LDH). Then the still beating heart was excised for measurement of the infarction size.

1-Measurement of the mean systemic arterial blood pressure:

The rats were anesthetized by urethane in a dose 1.25g/kg body weight dissolved in saline, half the dose was injected i.p. for a rapid action and the other half was injected S.C for a slow sustained action [12] then a longitudinal incision was done in the anterior aspect of the neck through which the trachea was detected and elevated by a lever for exploration of carotid artery in which the carotid cannula connected to the oscillograph (washing Ton, 400 MD4c oscillograph) was inserted for measurement of both systolic and diastolic arterial blood pressure [13] then mean systemic arterial blood pressure was calculated.

2-Measurement of Hematocrite value:

Blood sample was collected from the right ventricle and rapidly placed in wintrobe tube to be centrifugated at 5000 rpm for 15 minutes. The R.B.Cs will be separated at the bottom of the tube leaving clear plasma above. Hct value was calculated by measuring the percentage ratio of R.B.Cs column on the gradation to the total blood column [14].

3-Measurement of plasma cardiac enzymes and Nitric oxide:

The clear supernatant was used for the estimation of the following biochemical parameters; plasma CPK [15], LDH [16] and NO using standard commercial kits [17].

4-Measurement of the infarction size:

After withdrawal of intracardiac blood sample, the 6th costal cartilage is transected with fine pointed scissors [18]. The excised beating heart was submerged in cold (8°C) 30 mmol KCl to achieve diastolic arrest. The right ventricle and both atria were excised to isolate the left ventricle (the septum and free wall) which is then sectioned by a sharp surgical blade into transverse slices, each of about 1.5mm thick which was then submerged in a 1.5% triphenyl tetrazolium chloride (TTC) stain in phosphate buffer, PH 7.4, for 10-15 minutes at 37°C. This stain form red colour precipitates in the presence of intact dehydrogenase enzyme system thus the necrosed areas fail to stain [19]. The slices were washed with saline and then clear glass plates were placed over both sides of each slice. Epicardial and endocardial outlines as well as the TTC stained and non- stained areas were traced on clear plastic sheets. The plastic sheet was then fixed on an E.C.G paper and the small squares occupying the stained and non-stained areas were counted giving each in mm². The sum of the stained and non-stained areas give the surface area of the whole heart slices and the infraction size was calculated as percentage of the sum of infract areas to the sum of surface areas of all the slices [20].

Results:

Table (1): Shows the values of infarction size (% LV), plasma CPK (U/L), plasma LDH (U/L), plasma NO ($\mu\text{mol/ml}$), MSABP (mmHg) and hematocrite value (%) as mean \pm standard deviation. Then P values are calculated using Student t- test.

	Group I (Control)	Group II (ISO)	Group III (ISO + rhEPO Single dose)	Group IV (rhEPO 10 days + ISO)
Infarction size	0	49.71 \pm 5.47	16.29 \pm 3.77	14.57 \pm 3.21
Plasma CPK	107.71 \pm 4.64	437.43 \pm 8.87	142.14 \pm 3.24	125.71 \pm 8.79
Plasma LDH	148.57 \pm 3.99	524.71 \pm 26.98	155.43 \pm 4.24	149.14 \pm 6.23
Plasma NO	29.43 \pm 5.74	9.86 \pm 3.98	46.14 \pm 7.34	11.14 \pm 2.41
MSABP	95.86 \pm 4.22	91 \pm 3.74	91.14 \pm 5.01	117.29 \pm 10.03
Hct	47.43 \pm 3.35	48.57 \pm 2.88	49.71 \pm 2.82	76.29 \pm 7.95

Table (1) & Fig. (1): Show that isoproterenol injection significantly increases the infarction size from 0 in control group to 49.71 \pm 5.47 in ISO group (group II) ($P < 0.001$). Injection with a single dose of rhEPO (5000 U/kg body weight), 2 hours after induction of myocardial infarction significantly decrease it from 49.71 \pm 5.47 in ISO group to 16.29 \pm 3.77 in group III ($P < 0.001$). Also daily injection with rhEPO (5000 U/kg body weight/day for 10 days before induction of myocardial infarction) significantly decreases it from 49.71 \pm 5.47 in ISO group to 14.57 \pm 3.21 in group IV ($P < 0.001$) but there was non-significant change in infarction size ($P > 0.05$) in rats injected daily with rhEPO for 10 days before induction of myocardial infarction (group IV) when compared with rats injected with a single dose of rhEPO, 2 hours after induction of myocardial infarction (group III).

