RESEARCH ARTICLE

F.U.Med. J. Health Sci.
2018; 32 (1): 41 - 45
http://www.fusabil.org

The Effect of Cardiopulmonary Bypass Surgery on Arginase Activity and Nitric Oxide Levels

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Received : 04.01.2018
Accepted : 12.06.2018

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Objective: The aim of the study was to assess the endothelial function with arginase activity and nitric oxide (NO) levels in patients undergoing coronary artery bypass grafting (CABG) surgery.

Materials and Methods: The study was conducted on 26 patients undergoing CABG surgery. Blood samples were collected from the patients in the preoperative, peroperative, and postoperative periods. Arginase activity and NO levels were measured spectrophotometrically from the collected samples.

Results: Arginase activity decreased significantly in patients in the peroperative period compared to the preoperative period. There was no significance in terms of arginase activity in the postoperative period compared to the preoperative and peroperative periods. On the other hand, NO levels increased significantly in patients in the peroperative period. There was no significant increase in terms of NO level in the postoperative period; whereas, there was an insignificant increase compared to the preoperative period.

Conclusion: Complications that may occur after the bypass surgery can be avoided by determining the treatment methods that will prevent the decrease in NO level and the increase in arginase enzyme activity occurring as a result of the bypass surgery and it can be ensured these patients to have a more enhanced quality of life and to be healthy after the surgery.

Key Words: *Cardiopulmonary bypass, arginase, nitric oxide*

Kardiyopulmoner Bypass Cerrahisinin Arginaz Aktivitesi ve Nitrik Oksit Seviyeleri Üzerine Etkisi

Amaç: Çalışmanın amacı, koroner arter bypass greftleme (CABG) cerrahisi uygulanan hastalarda, arginaz aktivitesi ve nitrik oksit (NO) düzeyleri ile endotel fonksiyonlarını değerlendirmektir.

Gereç ve Yöntem: Çalışmada, CABG ameliyatı geçiren 26 hasta yer aldı. Hastalardan preoperatif, peroperatif ve postoperatif dönemlerde kan örnekleri alındı. Alınan kan örneklerinde arginaz aktivitesi ve NO seviyeleri spektrofotometrik olarak ölçüldü.

Bulgular: Arginaz aktivitesi, peroperatif dönemdeki hastalarda preoperatif döneme göre anlamlı olarak azaldı. Postoperatif dönemde arginaz aktivitesi bakımından preoperatif ve peroperatif dönemlere göre anlamlı bir fark tespit edilmedi. Ayrıca, peroperatif dönemdeki hastalarda NO düzeyleri anlamlı bir şekilde arttığı belirlendi. Postoperatif dönemde ise NO düzeyinde istatistiksel olarak anlamlı bir artış olmamasına rağmen, preoperatif döneme göre artış beirlendi.

Sonuç: Bypass cerrahisinden sonra meydana gelebilecek komplikasyonlar, bypass ameliyatı sonucunda ortaya çıkan NO düzeyinde azalmayı ve arginaz enzim aktivitesindeki artışı engelleyecek tedavi yöntemlerinin belirlenmesi ile önlenebilir ve bu hastaların operasyon sonrası daha kaliteli ve sağlıklı bir hayat yaşamaları sağlanabilir.

Anahtar Kelimeler: *Kardiyopulmoner bypass, arginaz, nitrik oksit*

Introduction

Being an essential part of many cardiothoracic procedures, cardiopulmonary bypass (CPB) is known to be associated with an excessive undesirable systemic inflammatory response and cardiac biomarkers released in reaction to CPB and surgical trauma (1). Consequently, a systemic inflammatory response arises from 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 significantly associated with a number of postoperative complications such as respiratory failure, pulmonary damage, and brain damage (1).

Arginine is fundamental in the synthesis pathway of NO; therefore, L-arginine plays the most important role in the regulation of vascular health and homeostasis (2). NO is synthesized from arginine by the NO synthase (NOS) isoforms: while neuronal NOS (nNOS) is primarily present in neuronal cells, endothelial NOS (eNOS) is primarily present in endothelial cells, and cytokine-inducible NOS (iNOS) is present in various cell types including macrophages, hepatocytes, muscles, and chondrocytes. The eNOS have a role in regulation of the physiological vascular tone (3). Both NOS and arginase use arginine as a common substrate. On the other hand, arginase may decrease NO production by competing with NOS for arginine (4).

Reduced biological effectiveness of NO causes the cardiovascular pathology-related vasodilatation, anti-thrombotic, anti-inflammatory and anti-apoptotic actions to be impaired (5).

In the ischemic/reperfused heart, low regulation of eNOS occurs 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. In conclusion, the reduction of the intracellular arginine pool by arginase activation may protect cells under the conditions in which NOS is not able to synthesize NO. This advantage brought by high arginase activity during ischemia may turn into a serious disadvantage when the tissue is reperfused (6).

The aim of the study was to assess the endothelial function with arginase activity and NO level in patients undergoing coronary artery bypass grafting (CABG) surgery.

