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Research Article

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The Effect of On-Pump and Off-Pump Bypass Surgery on Arginase Activity and Nitric Oxide Level

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Abstract It is aimed to investigate the effect of on-pump and off-pump bypass surgery on arginase activity and nitric oxide level. 28 (13 off-pump bypass and 15 on-pump bypass) patients undergoing CABG surgery were included in the study. Blood samples of the patients were collected during the preoperative, peroperative, and postoperative periods. The collected samples were used to spectrophotometrically measure arginase activity and NO levels. No statistically significant difference was observed when the preoperative, peroperative, and postoperative periods of the patients, who underwent on-pump and off-pump bypass surgery, were compared. A statistically significant difference was observed between on-pump and off-pump bypass surgeries in terms of the arginase enzyme activity in the preoperative period. It is possible to prevent complications associated with the bypass surgery by determining the treatment methods for the decreased NO level and the increased arginase enzyme activity due to the bypass surgery so that these patients can have an enhanced quality of life and be healthy after the surgery.

Keywords Cardiopulmonary bypass, Arginase, Nitric oxide, Off-pump, On-pump

Introduction

It is a known fact that cardiopulmonary bypass (CPB), which is a significant part of numerous cardiothoracic procedures, is induced by an excessive undesirable systemic inflammatory response and cardiac biomarkers released in response to CPB and surgical trauma [1]. As a result, a systemic inflammatory response is associated with the activation of leukocytes and production of free oxygen radicals, arachidonic acid metabolites, platelet-activating factor (PAF), and nitric oxide (NO). The systemic inflammatory response is mainly due to numerous postoperative complications such as respiratory failure, pulmonary damage, and brain damage [1].

Arginine is essential for the synthesis pathway of NO; therefore, the most important role of L-arginine is to regulate vascular health and homeostasis [2]. The synthesis of NO is realized from arginine by the NO synthase (NOS) isoforms. While neuronal cells have neuronal NOS (nNOS), endothelial NOS (eNOS) is primarily present in endothelial cells and a variety of cell types including macrophages, hepatocytes, muscles, and chondrocytes have cytokine-inducible NOS (iNOS). One of roles of the eNOS is to regulate the physiological vascular tone [3]. While both NOS and arginase employ arginine as a common substrate, arginase may decrease NO production by competing with NOS for arginine [4].

Reduced biological effectiveness of NO is responsible for impairment of the cardiovascular pathology-related vasodilatation, anti-thrombotic, anti-inflammatory, and anti-apoptotic actions [5].

In the ischemic/reperfused heart, low regulation of eNOS is observed against arginase I induction [6]. As a molecular response to hypoxia, the consumption of arginine with production of NO against other metabolic pathways used by arginine may be a defense strategy of cells to withstand hypoxic stress. As a result, the fact that arginase activation reduces the intracellular arginine pool may is associated with protection of cells under the conditions in which NOS may not synthesize NO. This advantage of high arginase activity during ischemia can become a serious disadvantage during reperfusion of tissue [6].

A number of studies, it has been reported that arginase uses arginine against NO production in case of hypoxia; however, there is a limited number of studies regarding the effect of bypass surgery on arginase activity and NO level. Also there is no study regarding the effect of the use of pump in the bypass surgery on arginase activity and NO level; thus the aim of this study was to investigate the effect of on-pump and off-pump bypass surgery on arginase activity and nitric oxide level.

Material and Methods

The Population of The Study

The population of the study consisted of totally 28 patients undergoing on-pump bypass operation (12 women and 16 men and 13 off-pump bypass patients and 15 on-pump bypass patients). This study included all the patients who underwent the elective cardiopulmonary bypass. Being informed about the purpose of the study, these patients signed the informed consent form. Before the study, the related protocol was reviewed and approved of the ethics committee of Fırat University (Reg. No. 2015015) was obtained.

In the present study, blood samples were taken 3 times from each patient: before the surgical intervention (preoperative period), at the end of the surgical intervention (peroperative period), and approximately 24 hours after the surgical intervention (postoperative period). These blood samples were used to examine the biochemical parameters (nitric oxide (NO) level and arginase activity).

Surgical Technique

All the patients were operated by the same surgical and anesthetic team in the same operation room. Six-channel electro cardio gram (ECG) and non-invasive arterial pressure monitoring were applied to the patients onto the operating table. Radial artery catheter was inserted under local anesthesia prior to anesthesia induction. Blood samples of the patients were taken along with the initial blood gas in the preoperative period. Afterwards, invasive pressure monitoring was realized. 100 mg lidocaine intravenous (iv), 300 mg magnesium iv., 100 µgr fentanyl iv., 0.60-1.2 mg/kg esmeron iv., and 2 mg/kg propofol iv., were used to realize anesthesia induction. Following the anesthesia, central venous cannula and urine catheters were inserted. General anesthesia was maintained by adding 20 mg esmeron and 100 µgr fentanyl iv in the oxygenator reservoir with 30-minute intervals.

