RESEARCH ARTICLES

TIMING OF CARDIOPULMONARY BYPASS TERMINATION: EFFECTS ON BIOCHEMICAL MARKERS OF EARLY MYOCARDIAL INJURY

KARDİYOPULMONER BYPASS SONLANDIRMASINDA ZAMANLAMA: ERKEN MİYOKARD HASARINDAKİ BİYOKİMYASAL BELİRLEYİCİLER ÜZERİNE ETKİSİ

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ABSTRACT

Purpose: *Major concern during cardiopulmonary bypass (CPB) is myocardial protection. The purpose of this study was to compare the effects of two techniques of terminating CPB on the biochemical markers of early myocardial injury.* ***Method:*** *Twenty-two adult patients with three or four vessel diseases undergoing elective coronary artery bypass grafting surgery were studied in a prospective randomized clinical design. All distal anastomoses were performed during a single period of cross clamping. Then, patients were randomized in terms of terminating CPB. In group 1 (n=10), CPB was terminated before the proximal anastomoses were completed and in group 2 (n=12), CPB was terminated after completion of all proximal anastomoses. Blood samples were collected from the coronary sinus as baseline (t1), at the time of 0 (t2) and 5 minutes after (t3) cross-clamp removal, 5 (t4) and 15 minutes (t5) after CPB termination.* ***Results:*** *Plasma lactate, hypoxanthine and malondialdehyde (MDA) levels significantly increased over time in both groups. MDA levels at t4 and t5 were higher and pH, base excess, ionized calcium and bicarbonate levels at t2 were significantly lower in group 2 when compared to group 1 ($p< 0.05$).* ***Conclusion:*** *Two techniques of terminating CPB did not differ in terms of end-products of energy metabolism and acid-base analysis as biochemical markers of myocardial status.*

Key Word: *Cardiopulmonary Bypass; Technique; Myocardial Protection*

ÖZET

Amaç: *Kardiyopulmoner bypass (KPB) sırasında temel hedeflerden biri miyokardın korunmasıdır. Bu çalışmada amaç, iki farklı zamanda KPB sonlandırma tekniğinin, erken miyokard hasarında biyokimyasal belirleyiciler üzerine etkisini karşılaştırmaktır.* ***Metod:*** *Üç-dört damar hastalığı olup koroner arter bypass greft cerrahisi planlanan, 22 erişkin hasta prospektif randomize düzende çalışmaya alındı. Tüm hastalarda distal anastomozlar aortik klemp altında yapıldı. Hastalar KPB'ı sonlandırma aşamasında randomize olarak iki gruba ayrıldı. Birinci grupta (n=10), KPB proksimal anastomozlar tamamlanmadan sonlandırıldı ve ikinci grupta (n=12), KPB proksimal anastomozlar tamamlandıktan sonra sonlandırıldı. Koroner sinüsten kan örnekleri, başlangıçta (t1), aortik klemp kaldırıldıktan hemen sonra (t2), 5 dakika sonra (t3), KPB sonlandırıldıktan 5 dakika (t4), ve 15 dakika sonra (t5) alındı.* ***Bulgular:*** *Plazma laktat, hipoksantin ve malondialdehit (MDA) düzeyleri her iki grupta da zaman içerisinde anlamlı olarak artış gösterdi. İkinci grupta, t4 ve t5'de MDA düzeyleri anlamlı olarak yüksek, t2'de ise pH, baz açığı, ionize kalsiyum ve bikarbonat düzeyleri anlamlı olarak düşük bulundu ($p< 0.05$).* ***Sonuç:*** *KPB'ı sonlandırma zamanı olarak karşılaştırılan iki farklı teknik arasında, miyokardın durumunun biyokimyasal belirleyicilerinden olan enerji metabolizması ürünleri ve asid-baz analizi bakımından fark bulunmadı.*

Anahtar Kelimeler: *Kardiyopulmoner Bypass; Cerrahi Teknik; Miyokard Korunması.*

INTRODUCTION

Although various methods are used during coronary artery bypass graft surgery (CABG), the most important end-point in all cardiopulmonary bypass (CPB) techniques is myocardial protection. Many studies in recent years focus on the reperfusion injury (1). This report presents a method for performing proximal anastomoses after termination of CPB under side clamping of the aorta and evaluates the effects of this method on myocardial biochemistry.