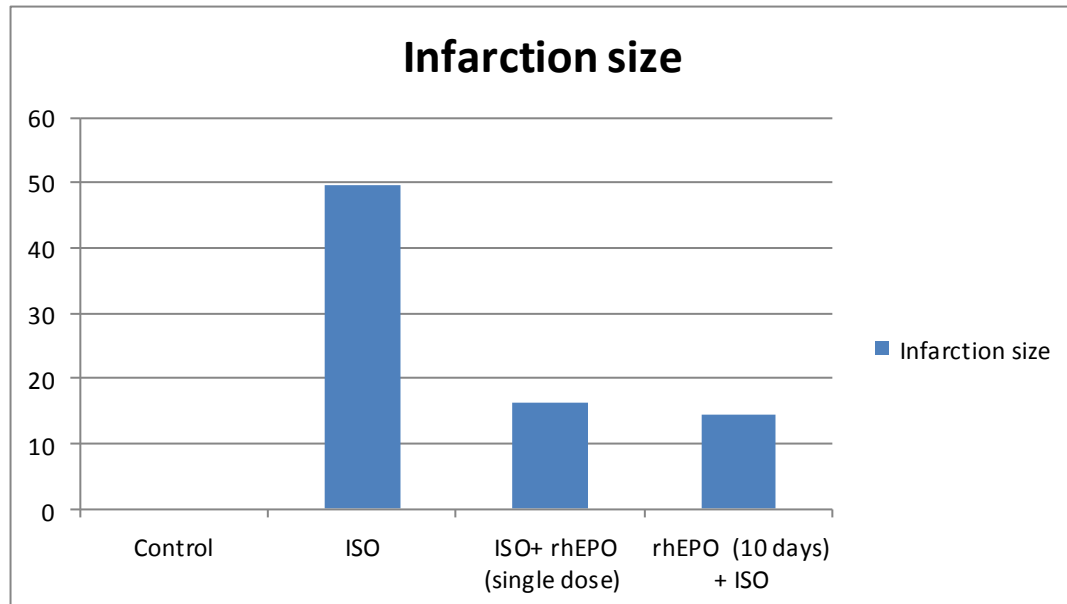


Fig. (1)

Table (1) and Fig. (2 & 3): Show that isoproterenol injection significantly increases the cardiac enzymes (CPK and LDH) from 107.71 ± 4.64 and 148.57 ± 3.99 in control group to 437.43 ± 8.87 and 524.71 ± 26.98 respectively in ISO group ($P < 0.001$). Injection with a single dose of rhEPO 2 hours after induction of myocardial infarction significantly decrease them from 437.43 ± 8.87 and 524.71 ± 26.98 in ISO group to 142.14 ± 3.24 and 155.43 ± 4.24 respectively in group III ($P < 0.001$). Also daily injection with rhEPO for 10 days before induction of myocardial infarction significantly decrease them from 437.43 ± 8.87 and 524.71 ± 26.98 in ISO group to 125.71 ± 8.79 and 149.14 ± 6.23 in group IV ($P < 0.001$). There was significant decrease in plasma CPK and LDH in rats injected daily with rhEPO for 10 days before induction of myocardial infarction (group IV) when compared with rats injected with a single dose of rhEPO, 2 hours after induction of myocardial infarction (group III) as they were decreased from 142.14 ± 3.24 and 155.43 ± 4.24 to 125.71 ± 8.79 and 149.14 ± 6.2 ($P < 0.001$ and < 0.05) respectively.

