Evaluation of a single bolus of erythropoietin effects on reducing ischemia-reperfusion injuries during coronary artery bypass graft surgery. A randomized, double blinded placebo control study.

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Abstract: Introduction: erythropoietin (EPO) is known as a regulating hormone for production of red blood cells called Erythropoiesis. Some studies have shown that erythropoietin have some non-hematopoietic protective effects on ischemia-reperfusion injury in myocytes. We evaluated the effect of EPO infusion on reducing ischemia-reperfusion injuries and improvement of cardiac function by echocardiography shortly after coronary artery bypass graft surgery. **Material and methods:** 43 patients were joined the study and randomly divided in two groups, EPO group: receiving standard medication and CABG surgery plus 700 IU/kg erythropoietin and control group: receiving standard medication and CABG surgery plus 10cc normal saline as placebo. The cardiac functions were assessed by Echocardiography at before, 4 days after and also 30 days after CABG operation. **Results:** Echocardiography indicated that EF had no differences between EPO and control group at 4 days (47.05±6.29 vs 45.90±4.97, P=0.334) or 30 days after surgery (47.27±28 vs 46.62±5.7, P=0.69). There were no differences between EPO and control group in wall motion score index at 4 days (P=0.83) or 30 days after surgery (P=0.902). In EPO group: Left ventricle end systolic and diastolic diameter (LVESD, LVEDD) had reduction, as compared to control group. **Conclusion:** we suggest that peri-operatively exogenous EPO infusion can't improveventricular function and Wall motion index in first weeks after surgery. But as compared to control group, reduction in LVEDD and LVESD at 4 days or 30 days after CABG surgery in EPO group suggested that EPO had correlation with reduction of myocytes remodeling and reperfusion injury early after CABG surgery.
[Shervin Ziabakhsh-Tabary, Mohammad Reza Habibi, Rozita Jalalian, Farzad Mokhtari-Esbuie. **Evaluation of a single bolus of erythropoietin effects on reducing ischemia-reperfusion injuries during coronary artery bypass graft surgery. A randomized, double blinded placebo control study.** *Biomedicine and Nursing* 2016;2(2): 96-101]. ISSN 2379-8211 (print); ISSN 2379-8203 (online). http://www.nbmedicine.org. 16. doi:10.7537/marsbnj02021616

Keywords: erythropoietin, ischemia, reperfusion injury, coronary artery bypass graft

Introduction:

Erythropoietin (EPO) is a glycoprotein hormone that producing by kidney and has main role in hematopoiesis (J.W Adamson, et al. 2008). Besides these hematopoietic effects, erythropoietin has non-hematopoietic effects on some tissues like brain (Brines ML, et al. 2000), kidney (Vesey DA, et al. 2004), retina (Junk AK, et al. 2002) and muscles (Ogilvie M, et al. 2000) and this is noticeable that both ventricular myocytes and endothelial cells have erythropoietin receptors (Westenbrink, et al. 2008). Erythropoietin protective effects on myocardial cells are performed by some different pathways such as: stimulation of neovascularization, activation of PI3K and 2.1 ERK pathways (Mudalagiri NR, et al. 2008 and R Schoemaker, et al. 2006) and Endothelial progenitor cells (EPCs) synthesis stimulation from bone marrow (Westenbrink BD, et al. 2007 and 2008).

Coronary artery bypass graft (CABG) causes increase myocardial perfusion and Ejection fraction in patients with coronary artery diseases (Elhendy A, et al. 2000) and it became an important treatment modality in ischemic patients. Although the rapid reperfusion by CABG had significant success and it caused decrease mortality and morbidity (David A, et al. 2008) but this reperfusion paradoxically can cause ionic and metabolic damage that lead to myocardial damage and myocytes death (Yellon DM, et al. 1999). Therefore new treatments should focus on decreasing damage after reperfusion.