While propofol (%1) was infused as 20 ml/hour iv., out of CPB; it was reduced to 10 ml/hour iv., during CPB. All the patients were applied with a median sternotomy. Heparin of 350-400 unit/kg was administered to left internal mammary artery (LIMA) before cannulation. Then, routine aortic and right atrial cannulation was performed. Cardiopulmonary bypass (CPB) was realized by using membrane oxygenators and moderate systemic hypothermia. Myocardial protection was provided by antegrade mild hypothermic blood cardioplegia (32˚C), repeated with 20-minute intervals. 2 mmol/lt magnesium sulphate, 5 mmol/lt potassium chloride, 1.6 gr/1000 cc sodium bicarbonate were added in every 1000 cc blood taken from the reservoir to prepare cold blood cardioplegia. Activated clotting time was >400 sec during the procedure. Mean blood pressure was maintained at 60 mmHg and over during the procedure. A single clamp technique was used to realize all the proximal saphenous vein anastomoses through cross clamp. Air was discharged from the proximal anastomoses and then cross clamp was removed. Once adequate cardiac performance was ensured, pump flow was decreased and CPB was terminated. Protamine at the ratio of 1:1.3 was used for 10 minutes following CPB procedure to neutralize heparin.

In the off-pump operation; the patients were performed and anesthesia induction was performed in the same way, its dosage was adjusted in order for the active clotting time (ACT) to be 250 following LIMA harvesting and for heparin to be administered at 80-100 unit/kg dosage after median sternotomy. The side clamp was used mainly to perform proximal saphenous vein graft anastomoses. Then, a stabilizer (Estech hercules) was used to realize distal coronary anastomoses through an intracoronary shunt. Once the left anterior descending (LAD) artery was filled with blood using LIMA or saphenous vein graft, anastomoses of other coronary arteries were realized. In the present study, the patients did not have any bloody circumflex artery and branches. Esmelol (brevibloc premix 10 mg 250 ml) infusion was administered in order for the heart rate to be 70 beats per minute and for the blood pressure to be over 60 mmHg. Protamine and closure were administered routinely to reverse the heparinized state.

All of on-pump and off-pump patients were followed up in the intensive care unit in the postoperative period**.** After they were taken into the intensive care unit, their second (peroperative) blood samples were taken from the radial artery catheter together with the blood gas. Third (postoperative) blood samples were taken in the approximately postoperative 24 hours during the intensive care follow-up. All the samples were sent immediately to the laboratory so that they were properly prepared and stored.

Sample Collection

After blood samples were taken in two test tubes containing heparin via the cannula inserted in radial artery, they were taken to the laboratory to perform invasive blood pressure monitoring**.** One of the heparinized bloods was used as the full blood; whereas, the other heparinized blood was centrifuged at 3000 rpm for 5 minutes. Its plasma was separated and washed three times by physiological saline solution. Afterwards, it was stored at the deep-freezer at -80ºC until biochemical analyses.

Arginase activity and NO level

The increase in the amount of urea (the reaction product) was determined to measure arginase activity [7]. One unit (U) of enzymatic activity was defined as µmol of the product per hour at 37 ºC. The results were given as units/mg of protein.

The NO level of the tissue samples was assayed based on the method of Griess [8].

The protein content of the tissue samples was assayed based on the method of Lowry et al. [9]. Bovine serum albumin was used as the standard.

Statistical Analysis

The SPSS package program (15.0 for Windows) was used to perform statistical analysis. Normality analyses were carried out by Kolmogorov-Smirnov Goodness of Fit Test. As a result of this test, it was specified that the groups were normally distributed at a significance level of α=0.01. However, while NO showed a normal distribution only in the postoperative period for the significance of α=0.05, there was no normal distribution in preoperative and peroperative periods. Although arginase showed a normal distribution in preoperative and postoperative periods at significance level of α=0.05, it had no normal distribution in peroperative period (Table 1, Table 2). Thus in this group test, Mann Whitney –U test was preferred. Mean ± standard error mean (SEM) were used to illustrate all the results.

Table 1: Normality distribution for NO in preoperative, peroperative and postoperative periods.

	Kolmogorov-Smirnov Goodness of Fit Test		
	Statistic value	**df**	**p-value**
Peroperative	0.181	28	0.019
Postoperative	0.148	28	0.118*
Preoperative	0.181	28	0.019

*: df; degree of freedom

Table 2: Normality distribution for arginase in preoperative, peroperative and postoperative periods

	Kolmogorov-Smirnov Goodness of Fit Test		
	Statistic value	**df**	**p-value**
Preoperative	0.120	28	0.200*
Peroperative	0.234	28	0.000
Postoperative	0.083	28	0.200*

*: df; degree of freedom

Results

No statistically significant difference was found when the preoperative, peroperative and postoperative periods of the patients, who underwent on-pump and off-pump bypass surgery, were compared (p>0.05) (Table 3). When the preoperative, peroperative and postoperative periods of the patients undergoing on-pump and off-pump bypass surgery were compared in arginase enzyme activity; a statistically significant difference was found between the on-pump and off-pump bypass surgeries in the preoperative period (p<0.05) (Table 4). The biochemistry data of all of the patients undergoing on-pump and off-pump bypass surgery revealed no statistical significance (p>0.05) (Table 5).