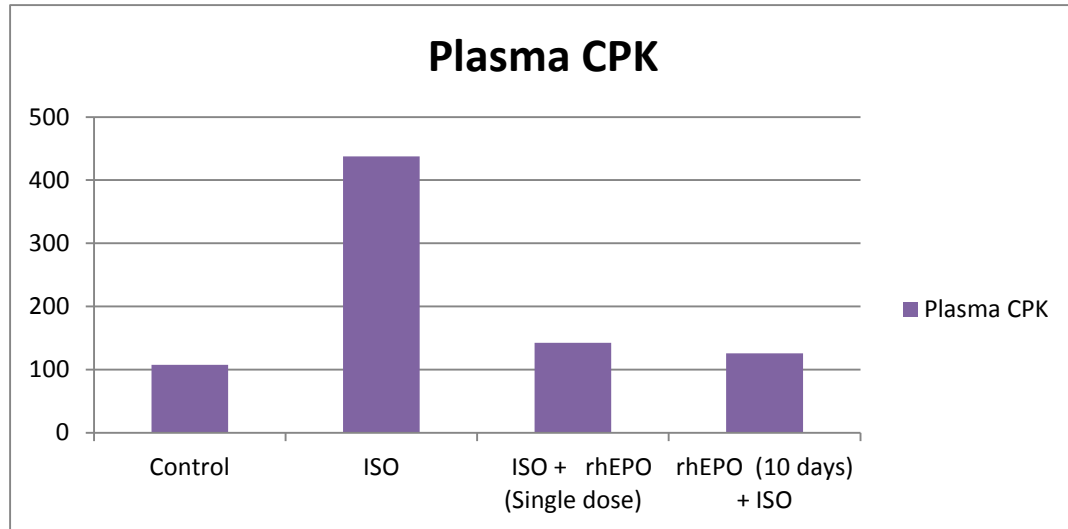


Fig. (2)

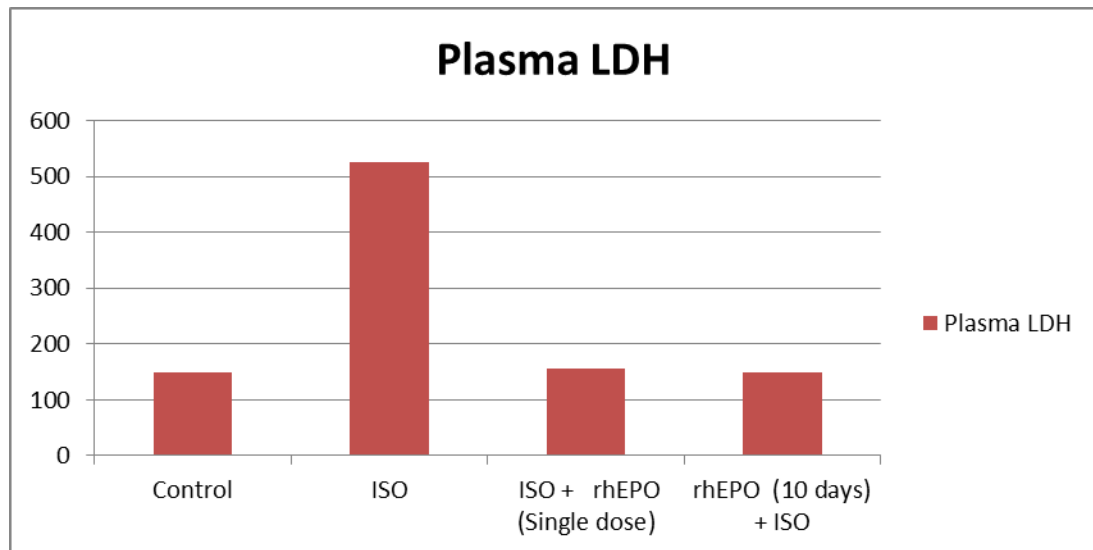


Fig. (3)

Table (1) & Fig. (4): Show that isoproterenol injection significantly decreases plasma nitric oxide (NO) from 29.43 ± 5.74 in control group to 9.86 ± 3.98 in ISO group ($P < 0.001$). Injection with a single dose of rhEPO, 2 hours after induction of myocardial infarction significantly increases it from 9.86 ± 3.98 in ISO group to 46.14 ± 7.34 in group III ($P < 0.001$) while daily injection with rhEPO for 10 days before induction of myocardial infarction causes non-significant change in plasma NO when compared with ISO group as it was increased from 9.86 ± 3.98 in ISO group to 11.14 ± 2.41 in group IV ($P > 0.05$). There was significant decrease in plasma NO in

rats injected with rhEPO for 10 days before induction of myocardial infarction (group IV) when compared with rats injected with a single dose of rhEPO, 2 hours after induction of myocardial infarction (group III) as it was decreased from 46.14 ± 7.34 in group III to 11.14 ± 2.41 in group IV ($P < 0.001$).