METHODS

Patient population:

Twenty-two adult patients with three- or four- vessel coronary artery disease undergoing elective primary isolated CABG surgery were studied in a prospective randomized design. All patients had left anterior descending coronary artery (LAD) disease and none required inotropic support preoperatively. All were planned to receive both left internal mammary artery (LIMA) and saphenous vein grafting. Patients who had associated valvular or other systemic diseases, poor ventricular function (ejection fraction below 40%) and those undergoing emergency surgery were not included in the study. The protocol was approved by the Hospital Ethical Committee and informed consent was obtained from the patients.

Anesthetic and the surgical technique:

All patients were premedicated with diazepam and famotidine orally one hour before surgery. Upon arrival in the operating room, non-invasive monitors were placed; radial artery and central venous catheters were inserted after induction of anesthesia. Anesthesia was induced with low dose thiopenthal (2-4 $mg.kg^{-1}$), fentanyl (7-10 $\mu g.kg^{-1}$), and vecuronium (0.1 $\mu g.kg^{-1}$); and maintained with isoflurane 0.7%, air/O_2 50% and fentanyl infusion (10 $\mu g.kg^{-1}.h^{-1}$) throughout the surgery. Methylprednisolone (10 $mg.kg^{-1}$) was administered at the beginning of CPB and the renal dose of dopamine infusion was used during the surgery. A coronary sinus catheter was inserted in all the patients before the initiation of CPB. Patients underwent standard total cardiopulmonary bypass with the use of membrane oxygenator (Cobe VPML-plus) and crystalloid prime. Pump flow was maintained at 2.2-3.0 $l.min^{-1}.m^{-2}$ and perfusion pressures at about 60 mmHg throughout the CPB period. CPB was conducted at systemic hypothermia (core temperature, 28°C) and moderate hemodilution (hematocrit, 26%). Cardiac arrest was initiated by aortic cross-clamp; and cold cardioplegia and topical cooling were used for myocardial protection.

The same anesthetic and surgical team performed all operations. LIMA anastomosis to LAD and all distal anastomoses were performed during a single period of cross clamping the CPB. After these anastomoses were performed, the cross-clamp was removed and the LAD perfusion was provided. Then rewarming was completed and the heart was allowed to beat either spontaneously or by the use of defibrillation. By this time, patients were randomized in one of the two groups in terms of terminating the CPB. In group 1 (n=10), CPB was terminated before the proximal anastomoses were completed. Proximal anastomoses were performed using a partial-occluding side clamp and the heart was perfused through the LAD during this time. In group 2 (n=12), CPB was terminated after completion of all proximal anastomoses.

Biochemical markers of myocardial injury:

Blood samples were collected from the coronary sinus to measure blood gases, acid-base status and plasma levels of lactate, hypoxanthine, malondialdehyde (MDA) and uric acid as biochemical markers of myocardial injury. Parameters were evaluated at five time intervals: first sample (control) after initiation of CPB and before aortic cross clamping (t1), at the time of aortic cross clamp removal (t2), 5 minutes after cross clamp removal (t3), 5 minutes (t4) and 15 minutes (t5) after CPB termination.

The levels of thiobarbituric acid reacting substances in serum, as a measure of lipid peroxidation, was determined as described by Wade and Van Rij (2). For determination of hypoxanthine, one milliliter of serum was deproteinized with an equal volume of ice-cold perchloric acid (8%). After centrifugation, the supernatants were neutralized using 0.7 M K_3PO_4 and hypoxanthine levels were determined using xanthine oxidase (3). Taking the molar absorptivity of uric acid (12200 $M^{-1}cm^{-1}$), xanthine oxidase-catalysed formation of uric acid was measured at 293 nM. Hypoxanthine concentration was calculated as nmol/ml of serum.

Statistical analysis:

Data are presented as mean ± standard deviation (SD). Mann Whitney-U test was used for comparisons between groups and Friedman and Wilcoxon tests were used for repeated measurements of dependent variables within groups. Values of $p<0.05$ was defined as significant.