Besides the protective effect of Erythropoietin on myocardial ischemia, studies on animal models showed that erythropoietin also can reduce reperfusion

tissue injury. (Doue T, et al. 2008 and Lipsic E, et al. 2004 and Leila Javadi, et al. 2010) but studies on human models showed some controversy (Lipsic E, et al. 2006 and Mocini D, et al. 2008). One human model study showed erythropoietin protective effect against hypoxia/reoxygenation injury (Yellon DM, et al. 1999) but Mocini in a different model that performed on patients who had been undergone CABG, showed that erythropoietin had no association with reduction of myocardial biomarker: Troponin I and CKMB levels after CABG surgery (Mocini D, et al. 2008). To justify this result they explained: Erythropoietin induces tissue protection with anti-apoptotic mechanism but they assessed the effects of EPO by two indicator of necrosis (Troponin I and CKMB).

Left ventricular function has usually been described in term of the ejection fraction (EF) (Taylor GJ, et al. 1980). By considering the controversy of these studies and importance of injury after ischemia and reperfusion in coronary artery bypass graft surgery, we designed a double-blinded case-control study by assessment of echocardiography parameters before and after CAGB operation to evaluate erythropoietin protective effects on reperfusion injury after CABG.

Material and methods:

Studypopulation:

This is a randomized, double blinded, clinical trial study that Study population was consisted of all patients that were referred to Fatemeh Zahra hospital (in Sari, Iran) for elective CABG. 43 patients who had inclusion criteria and passed the exclusion filter were divided into two groups randomly. Patients in erythropoietin group were treated by common medical therapies and CABG plus 700IU/kg erythropoietin (PD Poietin, puyeshdaroo, Iran), intravenously infusion, exactly 5 min after termination of cross clamp: at the start of reperfusion and patients in control group were treated by common medical therapies and CABG surgery plus 10cc normal saline as placebo.The study method was approved by ethical committee of our organization and written informed agreement was taken from all patients.

Inclusion criteria:

- Revascularization requirement according to angiographic evidences.

Exclusion criteria:

- history of MI in recent 3 months
- Previous myocardial trauma or major surgery in recent 3 months
- EF<30%
- Cr>2.5
- receive streptokinase or previous reperfusion treatments
- erythropoietin intake in recent 6 months
- polycythemia

Study design:

Transthoracic echocardiography(using vivid S5 echocardiograph) with simpson method andalsodoppler echocardiography were performed in all patients before, 4 days and 30 days after CABG operation.Regional wall motion was evaluate by 16-segment model recommended by American society of echocardiography.Other variables that were measured included age, gender, BMI, blood pressure, cholesterol, BUN, Cr, BS, Hgb, Hct, plt, Retic, Na, K, (by pars test kits) ejection fraction and cross clamping time.

Statisticalanalysis:

Group differences for continuous variables were examined by T-test. The data distributions were checked with Kolmogorov-Smirnov test. Mann-Whitney test was performed for data that don't follow normal distribution. In the case of categorical variables, group differences were examined by the $\chi 2$ test. Results were considered statistically significant when the variability level was < 0.05. Statistical analysis was performed with SPSS software (version 16) to. We used SPSS (version 16) to data analysis.

Results:

There were no differencesbetween EPO and control group in the number of impaired vessels (2.27±0.787 vs 2.29±0.784, P=0.863)andage(59.73 ±7.73 vs 62.57 ±8.6, P=1.878). Other patient's information is shown in table 1.

As shown in table2, there were no significantly differences between EPO and control group in EF value at 4 days after surgery (47.05±6.29 vs 45.90±4.97, P=0.334) and also 30 days after surgery (47.27±28 vs 46.62±5.7, P=0.69).

Mean level of Wall motion score index (WMSI)Also had no differences between EPO and control group at 4 days after surgery (1.08±0.09 vs 1.07±0.10, P=0.83) and also at 30 days after surgery (1.10±0.13 vs 1.10±0.16, P= 0.902) (figure1). Mean level of left ventricle end diastolic diameter (LVEDD) and left ventricle end diastolic diameter (LVESD)are shown in table3.

Discussion:

The early postoperative period could be considered suboptimal for assessment of ventricular function due to perioperative ischemia and superimposed reperfusion injury with a possible prolonged negative effect on contractile function (Søraas CL, et al. 2011) and Present study evaluated the effect of single bolus of erythropoietin, at the start of reperfusion after myocardial ischemia during CABG surgery.