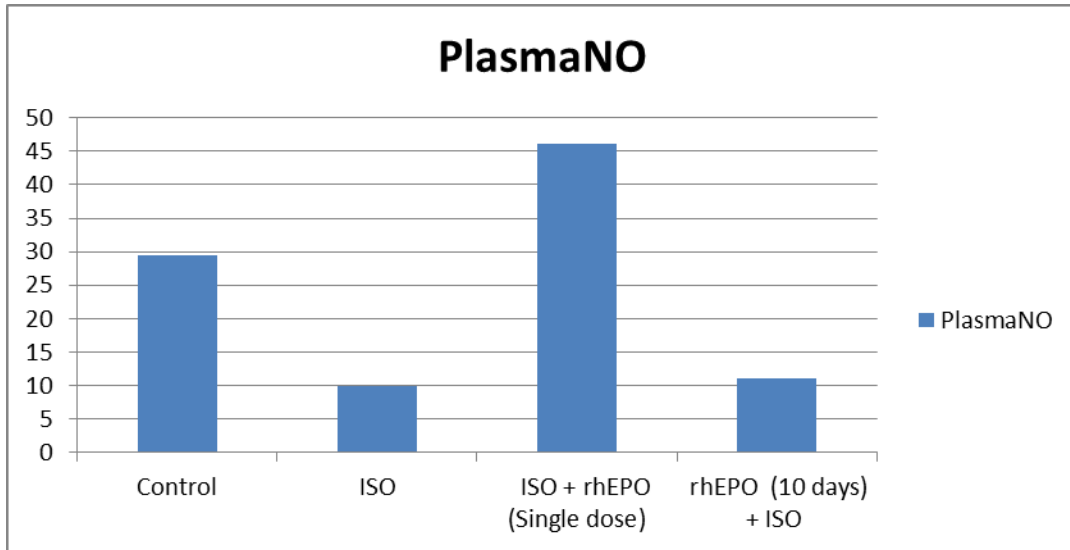


Fig. (4)

Table (1) & Fig. (5): Show that isoproterenol injection significantly decreases the mean systemic arterial blood pressure (MSABP) from 95.86 ± 4.22 in control group to 91 ± 3.74 in ISO group ($P < 0.05$). Injection with a single dose of rhEPO, 2 hours after induction of myocardial infarction caused non-significant change in MSABP as it was changed from 91 ± 3.74 in ISO group to 91.14 ± 5.01 in group III ($P > 0.05$) while daily injection with rhEPO for 10 days before induction of myocardial infarction caused significant increase in MSABP from 91 ± 3.74 in ISO group to 117.29 ± 10.03 in group IV ($P < 0.001$). Also there was significant increase in the MSABP in rats injected daily with rhEPO for 10 days before induction of myocardial infarction (group IV) when compared with rats injected with a single dose of rhEPO, 2 hours after induction of myocardial infarction (group III) as it was increased from 91.14 ± 5.0 in group III to 117.29 ± 10.03 in group IV ($P < 0.001$).