RESULTS

Patient and surgical characteristics of the groups were similar (Table 1). Lactate levels at five time intervals in group one were 2,1±0,8 mmol/L; 3,4±0,9 mmol/L; 4,1±1,2 mmol/L; 3,8±1,5 mmol/L; 3,5±1,1 mmol/L and in group two, 1,6±0,5 mmol/L; 4,0±0,8 mmol/L; 3,9±0,9 mmol/L; 3,3±1,0 mmol/L; 3,5±1,1 mmol/L respectively. Hypoxanthine levels in groups were 7,2±4,6 nmol/ml; 14,1±9,3 nmol/ml; 18,9±7,1 nmol/ml; 22,8±4,4 nmol/ml; 30,1±8,9 nmol/ml and 9,3±4,5 nmol/ml; 15,0±3,7 nmol/ml; 23,4±10,5 nmol/ml; 28,9±8,5 nmol/ml; 35,0±10,2 nmol/ml respectively. Plasma MDA levels were 0,76±0,12 µmol/L; 0,86±0,11 µmol/L; 0,84±0,11 µmol/L; 0,83±0,13 µmol/L; 0,86±0,13 µmol/L and 0,83±0,07 µmol/L; 0,83±0,11 µmol/L; 0,90±0,09 µmol/L; 1,00±0,09 µmol/L; 0,98±0,12 µmol/L respectively. Plasma uric acid levels in groups were 4,63±1,74 mg/dl; 4,35±1,33 mg/dl; 4,68±1,46 mg/dl; 4,65±1,76 mg/dl; 4,71±1,56 mg/dl and 4,90±1,07 mg/dl; 4,23±1,12 mg/dl; 4,58±0,91 mg/dl; 5,16±1,16 mg/dl; 5,42±1,24 mg/dl respectively.

Plasma lactate and hypoxanthine levels were significantly increased over time in both groups ($p< 0.01$) (Fig. 1 and 2). Lactate levels in all time measurements were higher than the baseline levels in both groups. However the values were insignificant between groups ($p> 0.05$). Hypoxanthantine levels demonstrated an increasing trend over time in both groups but the changes were also similar between groups. There were no significant differences in levels of MDA in group 1 but the changes in group 2 were significant over time ($p< 0.01$) (Fig. 3). MDA levels measured after termination of CPB (t4 and t5) were significantly higher in group 2 compared to those in group 1 ($p=0.001$ and $p=0.034$, respectively). Uric acid values did not differ between groups or within groups over time ($p> 0.05$) (Fig. 4).

Regarding the acid-base status; there were no differences with respect to changes in pO_2 and

Table - 1: Patients' and surgical characteristics

	Group 1	Group 2
Age (year)	61,4 ± 7,9	59,6 ± 9,4
Gender (M/F)	9 / 1	11 / 1
Weight (kg)	73,3 ± 9,1	75,1 ± 10,2
XC duration (min)	36,0 ± 6,4	35,6 ± 8,9
CPB duration (min)	67,2 ± 15,8	93,8 ± 29,5 *
Operation duration (min)	345,0 ± 27,3	315,9 ± 28,8

Mean ± SD. (XC: aortic cross-clamp; CPB: cardiopulmonary bypass)
* $p< 0.05$ compared to group 1.

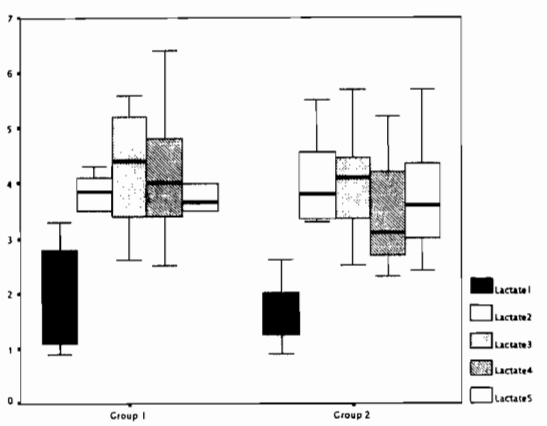

Fig. 1: Plasma lactate levels (mmol/L).

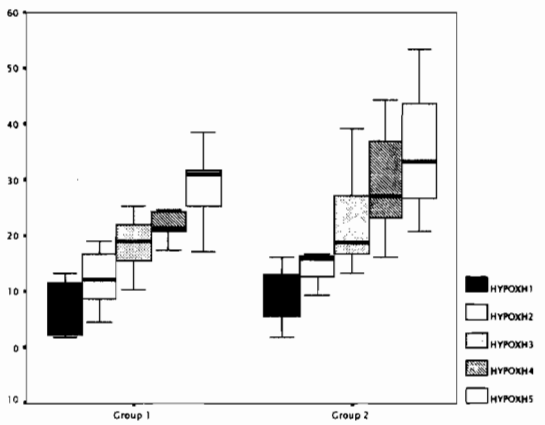

Fig. 2: Plasma hypoxanthine levels (nmol/ml). Hypoxh: hypoxanthine.