Left ventricular function has usually been described in term of the ejection fraction (EF) (Taylor GJ, et al. 1980). In present study there were no significantly differences between EPO and control group in EF value at 4 days and also 30 days after surgery, it mean that EPO had no effect on improving ventricular function in first 4 weeks after CABG surgery.

It's not clear whether EF is the most meaningful index of left ventricle function in ischemic and infarct situation. Low EF may be caused by poor contractile function due to extensive myocardial damages or continuing ischemia. Thus some study told that end-systolic volume or end-diastolic volume might be better predictor of prognosis than EF (White HD, et al.1987).In this study as compared to control group, EPO was correlated with slightly reduction in LVEDD and LVESD at 4days after surgery and also 30 days after surgery from baseline, although it was not significant, and it means that EPO infusion can reduce reperfusion injuries, myocytes remodeling and improves prognosis in ischemic situation like CABG surgery.

Our result showed that as Compared to control group, EPO did not effect on reduction of WMSI at 4days and also 30 days after surgery. WMSI indicates ventricular septum dysfunction and Echocardiographic determination of wall motion is a useful tool to observe LV function (White HD, et al. 1987).In this study WMSI had no differences in two groups; this result means that administration of erythropoietin during CABG had not effects on reduction of remodeling and stunning of ventricular septum at4 days and 30 daysafter surgery and maybe long term evaluation of EPO effectiveness would be different. WMSI, LVESD and LVEDD in previous study did not assessed to evaluate protection effects of EPO against ischemia-reperfusion injuries postoperatively and our result is consequential. previous study like Mocini's study (Mocini D, et al. 2008), evaluated EPO efficacy by measuring Troponin I and CKMB levels.

Time needed for LV function improvement depends on level of degeneration and connective tissue proliferation (Elsässer A, et al. 1997) and the time course and degree of improvement of function recovery shortly after revascularization is differ. In this study we examine the effect of EPO in first 4weeks after CABG procedure.Some studies have found no change or deterioration in segmental wall motion with in the first week postoperatively (Shepherd RL, et al. 1974 and Awan MA, et al. 2007) but other studies elucidates improves myocardial contractibility within first days postoperatively (White HD, et al. 1987), already intra-operatively or within first weeks postoperatively (Lorusso R, et al. 2001 and Knapp M, et al. 2007). Maybe we need further and long-term follow up in these patients to determine whether EPO has efficacy in WMSI changes after CABG.

The next point is that most of our patients had EF>30% and just 6patients had EF<30% and maybe efficacy of EPO on ventricular function in patient with lower EF would be more than well patients. We suggest for future study to conclude patients with lower EF to examine effect of EPO on this patients.

In recent studies effective dosage of erythropoietin to decrease damages of ischemia-reperfusion is argued. In animal experimental models there been used higher dose than human models. Such study of L. Javadi (2008), which in that study EPO with dosage of 5000 IU/kg was used and as a result: they explained that erythropoietin can reduce infarc area and minimize cell damage and reduce myocytes apoptosis tissue damage and it controlled the apoptosis of myocytes. In E lipsic study (2004), also the same dosage was used and results were similar, but in human experiments, dosage was used lesser than animal models, and maybe this caused more consent results in animal models.In a case-control study (Mocini D, et al. 2008), Mocini used 40000 IU of erythropoietin and as a results there were no differences in troponin I and CKMB levels in both EPO and control groups. They mentioned that maybe this result correlated with EPO dosage. In present study we used 700IU/kg PD poietin that was estimatly as same as EPO dosage in Mocini's study.

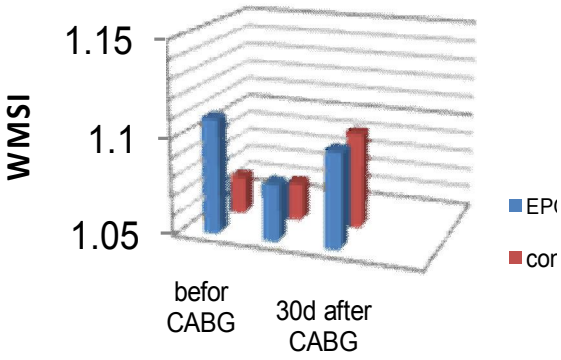

Figure 1. Wall motion score index at before and after CABG surgery in both groups.