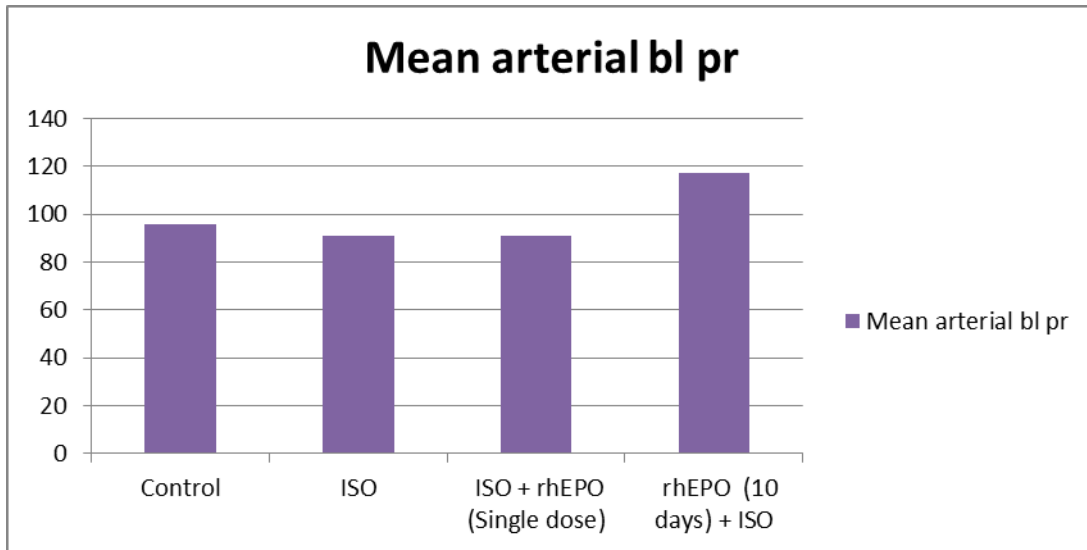


Fig. (5)

Table (1) & Fig. (6): Show that isoproterenol injection caused non-significant change in hematocrit (Hct) value as it was changed from 47.43 ± 3.35 in control group to 48.57 ± 2.88 in ISO group ($P > 0.05$). Injection with a single dose of rhEPO, 2 hours after induction of myocardial infarction had no significant change on Hct value as it was changed from 48.57 ± 2.88 in ISO group to 49.71 ± 2.82 in group III ($P > 0.05$) while daily injection with rhEPO for 10 days before induction of myocardial infarction caused significant increase in Hct value when compared with ISO group as it was increased from 48.57 ± 2.88 in ISO group to 76.29 ± 7.95 in group IV ($P < 0.001$). There was significant increase in Hct value in rats injected daily with rhEPO for 10 days before induction of myocardial infarction (group IV) when compared with rats injected with a single dose of rhEPO, 2 hours after induction of myocardial infarction (group III) as it was increased from 49.71 ± 2.82 in group III to 76.29 ± 7.95 in group IV ($P < 0.001$).

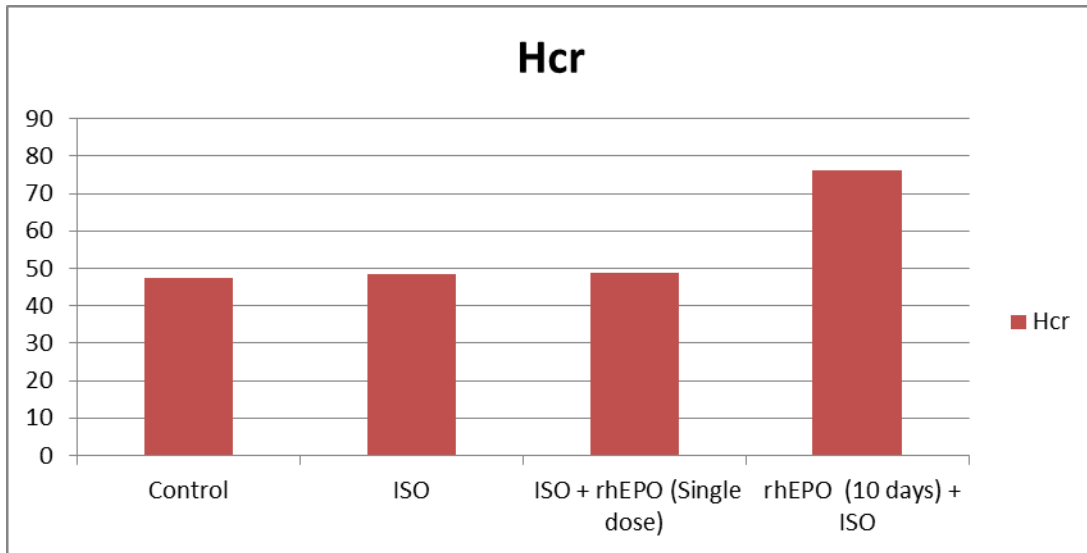


Fig. (6)

Discussion:

Myocardial infarction (MI) continues to be a major public health problem and the leading cause of morbidity and mortality worldwide [21]. The hormone erythropoietin (EPO), produced by adult kidney and the fetal liver, is well known in regulating mammalian erythropoiesis. Exogenous EPO, the recombinant human EPO (rhEPO), was introduced approximately two decades ago for the treatment of anemia resulting from variety of conditions such as chronic renal failure and chemotherapy. However during the last decade, EPO and its receptor (EPOR) were found to present outside the liver and the kidney as in the brain and heart. At the same time, several experimental studies using rhEPO have shown its potential neuroprotective and cardioprotective role against ischemia occurring independently on its hematopoietic action [22].

