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Effect of Citrate Phosphate Dextrose Solution on Reperfusion Injury in Coronary Artery Bypass Surgical Patients Undergoing Cardiopulmonary Bypass

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ABSTRACT

Introduction: Reperfusion injury is one of the most common phenomena associated with coronary artery bypass graft (CABG) .The mechanism of ischemia and reperfusion injury is not known precisely, but may be free radicals and other activated oxygen metabolites have an important role in tissue damage following reperfusion injury. This study was to evaluation of citrate solution effects on oxidative stress and cardiac function and Cardiac enzymes in patient's candidate to CABG. ***Methods:*** In Double blind clinical trial study in Tabriz University of medical science, 50 patients candidate to CABG randomly divided in two groups and matched together according to sex, age and NYHA class. In intervention group after surgery and before the opening of the aortic clamping solution warm blood containing citrate phosphate dextrose (CPD; 3cc/100cc), value (100cc/min/m2BSA) for three minutes was administered. In control group, only pure blood administered. Oxidative stress markers measured in five stages and cardiac enzymes measured in three stages of surgery. ***Results:*** Mean age 62.3±9.1 years including 30(60%) men and 20(40%) women. Ejection fractions between two groups were not significant before and after treatment. Administration of CPD was not significant effects on cardiac enzyme. Measurement of oxidative stress in different time were not different in Malonil Di Aldehid, superoxide dismutase and GPx but total antioxidant status were improved after intervention in compared with control group ($p<0.001$).***Conclusion:*** Results showed that CPD were positive effects of increasing in total antioxidant status after CABG. but in reduction of other oxidative markers were unlabeled.

Introduction

Coronary artery disease is one of the leading causes of death in the world so that 8. 3 million men and 4.3 million women die each year due to coronary artery disease.[1] Bypass is one of the most essential parts of cardiopulmonary surgery on the heart, which can have harmful effects and may cause various tissue damages. Although using cardiopulmonary bypass procedure is routinely performed without any particular problem in patients undergoing cardiac surgery, we see some of the problems caused by ischemia and reperfusion injury in many organs such as renal, pulmonary, cardiovascular system and central nervous system. Besides the damage caused by cardiopulmonary bypass, reperfusion injury after a period of ischemia can lead to severe tissue damage ,that is defined as the phenomenon under reperfusion injury. It can be affected paradoxically our treatment methods also can worsen clinical outcomes for patients.[2,3]

The mechanism of ischemia and reperfusion injury is not known precisely, but several studies have suggested the theory that free radicals and other activated oxygen metabolites (ROS) are involved in many human diseases .Reperfusion injury after ischemia is the classic example. Recent studies have emphasized the role of oxygen free radicals and oxidative stress in the damage caused by ischemia / reperfusion.[4] Experimental studies by Zweier *et al.* indicated the production of reactive oxygen and free radicals during ischemia. During this process, oxidative stress is responsible for damage of important part of the process. Their role is by reducing the ability of cell biology and reduction of intracellular molecular signals.[5]according to the studies, calcium has an important role in a variety of complications and tissue damage fol-

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lowing ischemia and reperfusion injury. Ischemic heart is prone to rapid flow of calcium effusion into the myositis that occurs in the initial minutes after aortic clamping removal or in the last minutes of cardiopulmonary bypass and case to increase in cytosol calcium concentration in cardiac cells myositis.[6, 7] Ways to reduce the concentration of ionized calcium are using calcium channel blockers, sodium hydrogen ion exchange inhibitors and calcium solutions as the citrate phosphate dextrose (CPD). Increasing in ionized calcium can be easily controlled by using cardioplegic solutions containing large amounts of potassium or magnesium and acting through inhibition of calcium entry into cells.[5]

The purpose of this study is to determine the impact of CPD solution at the end of cardiopulmonary bypass (CPB) on left ventricular ejection fraction (EF) and on antioxidants superoxide dismutase, malondialdehyde total antioxidant capacity in patients after coronary artery bypass graft surgery.

Materials and methods

During a year, in double blind clinical trial study at Tabriz University of medical science patients according to our inclusion criteria whom undergoing elective CABG in Madani heart hospital, Tabriz, Iran were enrolled in this study. The number of sample size has been determined 50 cases based on other studies. Patients randomly were divided to one of the study or control groups according to the following site (http://www.grophpad.com/quickalcs/randomized.cfm), also were matched together according to sex, age and New York Heart Association (NYHA). Before the surgery, all of the patients in both groups were informed about the benefits of this research and then if they signed the consent form will entrance to the study. Patients were assured that all information will be confidential and they can come out of research each time.

