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The Effect of Statin Therapy on Stimulation of Endothelium-Derived Nitric Oxide before and after Coronary Artery Bypass Surgery

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ABSTRACT

Background: The purpose of this study was to determine the effects of statins on endothelium-derived nitric oxide (NO) levels during coronary artery bypass grafting (CABG) surgery.

Methods: In a prospective study, 130 patients with coronary artery disease were randomized according to preoperative atorvastatin treatment. The patients in group 1 took 40 mg atorvastatin daily for at least 1 month preoperatively, and those in group 2 took no atorvastatin preoperatively. Plasma nitrite and nitrate were measured at baseline and after inducing reactive hyperemia, both before and after surgery. Reactive hyperemia was induced by placing a blood pressure cuff on the upper forearm, inflating it for 5 minutes at 250 mm Hg, and then rapidly deflating the cuff. Blood was collected from the radial artery on the same side 2 minutes after cuff deflation. Plasma levels of total cholesterol, triglycerides, and high- and low-density lipoproteins were measured and analyzed for correlations with NO.

Results: The mean (±SD) baseline plasma NO levels before operation were as follows: group 1, 33.97 ± 18.27 nmol/L; group 2, 24.24 ± 8.53 nmol/L ($P < .001$). A significant difference between the 2 groups in plasma NO levels was observed after preoperative reactive hyperemia induction: group 1, 56.43 ± 15.03 nmol/L; group 2, 43.12 ± 10.67 nmol/L ($P < .001$). Two hours after cardiopulmonary bypass (CPB), we observed no significant differences in plasma NO levels, either at baseline (group 1, 11 ± 3.41 nmol/L; group 2, 9 ± 5.51 nmol/L) or after reactive hyperemia (group 1, 17.98 ± 6.77 nmol/L; group 2, 18.00 ± 6.47 nmol/L). A correlation with preoperative nitroglycerine use was observed ($P = .007$; $r = 0.23$). Linear regression analysis ($F = 1.463$; $R = 0.314$; $R^2 = 0.099$; $P = .16$) indicated that the only significant correlation was with preoperative nitroglycerine use ($P = .007$; t = 2.746).

Conclusions: Preoperative atorvastatin treatment in patients with coronary artery disease increases plasma NO levels before and after reactive hyperemia prior to surgery. CABG surgery with CPB significantly impairs endothelial-derived NO levels, with or without preoperative atorvastatin treatment. Preoperative nitroglycerine use is correlated with higher NO levels after CABG.

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INTRODUCTION

Coronary artery bypass grafting (CABG) surgery with cardiopulmonary bypass (CPB) has adverse effects on endothelial functions, including hypoxia during cardioplegic arrest, inflammatory effects of cytokines, and direct physical damage [Levy 2003]. CPB thus reduces the ability of the endothelium to synthesize and release nitric oxide (NO), leading to an increased risk of postoperative complications [Beghetti 1998; Krishnadasan 2000].

The 3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitors, known as "statins," are widely used to control hypercholesterolemia. Statin use also improves endothelial dysfunction. These beneficial effects of statins are related to other mechanisms independent of cholesterol lowering [Rosenson 1998]. Recent studies have demonstrated that atorvastatin treatment is associated with a considerable improvement in forearm blood flow after reactive hyperemia [Simons 1998; Masumoto 2001].

Shear stress is the strongest physiological stimulus of endothelial NO synthase (eNOS) activity. Reactive hyperemia is a provocative test for eNOS activity and is induced by placing a blood pressure cuff around the forearm of the patient and inflating it to 250 mm Hg for 5 minutes [Rassaf 2006]. For this study, we measured the levels of endothelial-derived NO levels at baseline and after reactive hyperemia, both before and after surgery. In this regard, biochemical data comparing endothelial-derived NO concentrations before and after CABG with CPB have not previously been published.

MATERIALS AND METHODS

Study Protocol

In a prospective study, we randomized 130 patients with coronary artery disease from January 1, 2008, to May 31, 2009, to preoperative treatment with 40 mg atorvastatin daily (group 1, n = 65) or to placebo (group 2, n = 65). Both the patients and the physicians were blinded to the drug-assignment group. Inclusion criteria included the following: an age between 45 and 75 years, New York Heart Association (NYHA) class I or

II, coronary artery disease confirmed by angiographic study, and hypercholesterolemia. Exclusion criteria included the following: diabetes mellitus, renal or hepatic impairment (blood creatinine >1.5 mg/dL; blood aspartate aminotransferase and blood alanine aminotransferase levels >2-fold higher than normal values), congestive heart failure, active inflammatory diseases, and a history of myocardial infarction within the previous 6 months. The study was approved by the local ethics committee, and consent was obtained from all patients. Treatment with β-adrenergic blocking agents, nitrate, vasodilators, and calcium channel blocking agents was continued preoperatively until the day of surgery.