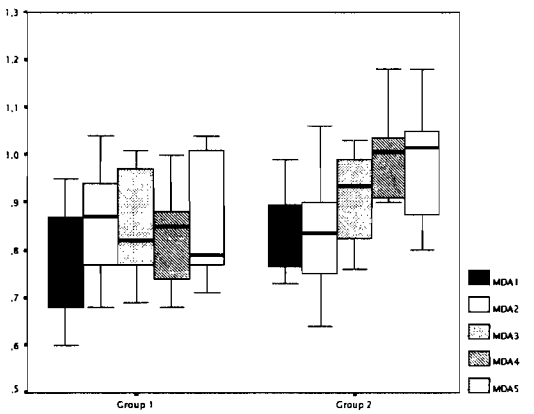

Fig. 3: Plasma malondialdehyde levels (μmol/L). *p<0.05 compared to group 1. MDA: malondialdehyde.

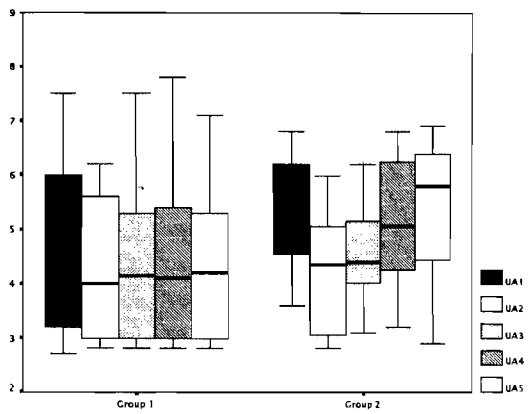

Fig. 4: Plasma uric acid levels (mg/dl). UA: uric acid.

Table - 2: Acid-base status in two groups.

	Group 1					Group 2				
	T1	T2	T3	T4	T5	T1	T2	T3	T4	T5
PaO_2 (mmHg)	34,4±7,1	39,1±7,6	33,9±5,5	35,3±6,8	35,9±7,0	24,4±5,0	32,3±8,3	22,4±6,0	25,6±5,5	27,4±6,9
$PaCO_2$ (mmHg)	38,2±6,7	37,6±9,0	32,3±4,1	35,4±6,8	40,1±8,6	43,4±8,2	47,0±16,0	41,7±8,1	38,0±6,2	40,7±6,7
pH	7,40±0,05	7,42±0,12	7,48±0,06	7,47±0,06	7,44±0,06	7,38±0,05	7,25±0,1*	7,40±0,07	7,46±0,05	7,44±0,06
Base excess	-1,1±3,5	-0,4±3,3	0,6±2,3	1,5±0,9	2,3±2,0	-0,5±1,0	-6,5±5,0*	-0,2±2,0	2,2±1,4	2,3±2,3
HCO-3 (mEq/L)	23,9±3,5	24,0±2,3	23,9±1,7	25,5±1,1	26,9±2,6	25,8±1,8	20,0±4,0*	25,5±1,8	26,9±1,8	27,5±2,4
iCa++ (mmol/L)	0,96±0,19	0,81±0,13	0,76±0,12	0,93±0,12	0,89±0,15	0,95±0,11	0,73±0,18*	0,84±0,11	0,88±0,14	0,90±0,08

Mean ± SD.* p< 0.05 compared to group 1.

pCO_2 levels in the two groups over time (Table 2). Comparison between groups demonstrated that pH, base excess, ionized calcium and bicarbonate levels from coronary sinus samples taken at the time of cross-clamp removal (t2) decreased significantly in group 2 when compared to group 1 ($p<0.05$).

Three patients in group 1 and none in group 2 required inotropic support after termination of CPB ($p>0.05$). There were no differences with respect to administration of defibrillation after cross-clamp removal to produce the heart beat.

DISCUSSION

With the introduction of coronary artery bypass grafting, surgeons have developed their own techniques for performing the operation, with uniform success. In this study two techniques of terminating CPB were compared with respect to their effects on biochemical markers of early myocardial injury.