Best time for EPO infusion is not clear. In some studies erythropoietin was infused 24 hours before ischemia and reperfusion (Leila Javadi, et al. 2010 and Lipsic E, et al.2006). Based on Lipsic study in 2004 that they measured effectiveness of erythropoietin by apoptosis rate and percentage of active Caspase-3 enzyme, time of EPO prescription was evaluated and the best time was after the onset of reperfusion after

ischemia during surgery. In D Mocini study (Mocini D, et al. 2008) erythropoietin was injected in the immediate pre-surgical period. In our present study, we used erythropoietin at the start of tissue reperfusion after aorta clamping. So the best time for EPO prescription in human experiments is still unknown. Maybe controversies about effectiveness of erythropoietin resulted in this study depended on time of infusion and perhaps there will be better results if we inject EPO 24 hours before CABG.

A small study group was our limiting factor in this study and further study with more cases may clear out the controversies.

Table 1. Primary characteristic of patients

	EPO group	Control group	P
Number	22	21	
Gender	13	8	0.1
Age(year)	59.73±7.73	62.57±8.60	1.878
Smoking	14	13	0.4
Diabetic history	8	10	0.9
BMI(kg/m2)	25.82±1.83	24.36±2.12	0.009
Creatinine (mg/dl)	0.94±0.18	0.86±0.27	0.149
Impaired vessels(n)	2.27±0.787	2.29±0.784	0.863
EF before operation(n)	46.36±8.04	45.90±8.41	0.178
Hgb(g/dl)	12.64±2.10	13±1.20	0.955
Retic(%)	0.85±0.33	0.61±0.25	0.166
Na(mEq/L)	141.36±1.98	140.95±4.11	0.67
K(mEq/L)	4.20±0.35	4.45±0.44	0.594
FBS(mg/dl)	132.68±55.19	158.95±79.98	0.132
pack cell(n)	1±1.06	0.48±0.68	0.021
Graft(n)	3.14±0.88	3.38±0.74	0.33
pomp time(min)	78.21± 18.8	79.76±12.25	0.6
cross clamping time(min)	50.95±10.85	53.86±9.13	0.08

EPO: erythropoietin, BMI: body mass index, EF: ejection fraction

Table 2. Patient's EF before and after CABG in both groups

	EPO group	Control group	p
EF Before surgery	46.36±8.04	45.90±6.42	0.178
EF 4 days after surgery	47.05±6.29	45.90±4.97	0.334
EF 30 days after surgery	47.27±28	46.62±5.7	0.69

EF: ejection fraction, EPO: erythropoietin, CABG: Coronary artery bypass graft.

Table 3. Patients' echocardiography parameters in both groups.

	EPO group	Control group	p
WMSI before CABG	1.11±0.12	1.07±0.10	0.15
WMSI 4d after CABG	1.08±0.09	1.07±0.10	0.83
WMSI 30d after CABG	1.10±0.13	1.10±0.16	0.902
LVEDD before CABG	5.09± 0.70	4.68±0.94	0.314
LVEDD 4d after CABG	4.86±0.74	4.73±0.59	0.436
LVEDD 30d after CABG	4.95±0.68	4.79±0.61	0.434
LVESD before CABG	3.72±0.79	3.63±0.84	0.825
LVESD 4d after CABG	3.53±0.75	3.67±0.54	0.230
LVESD 30d after CABG	3.55±0.71	3.77±0.77	0.876

EPO: erythropoietin, 4d: 4 days, LVEDD: left ventricle end diastolic diameter, LVESD:left ventricle end systolic diameter.

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Conclusion:

Our data suggest that peri-operatively exogenous EPO infusion can't improve ventricular function and Wall motion index in first weeks after surgery. But as compared to control group, reduction of LVEDD and LVESD levels at 4 days and also 30 days after CABG in EPO group suggested that EPO had correlation with reduction in myocytes remodeling and reperfusion injury early after CABG surgery.

Suggestion: we need more long term evaluation to specify that erythropoietin prescription during surgery can lead to increased survival rate and LV function. By considering the result of this study, it is recommended to design next studies with more cases. We suggest for future study to conclude patients with lower EF to examine effect of EPO on this patients.

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6/25/2016