Inclusion criteria were as follows: lack of other heart attack or another heart surgery at same time; no previous heart surgery; positive history of past severe disease; on emergency surgery; the absence of high risk surgery; to abandon cases involving one vessel; uncontrolled diabetes; lack of severe left ventricular dysfunction; no MI with Q wave in past six weeks; lack of severe lesions of LM (greater than or equal to 50%); unstable angina; noncytotoxic drugs and radiation use; no lately blood transfusion in past month, and willingness to participate in the study. Exclusion criteria were as follows: the operation of the on pump switch to off pump; addition of other surgery such as left ventricular aneurysm; performed endarterctomy on the involved vessels; prolonged clamping more than 100 minutes; prolonged pump more than 130 minutes; hemolysis of samples obtained from patients; clotting of samples taken from patients; lack of proper storage and transport of samples at the usual time; less than 5 / 1 cc of blood serum sample size. After selecting the group of patients from on pump and off pump; according to the considerations on the status of involved vessels anatomic; with renal disease; cerebrovascular disease; a history of stroke and severe ascending aorta atherosclerosis associated with severe carotid artery stenosis. Random list was reserved and hidden beside perfusionist who was responsible of injection of CPD solution at the end of cardiopulmonary bypass. The patients were anesthetized during surgery and were not aware of CPD solution injection or infusion at the end of the pump. The surgeon and cardiologist who measured of ejection fraction (EF) before and after the operation and administration of the laboratory were not informed about the injection or not injection of CPD solution during surgery. In case group after surgery and before the opening of the aortic clamping, warm blood shot of CPD solution (3cc to 100 cc) amount of (100 cc/min/m2BSA) was injected for three minutes until perfusion pressure maintaining of 30 mmHg. After the ending of warm blood shot injection, calculating the net blood pressure amount of 50 to75 mmHg until the heart rate (7 to 10 minutes) continued. In control group only injected pure blood. But all routine procedures were performed for them. Blood samples (10 cc for each time) were measured respectively for measurement of cardiac enzymes and inflammatory factors in before opening the bypass, before clamping and 10 minutes after opening the clamping of the coronary sinus and venous blood, when patient's arrival in ICU and the first and also second morning after surgery was taken only from vein.

The main outcome measured were followed as: Measurement of serum malondialdehyde (MDA): Total Antioxidant Capacity and superoxide dismutase (SOD). Method of measurement of Total antioxidant capacity in serum by using a commercial kit Ltd Random Laboratories UK, CatNo.NX2332 .Ethics Committee of Tabriz University of Medical Sciences was approved this study. IRCT code is IRCT201108147325N1.

The statistical calculations were performed using SPSS version 17.0 (SPSS Inc, Chicago, IL, USA). All P values of < 0.05 (two-tailed) were considered statistically significant. Continuous variables with normal distribution are presented as mean ± SD. Categorical variables were analyzed with student's t-test. Repeated measurements have been used for evaluation of laboratory quantity variables that was serially measured. Before statistical analysis, Kolmogorov–Smirnov test was used for evaluation of normal distribution of quantitative variables. Finally, Chi-squared test was used for evaluation of qualitative variables, such as sex and other qualitative variables. In this study, P values less than 0. 05 are considered significant.

Results

The study was performed on 50 candidate patients for CABG included 30 men (60%) and 20 women (40%) with mean age of 62.3 ± 9.1 years (45-70; Table 1).

Patients were classified randomly in two groups. Administration of CPD was not any significant effects on cardiac enzyme.

Table 1. Demographic variables of study population

Group		control	intervention	P
Age		5 / 9 ± 2 / 60	3 / 8 ± 4 / 64	0/ 3
sex	Female	9(36%)	11(44%)	
	male	16(64%)	14(56%)	0/ 1
NYHA	I	0	0	
	II	(65.5%)	(40%)	0/ 4
	III	(37.5%)	(60%)	
EF		47.4±7.08	44.1±8.09	0/ 1

EF, Ejection Fraction

There was no significant difference between the two groups according to sex (P>0. 05), age (P>0. 05), NYHA functional classification of heart failure (P>0. 05), in the levels of glutathione peroxidase (GPx) (P>0. 05), cardiac enzymes such as CTNI (P>0. 05) ،CPK (P>0. 05), CK-MB (P>0. 05) and oxidative stress levels (P>0. 05).

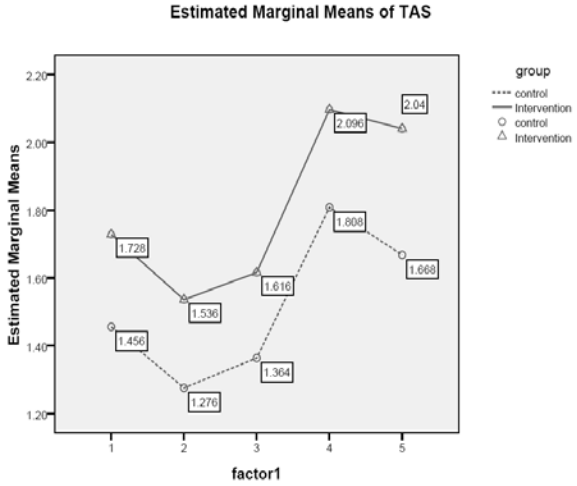

Fig. 1. The total antioxidant capacity status changes between the two groups.