All patients admitted for CABG were evaluated in the clinic preoperatively for their recommended medications, including statin therapy. All patients in group 1 received 40 mg atorvastatin daily for at least 30 days preoperatively. Group 2 patients received no treatment. Blood samples for NO measurement were withdrawn at baseline and after reactive hyperemia, both before surgery and 2 hours after surgery, for a total of 4 times.

The measurement of NO was based on the method introduced for reactive hyperemia [Masumoto 2001; Kerst 2004; Wassmann 2004]. A pressure cuff was placed at the brachial artery, inflated up to 250 mm Hg for 5 minutes, and subsequently deflated. This maneuver increases the shear stress exerted by the flow of blood over the surface of the endothelium in the brachial artery of the forearm circulation [Anderson 1995; Corretti 2002]. Shear stress over the endothelium increases NO production by stimulating eNOS. For the measurement of endothelium-derived NO, the arterial blood sample was withdrawn from the radial artery catheter on the same side 2 minutes after deflation of the cuff. The blood sample was placed into precooled tubes containing EDTA, immediately centrifuged for 15 minutes at 2000g, and stored at –70°C. NO-derived end products (NO_2, NO_3) were measured with the tri-iodide/ozone–based chemiluminescence assay, which is described elsewhere [Rassaf 2002]. In brief, the plasma sample was divided into 2 aliquots. One aliquot was processed with the NO chemiluminescence analyzer. The difference between the 2 aliquots was calculated to yield the nitrite concentration in the plasma sample. Plasma levels of total cholesterol, triglycerides, and high- and low-density lipoproteins were measured before and after the operation. The data collected for these parameters were analyzed for correlations with plasma NO levels.

Operative Procedure

All patients underwent CABG surgery with CPB via standard procedures. The ascending aorta was cannulated for arterial cannulation, and the right atrium was cannulated for venous cannulation. Induction and maintenance of anesthesia were similar for all of the patients, including weight-related doses of fentanyl, midazolam, and pancuronium bromide. A central venous catheter and a radial artery cannula were inserted. The pump was primed with 1500 mL of Ringer lactate solution plus 200 mL of 20% mannitol. The heart was exposed through a median sternotomy, and 300 U/kg of sodium heparin was administered intravenously before CPB to produce an activated clotting time >400 seconds. The hematocrit was maintained between 20% and 25%, and pump flows were kept between 2.0 and 2.5 L/min per m^2 to keep the mean arterial pressure between 50 and 70 mm Hg. All patients were cooled to moderate hypothermia (mean, 30°C), and cardioplegic arrest was achieved with cold blood cardioplegia (4°C). Antegrade blood cardioplegia or combined antegrade and retrograde blood cardioplegia was used for myocardial protection. The left ventricle was vented through the aortic root. After decannulation, protamine sulfate (10 mg/mL; Lilly, Indianapolis, IN, USA) was administered intravenously at a dose of 1 mg/300 U heparin to neutralize the heparin.

Statistical Analysis

A statistical software package for Windows (SPSS for Windows, version 15.0; SPSS, Chicago, IL, USA) was used for data analysis. A Student *t* test for paired samples was used for variables with a normal distribution. Independent-sample *t* tests, χ^2 tests, Pearson correlation analysis, and linear regression tests were performed. The data were expressed as the mean ± SD. Data for categorical variables were expressed as percentages. A *P* value <.05 was considered statistically significant.

RESULTS

The demographic characteristics of the 2 groups are listed in Table 1. A combination of β-adrenergic blocking agents, nitroglycerine vasodilators, and calcium channel blocking agents were used by all patients. None of the patients in either group were receiving corticosteroids or other nonsteroidal anti-inflammatory drugs. Patients who were receiving atorvastatin treatment preoperatively did not experience any side effects related to the drug. The 2 groups were similar with respect to types of procedures, CPB times, and aortic cross-clamp times (Table 2). In group 1, the preoperative lipid profile improved with atorvastatin treatment, and a significant increase in the concentration of high-density lipoprotein was

Table 1. Demographic Characteristics of Group 1 (Preoperative Atorvastatin Treatment) and Group 2 (Placebo)*