Table 3: Statistical comparison of on-pump and off-pump in terms of NO parameter for the preoperative, peroperative, and postoperative periods.

Periods	**On-Pump**	**Off-Pump**	**p**
Preoperative period	0.290±0.024	0.323±0.054	0.072
Peroperative period	0.314±0.044	0.300±0.023	0.496
Postoperative period	0.300±0.024	0.290±0.022	0.185

Table 4:. Statistical comparison of on-pump and off-pump in terms of arginase parameter for the preoperative, peroperative, and postoperative periods.

Periods	**On-Pump**	**Off-Pump**	**p**
Preoperative period	16.982±6.458	30.756±7.797	0.000
Peroperative period	20.228±8.806	19.947±6.239	0.964
Postoperative period	22.804±7.126	20.142±6.716	0.496

Table 5. Demographic data of the on-pump and off-pump patients.

Σn 28	**Groups**	**Urea**	**Creatinine**	**AST**	**ALT**	**CRP**
Presence of DM	Yes	42.66±5.52	1.07±0.09	22.00±4.93	23.77±4.96	7.57±0.50
		34.88±2.88	0.91±0.06	23.82±2.58	25.82±3.48	7.47±0.64
	No	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
	P					
Presence of HT	Yes	38.26±3.01	0.97±0.58	23.30±2.56	24.95±2.91	7.27±0.46
		32.33±4.48	0.96±0.31	22.33±6.56	26.33±11.46	9.32±1.31
	No	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
	P					
Smoking status	Yes	35.45±4.57	1.04±0.62	23.54±4.06	26.90±5.25	7.52±0.72
		39.13±3.39	0.92±0.76	22.93±2.90	23.80±3.08	7.50±0.59
	No	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
	P					
Presence of COPD	Yes	40.77±6.81	1.06±0.75	21.00±4.93	25.22±5.92	6.69±0.77
		35.88±2.20	0.92±0.66	24.35±2.55	25.05±3.07	7.94±0.54
	No	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
	P					

Discussion

Cardiopulmonary bypass-assisted surgery results in a systemic inflammatory response due to extrinsic and intrinsic factors like anesthesia, endothelial cell activation, tissue damage, contact activation with in the extracorporeal circuit, endotoxemia and ischemia reperfusion injury of the myocardium [10, 11].

L-arginine is a key chemical in cardiovascular health and a substrate for the generation of NO [12]. Dysfunction of the endothelial L-arginine-nitric oxide pathway is common in cardiovascular diseases.

Previous studies pointed out that L-arginine levels reduced in cardiovascular diseases [13]. Also, oxidative stress improved the activity of arginase enzyme converting arginine into ornithine and confining NO bioavailability in endothelial cells due to the increase in arginine consumption [12-14].

Even though the arginase activity decreased, the NO level increased. This may be a resistance strategy of the cells against hypoxic stress [16].

In this study, it was determined that arginase activity significantly increased in the preoperative period in the patients undergoing the off-pump bypass surgery; however no statistically significant difference was observed between the on-pump and off-pump bypass surgeries in the arginase activity in peroperative and postoperative periods, also no statistically significant change was found in the NO level of the patients undergoing on-pump and off-pump bypass surgeries in the preoperative, peroperative and postoperative periods. The reason of not determining any difference in the peroperative period and postoperative period may be associated with the fact that the blood samples were taken approximately after 24 hours especially in the postoperative period, a statistically significant result might have been obtained if blood was taken in the postoperative periods beyond 24 hours and the arginase activity and NO level were determined.

High arginase activity results in reduced NOS activity in post-ischemic tissues, low perfusion, and prolongation of ischemic time [16]. Additionally, low NO/cGMP levels increased reperfusion injury, loss of endothelial barrier function, and the susceptibility to cardiac arrhythmia [17]. This situation shows parallelism with the increase in the preoperative period in off-pump bypass surgery in the present study. It is considered that bypass surgery may lead to the development of different cardiovascular diseases in the future especially by increasing the arginase activity and decreasing NO level.

The postoperative period in the present study was approximately 24 hours after the patients undergoing on-pump and off-pump bypass surgery were taken to the intensive care unit. If blood samples were taken in the periods after the postoperative period, i.e. after about 24 hours, and arginase activity and NO level were measured in the blood samples, we could have found that the increase in the arginase activity and the decrease in NO level after the on-pump or off-pump bypass surgery were statistically significant. Also it was considered that the number of patients in this study was not sufficient since the patients were selected from a single center and there is a limited number of off-pump bypass operation conducted. Thus, it is intended to determine the arginase activity and NO level in the blood samples taken from more number of patients in our further studies. Also, in addition to the samples taken approximately after 24 hours in postoperative period, it is planned to take samples beyond these 24 hours.

It is possible to prevent complications associated with the bypass surgery by determining the treatment methods for the decreased NO level and the increased arginase enzyme activity due to the bypass surgery so that these patients can have an enhanced quality of life and be healthy after the surgery.

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