In addition, ejection fractions between two groups were not significantly difference before and after treatment. Measurement of oxidative stress in different time were not different in SOD, MDA and GPx, but total antioxidant capacity status were improvement after intervention in compared with control group (p<0.001), Figure 1 highlights the antioxidant capacity had better situation in the intervention group and impacts of intervention were evident in the later stages of measurement of total antioxidant capacity level in the group of treated with CPD solution. In the measured markers include MDA , catalase (CAT) and C reactive protein with high sensitivity (SCRP) were observed positive significant difference between two groups with p-values 0 / 002, / 001 <0 and 0 / 001 ,respectively. According to Figures 2-4, it was not observed in the early stages of the measurement it was more evident in the last two measurements stages.

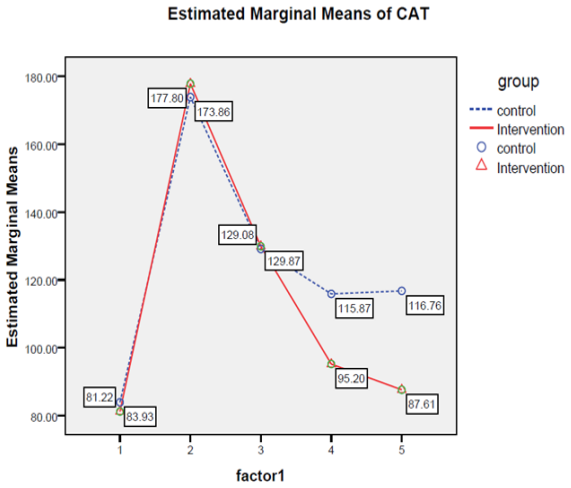

Fig. 2. The CATchanges between the two groups.

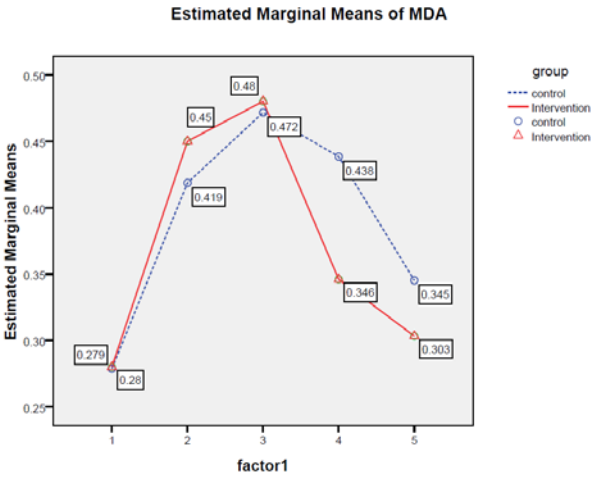

Fig.3. The MDA changes between the two groups.

Discussion

Reperfusion injury (RI) is one of the most common phenomena associated with CABG. This syndrome can present with a clinical arrhythmia, vascular damage, myocardial dysfunction, and often leads to decreased cardiac output syndrome.

MI may be not differentiated from re- perfusion syndrome, this is called a bad clinical outcome for the patient. [8,9] Pathogenesis and mechanism of injury is complex and not yet fully clear. However, calcium overload and free radical production are the major cause of the reperfusion injury syndrome.[10] Experimental studies on

animal models on the production of ROS and free radi-

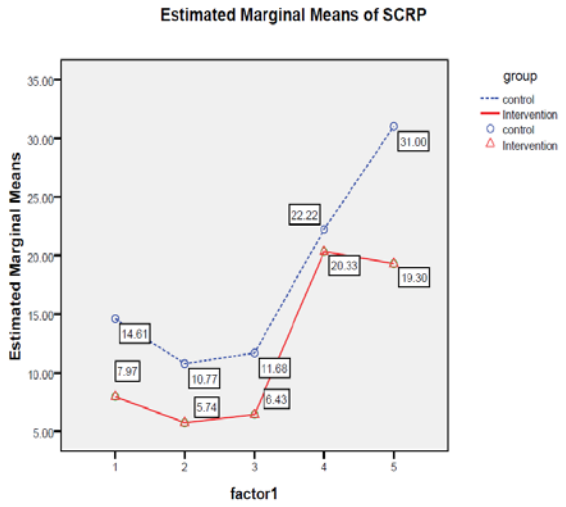

Fig.4. The SCRP changes between the two groups.