Material and Methods

The Population Study: A total of 26 patients undergoing on-pump bypass operation (5 women, 21 men) were included in the study. All the patients undergoing the elective cardiopulmonary bypass were included in this study. They were informed about the study and an informed consent form stating that they were voluntary to participate in the study was signed by them. The present study's protocol was reviewed and approved by the ethics committee of Fırat University (Reg. No. 2015015).

In the present study, blood was drawn 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). Nitric oxide (NO) level and arginase activity, which were the biochemical parameters, were examined in these blood samples.

Surgical Technique: All patients were managed by the same surgical and anesthetic team in the same operating room. Six-channel electrocardiogram (ECG) and non-invasive arterial pressure monitoring were applied to the patient taken to the operating table. Before anesthesia induction, the radial artery catheter was inserted under local anesthesia, preoperative blood samples were taken together with the initial blood gas, and an invasive pressure monitoring was performed. Anesthesia induction was performed by using 100 mg lidocaine intravenous (iv), 300 mg magnesium iv, 100 µg fentanyl iv, 0.60–1.2 mg/kg esmeron iv, and 2 mg/kg propofol iv. Central venous cannula and urine catheters were inserted in the post-anesthesia period. Maintenance of general anesthesia was performed by adding 20 mg esmeron and 100 µgr fentanyl iv in the oxygenator reservoir every 30 minutes.

While propofol infusion (%1) was performed as 20 mL/hour iv out of CPB, it was reduced to the infusion dose of 10 mL/hour iv during CPB. A median sternotomy was performed to all the patients. Heparin of 350-400 unit/kg was administered to left internal mammary artery (LIMA) prior to cannulation, which was followed by routine aortic and right atrial cannulation. Membrane oxygenators and moderate systemic hypothermia were used to carry out the cardiopulmonary bypass (CPB). Myocardial protection was achieved by using antegrade mild hypothermic blood cardioplegia (32 ºC) and repeated every 20 minutes. Cold blood cardioplegia was prepared by adding 2 mmol/L magnesium sulphate, 5 mmol/L potassium chloride, and 1.6 g/1000 cc sodium bicarbonate in every 1000 cc blood taken from the reservoir. Activated clotting time was maintained for >400 sec during the procedure. During the procedure, the mean blood pressure was kept at 60 mmHg and over. All the proximal saphenous vein anastomoses were performed via the cross clamp by using a single clamp technique. Air was discharged from the proximal anastomoses and the cross clamp was removed. After sufficient cardiac performance was provided, pump flow was reduced and CPB was ended. Heparin was neutralized with protamine at the ratio of 1:1.3 for 10 minutes after CPB.

After the operation, all patients were followed up in the intensive care unit**.** Second (peroperative) blood samples were taken from the radial artery catheter together with the blood gas right after the patient arrived to the intensive care unit. Third (postoperative) blood samples were taken approximately 24 hours after the surgery while the intensive care follow-up of the patient was ongoing. All the samples were sent to the laboratory as soon as possible and they were properly prepared and kept.

Sample Collection: Once blood samples were taken in two heparin-containing test tubes by means of the cannula inserted in radial artery, they were taken to the laboratory for invasive blood pressure monitoring**.** While one of the heparinized bloods was used as the full blood, the other heparinized blood was centrifuged at 3000 rpm for 5 minutes and its plasma was separated and then washed three times by using physiological saline solution. Then, it was kept at the deep-freezer at –80 ºC before biochemical analyses.

Arginase activity and NO levels: Arginase activity was measured by determining the increase of the amount of urea (the reaction product) (7). One unit (U) of enzymatic activity was defined as µmol of the product formed per hour at 37 ºC. The results were given as units/mg of protein.

The NO level of the samples was assayed according to the method of Griess (8). To determine the effect of several compounds on NO levels, treated with glutamate (1 mM) or co-treated with L-citrulline, L-arginine, and/or taurine (20 mM). At 24 h after the treatment, 100 µL of culture supernatant was collected from each sample and added to a 96-well micro-plate. Samples were then incubated with 100 µL of modified Griess reagent with 1% sulfanilamide in 5% phosphoric acid at room temperature for 7 min. Finally, 0.1 N-1-

napthylethylenediamine dihydrochloride in water was added followed by incubation at room temperature for 7 min. Absorbance at wave length of 550 nm was measured.

The protein content of the samples was assayed according to the method of Lowry et al. (9). Bovine serum albumin was used as the standard.

Statistical Analysis: Statistical analysis was carried out using the SPSS package program (15.0 for Windows). Once the difference between the groups was compared using Kruskal Wallis Test, which group was different was determined in pairs by applying to Mann Whitney-U test. All of the results were shown as mean ± standard error mean (SEM).

Results

Arginase activity decreased significantly in patients in the peroperative period compared to preoperative period. There was no significance in terms of arginase activity in postoperative period compared to preoperative and peroperative periods (Table 1).

On the other hand, NO levels increased significantly in patients in the peroperative period. However, there was no significant increase in NO levels in the postoperative period and there was insignificant increase compared to preoperative period (Table 1).

No statistical significance was found in the biochemistry data of all of the patients undergoing on-pump bypass surgery (Table 2).

Discussion

Cardiopulmonary bypass-assisted surgery leads to a systemic inflammatory response through 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).