So we aimed in our study to find out the efficacy of treatment with rhEPO against isoproterenol induced myocardial infarction, its possible mechanism of action and the dependence of this efficacy and safety on the time and duration of treatment. This was assessed by measuring plasma levels of cardiac enzymes (CPK and LDH) and Nitric oxide, infarction size, mean systemic arterial blood pressure and hematocrit value.

Our study revealed that isoproterenol, a non-selective β -adrenergic agonist induces myocardial infarction in rats which was confirmed by significant increased myocardial infarction size (% LV) and elevated plasma

levels of cardiac enzymes (CPK and LDH). This can be explained by its effect on increasing intracellular Ca^{++} and cAMP, increasing heart rate and cardiac contractility [23, 24]. Isoprotrenol injection also causes significant decrease in plasma Nitric oxide and mean systemic arterial blood pressure and non-significant change in hematocrite value. Our results was in agreement of other results which showed that isoproterenol injection caused unchanged or slightly increased systolic blood pressure, decreased diastolic blood pressure but the net result is decreasing the mean systemic arterial blood pressure through lowering the peripheral vascular resistance, enhances free radical formation and endothelial dysfunction and down-regulates the expression of endothelial nitric oxide synthase enzyme [25, 26]. In contrast to our results, isoproterenol injection increased plasma nitric oxide by increasing cardiac nitrite content and this might be the cause of decreased mean systemic arterial blood pressure [25, 27]. It also caused significant increase in RBCs packed cell volume [28].

In our study, Injection with a single dose of rhEPO, 2 hours after induction of myocardial infarction caused significant decrease in infarction size and plasma level of cardiac enzymes, significant increase in plasma NO but non-significant change in mean systemic arterial blood pressure and Hct value was noticed. On injecting the rats with a daily dose of rhEPO for 10 days before induction of myocardial infarction, a significant decrease in infarction size and plasma level of cardiac enzymes, significant increase in mean systemic arterial blood pressure and hematocrite value but non-significant change in plasma NO were noticed.

When we compared the results of the rats injected daily with rhEPO for 10 days before induction of infarction with those which were injected by a single dose of rhEPO, 2 hours after induction of infarction, there was significant decrease in cardiac enzyme, plasma NO, Significant increase in MSABP, Hct value and non- significant change in the infarction size.

In agreement with our results, EPO treatment lead to reduction in the infarction size and promote endothelial progenitor cell growth which increases angiogenesis whether it is injected before ischemia, during ischemia or during reperfusion [29, 30, 31]. However, its beneficial effect when given after reperfusion were better than when given before or during ischemia [31]. Exogenous rhEPO administration stimulates vascular NO production either directly by increasing the activity of nitric oxide synthase enzyme [32, 33, 34, 35] or indirectly through increased shear stress in

endothelial cells [35]. This may explain the cardioprotective role of EPO in myocardial infarction via increasing vasodilatation and facilitating effective collateral circulation [36, 37]. Epo administration in patients with an acute coronary syndrome is safe and feasible [31]. However, the main side effect and limiting factor of high and prolonged dose of rhEPO therapy resulted from increased hematocrit associated side effects such as increased blood viscosity, systemic hypertension, down regulation of nitric oxide synthesis and impaired tissue blood flow that results in thromboembolism [38, 39, 40].

In contrast to our results, performed trials showed that although short term treatment with EPO in AMI is safe, but it seems to have no clinical benefit concerning improvement in myocardial function or reduction in infarct size [41]. While other results showed that increase hematocrit which resulted from prolonged injection of 5.000 I.U/kg body weight of rhEPO associated is not harmful but it might improve cardiac function by improving delivery of oxygen to a hypoxic myocardium and that its protective effect against ischemic damage of myocardium is not associated with NO activation or NO mediated hemodynamic responses [42].

Conclusion:

Administration of EPO was not only beneficial when it is used in a single dose after induction of myocardial infarction but also when used as pretreatment before induction of myocardial infarction. However, Enhancement of erythropoietin cardiac tissue protective activity without increasing the number of red blood cells is the key in the management of heart disorders.

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