cals of oxygen in myocardial reperfusion syndrome has been sequenced of oxidative damage that leads to cellular dysfunction and damage the cellular structure.[11] In studies conducted on patients undergoing coronary artery surgery is achieved strong evidence of oxidant production during reperfusion. Also, reduce of total capacity of antioxidants occurs as protective agents oxidative damage that may have a major role to worsen oxidative damage. [5, 17]

In patients undergoing CABG using cardiopulmonary bypass and aortic clamping, can cause cardiac ischemia and increasing the chance of oxidative damage followed by reperfusion. Because of the unintended consequence of oxidative damage to endanger the patient's clinical outcome, the researchers aim has been focused to prevent this damage following surgery.According the important role of calcium in reperfusion injury different strategies have been suggested to prevent calcium overload, which include the following items[12] :

- Using cardioplegic solutions to reduce calcium content
- Using of calcium antagonists or calcium channel blockers
- Using chelators such as citrate for decreasing of calcium
- Using of hydrogen / sodium pump inhibitors

Mak and *et al.* examined calcium blockers and found that the dihydropyridine class of drugs had been highly successful in reducing the reperfusion syndrome caused by oxidative damage in CABG patients, especially tizanidine ،nifedipine, verapami and diltiazem, -four drugs have been known for this family.Results represent a decrease of glutathione peroxidase in 40% of cases, and have been observed significant and positive effect in preventing cell death .[13]

In this study, we tried to carefully designing and considering inclusion and exclusion criteria and carefully matched between two groups of patients for age, sex and functional class of heart NYHA to achieve reliable results. Based on the results, there is no difference in the level of catecholamine and cardiac enzyme CK-MB, which is indicative of myocardial injury between cases and controls groups in left and right ventricular function.[14] Complications of myocardial reperfusion affect the results of surgical and medical benefits. Our study is unique because citrates solution can be directly used in humans. Previous studies have been studied in animal models .According to our study, soluble citrates had no effect on the cardiac enzymes compared with the control group .In addition, changing of the course of these enzymes was the same between groups. Cardiac output was also similar between groups and did not see the difference.

Bixler *et al.* investigated the directly solution of CPD by using of 0.8 mg / kg at 15 minutes after the reperfusion in animal models of dogs, which had not significant effect in improving and maintaining in cellular and intra cellular function. Probably, these results have been because of chelators of solution and reducing other cations.[15] The inconsistent results of previous studies have not been showed any effect on the myocardial reperfusion injury syndrome.[16, 18]

Fukuhiro *et al.* assessed the performance of three strategies mentioned, on the animal model (rats) except for calcium blockers. These results indicate the beneficial effect of 2% solution of citrates in decreased blood's calcium flow was due to aortic clamping after reperfusion following ischemia.[12]

The researchers suppose that cardioplegic solutions containing potassium and magnesium are better effects than the citrates solutions in control of reperfusion syndrome. One reason is related to its mechanism of action that is as chelators, because the mechanism of action as chelators can reduce the magnesium content.[12, 13]

Solution containing calcium citrate may be reduced calcium, may have a role in controlling ischemia and also reducing tissue damage through binding to it. However, has not done any study in humans. Morishig *et al.* indicated that using whole blood containing citrate did not have any effect on cardiac output and cardiac enzymes just like our study. Evaluating the changes of various types of oxidative stress markers has significant effect on total antioxidant capacity in patients undergoing CABG. Based on this study, warm blood cardioplegic containing citrate has a significant effect in reducing oxidative stress and prevent to the damage of the miyosit membrane.[14]

As can be seen in Figure 2, this difference occurred in the later stages of measurement after the administration of the CPD. In addition, this difference had a little effect on catalase but did not show any differences on other

oxidative stress markers such as glutathione peroxidase, superoxide dismutase and MDA between groups. In previous studies [19, 20], course changing between the two groups was similar just like our study.

Our study only had improved of total antioxidants capacity levels and reduced of catalase levels, but other important oxidative stress marker did not change a lot. In animal models studies obtained as well as similar results.[14] The prescription of warm blood citrate containing compared with cold blood, has a significant effect on the glutathione peroxidase that is needed more studies in the future.

Conclusion

According to the results of this study, a solution of CPD is effective in improving the antioxidant status, but has little effect in reducing other markers of oxidative stress. It seems that the reduction of other cations may have a major role in reducing the beneficial effects of this drug that is needed more studies in the future.

Ethical issues

The local ethics committee of Tabriz University of Medical Sciences approved the study and all patients signed informed consent.

Conflict of interests

The authors declare no conflicts of interest

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