Being a substrate for the generation of NO L-arginine is a key chemical in cardiovascular health (12). Dysfunction of the endothelial L-arginine-nitric oxide pathway is a common mechanism seen in cardiovascular diseases.

Table 1. Statistical comparison of arginase and NO parameters in the preoperative, peroperative, and postoperative periods

Parameters	Preoperative period	Peroperative period	Postoperative period	P
Arginase (Unit)	22.80±1.55[a]	17.81±1.55[b]	21.50±1.36[ab]	P<0.05
Nitric Oxide (µmol/L)	0.28±0.003[a]	0.31±0.006[b]	0.29±0.004[ab]	P<0.05

Letters a and b show statistically different groups

Table 2. Demographic data of the on-pump patients

Σn 26	Groups	Urea	Creatinine	AST	ALT	CRP
Presence of Lima	Yes	33.90±3.27	0.99±0.10	26.45±3.51	26.45±4.34	7.78±0.66
	No	40.26±4.00	0.95±0.50	20.80±3.11	24.13±3.77	7.31±0.62
	P	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
Presence of DM	Yes	42.66±5.52	1.07±0.09	22.00±4.93	23.77±4.96	7.57±0.50
	No	34.88±2.88	0.91±0.06	23.82±2.58	25.82±3.48	7.47±0.64
	P	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
Presence of HT	Yes	38.26±3.01	0.97±0.58	23.30±2.56	24.95±2.91	7.27±0.46
	No	32.33±4.48	0.96±0.31	22.33±6.56	26.33±11.46	9.32±1.31
	P	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
Smoking status	Yes	35.45±4.57	1.04±0.62	23.54±4.06	26.90±5.25	7.52±0.72
	No	39.13±3.39	0.92±0.76	22.93±2.90	23.80±3.08	7.50±0.59
	P	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05
Presence of COPD	Yes	40.77±6.81	1.06±0.75	21.00±4.93	25.22±5.92	6.69±0.77
	No	35.88±2.20	0.92±0.66	24.35±2.55	25.05±3.07	7.94±0.54
	P	P>0.05	P>0.05	P>0.05	P>0.05	P>0.05

Previous studies revealed the reduced L-arginine levels in cardiovascular diseases (13). Also, oxidative stress enhanced the activity of arginase enzyme which converts arginine into ornithine and confined NO bioavailability in endothelial cells as a result of increased arginine consumption (12-14). Another study suggested that plasma levels of asymmetric dimethylarginine (ADMA), symmetric dimethylarginine (SDMA), L-arginine and L-arginine/ADMA ratio were reliable and feasible indicators for early ischemia reperfusion injury (15).

When the arginase activity and NO levels in the bloods of the patients, undergoing cardiopulmonary bypass surgery, in the preoperative, peroperative and postoperative periods were examined in the present study; it was found that while a significant decrease was observed in the arginase activity in the peroperative period compared to the preoperative period, there was a significant increase in the NO level in the peroperative period. However, even though it was not significant, an increase was observed in the arginase activity in the bloods taken after approximately 24 hours after the end of the operation, which is the postoperative period, compared to the peroperative period; whereas, a decrease was determined in the NO level although it was not significant. The increase in the NO level observed despite the decrease in the arginase activity can be a resistance strategy of the cells against hypotoxic stress (16) also in the present study, the NO level significantly increased in parallel to the significant decrease in the arginase activity in the peroperative period and these results can be interpreted as a strategy of the cells against hypotoxic stress. The results obtained from both other studies and the present study reveal that arginine mainly decreases regardless of the increase in the arginase activity or NO level and the decreased arginine level aims to protect hypoxic cells from irreversible damage (16).

High arginase activity leads to reduction of the NOS activity in post-ischemic tissues and thus low perfusion and prolongation of ischemic time (16). Furthermore, low NO/cGMP levels make contribution to reperfusion injury, loss of endothelial barrier function, and increase the susceptibility to cardiac arrhythmia (16). The fact that there was an increase in the arginase activity but not significant and also a decrease in the NO level but not significant in the postoperative period compared to peroperative period in the present study is parallel to other findings. We think that bypass surgery may cause the development of different cardiovascular diseases in the future especially by increasing the arginase activity and decreasing NO level.

Also we would like to state that the postoperative period in the present study covered approximately 24 hours after the patients were taken to the intensive care unit. Perhaps if blood samples were taken in the following periods after the postoperative period, i.e. after the period of approximately 24 hours, and arginase activity and NO level measurements were performed in the blood samples, we could have determined that the increase in the arginase activity and the decrease in NO level after the bypass surgery were statistically significant. In our further studies, by considering these periods, the blood samples would be taken for the periods after 24 hours after the surgery and arginase activity and NO level would be measured.

In conclusion, the results of the other studies (16, 17) and the present study reveal that these parameters observed are important in the development of postoperative complications. Complications that may occur after the bypass surgery can be avoided by determining the treatment methods that will prevent the decrease in NO level and the increase in arginase enzyme activity occurring as a result of the bypass surgery and it can be ensured these patients to have a more enhanced quality of life and to be healthy after the surgery.

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