In hospitals where the proximal anastomoses are completed first, the technique is to side-clamp the aorta without placing the patient on bypass unless hemodynamic instability or electrocardiogram changes suggesting myocardial ischemia are present. Core temperature is typically between 32 to 33°C. Once this part of the procedure is completed, extracorporeal circulation is initiated. In institutions where the distal anastomoses are performed first, the patient is placed on extracorporeal circulation and core temperature is reduced as quickly as possible before bypass grafting of native coronary arteries is attempted. As soon as the distal anastomoses are completed, the aortic cross-clamp is removed and the aorta is side-clamped to permit completion of the proximal anastomoses. The technique in our hospital also involves the distal anastomoses to be performed first. After removal of aortic cross clamp core temperature is usually about 34°C and typically increasing. In cases whose hemodynamic status and myocardial contractility

are suitable, CPB is terminated and the proximal anastomoses are performed using a side clamp on the aorta in order to shorten the duration of CPB.

Although CPB is fundamental for cardiac operations, it is associated with substantial postoperative morbidity (4). CPB can trigger the release of endotoxin, which can act as a powerful trigger for release of cytokines. Complement activation during CPB has also been suggested a contributing phenomenon to the release of cytokines. The release of cytokines during CPB can have deleterious effects on the heart and on other organs. The proinflammatory cytokines can significantly alter myocardial contractility. Studies in adults have shown that beside leading to intense complement activation, transient neutropenia with sequestration of the neutrophils in the pulmonary vasculature, and profound changes in both humoral and cellular immune function (5), prolonged duration of CPB is also associated with an increased risk of myocardial distention and damage to the already jeopardized heart, a potential need for additional blood products, and the augmented unfavourable "whole-body inflammatory response" including intrinsic coagulation, the classic complement pathway, fibrinolysis, kinin function, and neutrophil activation (6). Thus the main purpose of this study was to evaluate the myocardial effects of a technique that shortens the duration of CPB during CABG surgery so that all the above mentioned deleterious effects might decrease.

Hayashi et al (7) has shown that the duration of CPB is one of the factors associated with requirement for early inotropic support during emergence from CPB in patients undergoing CABG surgery. The use of inotropic agents is not free of risks. Injudicious use of these agents may be associated with tachycardia, dysrhythmias, hyperglycemia, increased reperfusion injury, and additional medical expense (8, 9). In this study two groups did not differ with respect to use of inotropic agents during and after CPB. The incidence of arrythmias or defibrillation requirements were similar.

Most cases of CPB are associated with aortic cross clamping, which results in global myocardial ischemia, whereas the release of the aortic cross-clamp results in myocardial reperfusion and subsequently more severe myocardial damage (4). The mechanisms are primarily related to ischemia-reperfusion of the myocardium and also other organs. Thus, the chief determinant factor in respect to postoperative functional outcome (cross-clamping vs duration of CPB) is controversial. Animal models have demonstrated that a severely decreased blood supply to the actively contracting myocardium results in a depression of high-energy phosphates (10-12), depressed systolic and diastolic function (13-15), and ultrastructural changes in mitochondria and tubular structures (16). This is the case where aortic clamping is removed and LAD perfusion has been provided but the other anastomoses are not completed, thus some part of myocardium is contracting with no reperfusion in our protocol. If reperfusion occurs early, all of these factors may be restored to normal (16-18). However, if reperfusion occurs too late, then some or all high-energy phosphates, myocardial function, or histological features may not return to normal (16, 19, 20). Because of the design of our study, only the first 30 to 45 minutes after LAD reperfusion could be studied. The products of energy metabolism in our study typically demonstrated similar changes. MDA levels in samples taken after termination of CPB were significantly higher in group 2. This is the only significant difference between the groups in terms of terminating CPB. However the peak levels as well as the duration of increased levels could not be determined so the clinical significance of comparison between groups could not be established. Immediate and exaggerated release of end-products of energy metabolism did not return to normal values within the observation time, though signs of cellular acidosis at the time of cross-clamp removal in group 2 were transient and gradually reversed to normal levels within five minutes. This statistically significant difference in blood gas analysis at the time of cross-clamp removal was unrelated to the technique of CPB termination. The lower levels of pH, base excess, ionized calcium and bicarbonate levels at this time indicates the role of higher pO_2 levels in the reperfusion injury.

In conclusion, the authors believe that despite minor differences between the groups the clinical significance remains controversial.

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REFERENCES

1. Ross S, Foex P. Protective effects of anesthetics in reversible and irreversible ischemia-reperfusion injury. Br J Anaesth 1999; 82: 622-632.
2. Wade CR, Van Rij AM. Plasma thiobarbituric acid reactivity: Reaction conditions and the role of iron, antioxidants and lipid peroxy radicals on the quantitation of plasma lipid peroxides. Life Sciences 1988; 43: 1085-1093.
3. Jong JW, Goldstein S. Changes in coronary venous inosine concentration and myocardial wall thickening during ischemia in the pig. Circ Res 1974; 35: 111-118.
4. Wan S, LeClerc JL, Vincent JL. Cytokine responses to cardiopulmonary bypass: lessons learned from cardiac transplantation. Ann Thorac Surg 1997; 63: 269-276.
5. Hauser GJ, Chan MM, Casey WF, Midgley FM, Holbrook PR. Immune dysfunction in children after corrective surgery for congenital heart disease. Crit Care Med 1991; 19: 874-881.
6. Manager WM, Frohlich ED, Gifford RW, Dustan HP. Norepinephrine infusion in normal subjects and patients with essential or renal hypertension: effect on blood pressure, heart rate and plasma catecholamine concentrations. J Clin Pharmacol 1976; 16: 129-141.
7. Hayashi H, Prabhu R, Kramer D, Oka Y. Predictors of inotropic support during weaning from cardiopulmonary bypass in coronary artery bypass grafting surgery. J Anesth 1997; 11: 3-9.
8. Chraemmer JB, Hoilund CP, Marving J, Christensen V. Left ventricular ejection fraction during anasthetic induction: comparison of rapid-sequence and elective induction. Can Anaesth Soc J 1986; 33:754-759.
9. Randall CC, Juli W, Stuart RH, John B. Pre-treatment with low-dose fentanyl for rapid-sequence induction. Anesthesiology 1983; 59: A344.
10. Jennings RB, Reimer KA, Hili ML, Mayer SE. Total ischemia in dog hearts, in vitro. Circ Res 1981; 49: 892-900.
11. Hearse DJ, Opie LH, Katzeff IE, Lubbe WF, Van der Werff TJ, Peisach M, Boulle G. Characterization of the "border zone" in acute regional ischemia in the dog. J Cardiol 1977; 40: 716-726.
12. Skinner FP, Scott RF, Morrison ES, Imai H, Jarmolych J, Lee KT. High energy phosphate compounds and mitochondrial function in ischemic myocardium of swine with advanced coronary atherosclerosis. J Mol Cell Cardiol 1973; 5: 515-526.
13. Tyberg JV, Yeatman LA, Parmley WW, Urschel CW, Sonnenblick EH. Effects of hypoxia on mechanics of cardiac contraction. Am J Physiol 1970; 218: 1780-1788.
14. Waters DD, DaLuz P, Wyatt HL, Swan HJC, Forrester JS. Early changes in regional and global left ventricular function induced by graded reductions in regional coronary perfusion. Am J Cardiol 1977; 39: 537-543.
15. Harden W, Barlow CH, Simson MB, Harken AH. Temporal relation between onset of cell anoxia and ischemic contractile failure. Am J Cardiol 1979; 44: 741-746.
16. Schaper J, Mulch J, Winkler B, Schaper W. Ultrastructural, functional and biochemical criteria for estimation of reversibility of ischemic injury. A study on the effects of global ischemia on the isolated dog heart. J Mol Cell Cardiol 1979; 11: 521-541.
17. Reimer KA, Jennings RB, Hill ML. Total ischemia in dog hearts in vitro. Cir Res 1981; 49: 901-911.
18. DeJong JW, Verdouw PD, Remme WJ. Myocardial nucleotide and carbohydrate metabolism and hemodynamics during partial occlusion and reperfusion of pig coronary artery. J Mol Cell Cardiol 1977; 9: 297-312.
19. Kannengiesser GJ, Opie LH, Van Der W. Impaired cardiac work and oxygen uptake after reperfusion of regionally ischemic myocardium. J Mol Cell Cardiol 1979: 197-207.
20. Mathur PP, Case RB. Phosphate loss during reversible myocardial ischemia. J Mol Cell Cardiol 1973; 5: 375-393