	Group 1 (n = 65)	Group 2 (n = 65)	*P*
Age, y	59.6 ± 7.2	61.9 ± 7.1	NS
Female/male sex, n	15/50	14/51	NS
Hypertension, n (%)	43 (66)	46 (70)	NS
β-Blocker use, n (%)	19 (24.6)	20 (30.7)	NS
Nitroglycerine use, n (%)	18 (27)	15 (23)	NS
Ca channel blocker use, n (%)	15 (23)	18 (27.6)	NS
Smoker, n (%)	23 (35.3)	26 (40)	NS
No. of grafts	2.77 ± 0.66	2.82 ± 0.63	NS
Aortic cross-clamp time, min	56.58 ± 9.01	56.44 ± 8.53	NS
CPB time, min	74.72 ± 9.02	73.33 ± 8.23	NS

*Data are presented as the mean ± SD where indicated. NS indicates not statistically significant; CPB, cardiopulmonary bypass.

Table 2. Baseline Laboratory Findings before Preoperative Atorvastatin Treatment*

	Group 1 (n = 65)	Group 2 (n = 65)	*P*
Total cholesterol, mg/dL	238 ± 41	222 ± 28	NS
HDL cholesterol, mg/dL	36 ± 19	39 ± 12	NS
Triglycerides, mg/dL	185 ± 83	213 ± 69	NS
LDL cholesterol, mg/dL	163 ± 33	158 ± 27	NS

*Data are presented as the mean ± SD. NS indicates not statistically significant; HDL, high-density lipoprotein; LDL, low-density lipoprotein.

Table 3. Laboratory Findings after Preoperative Atorvastatin Treatment*

	Group 1 (n = 65)	Group 2 (n = 65)	*P*
Total cholesterol, mg/dL	178 ± 33	224 ± 26	<.05
HDL cholesterol, mg/dL	46 ± 17	36 ± 13	<.05
Triglycerides, mg/dL	138 ± 49	209 ± 58	<.05
LDL cholesterol, mg/dL	108 ± 31	152 ± 33	<.05

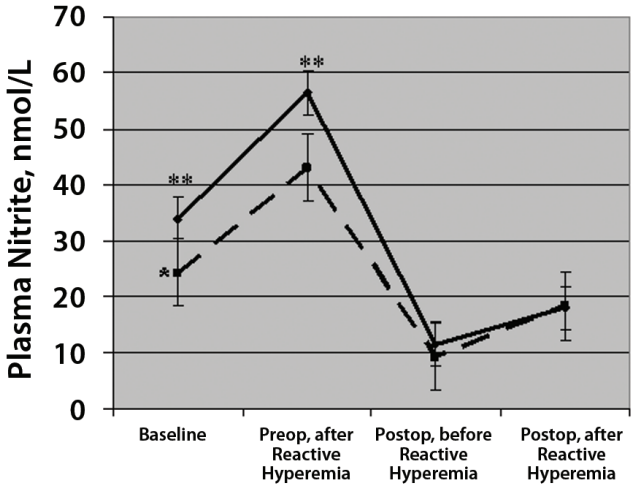
*Data are presented as the mean ± SD. HDL indicates high-density lipoprotein; LDL, low-density lipoprotein.

observed: group 1 before atorvastatin treatment, 36 ± 19 mg/dL; after atorvastatin treatment, 46 ± 17 mg/dL ($P < .05$). The total cholesterol level in group 1 decreased from 238 ± 41 mg/dL before atorvastatin treatment to 178 ± 33 mg/dL ($P < .001$) after atorvastatin treatment (Table 3).

NO levels were measured at baseline and after reactive hyperemia before the patient underwent operation. The baseline NO levels of the 2 groups were significantly different: group 1, 33.97 ± 18.27 nmol/L; group 2, 24.24 ± 8.53 nmol/L ($P < .001$). Preoperative plasma NO levels in the 2 groups were also significantly different following reactive hyperemia: group 1, 56.43 ± 15.03 nmol/L; group 2, 43.12 ± 10.67 nmol/L ($P < .001$). Two hours after the completion of CPB, however, the NO levels in the 2 groups of patients were not significantly different, either at baseline (group 1, 11 ± 3.41 nmol/L; group 2, 9 ± 5.51 nmol/L; $P > .05$) or after reactive hyperemia, (group 1, 17.98 ± 6.77 nmol/L; group 2, 18.00 ± 6.47 nmol/L; $P > .05$) (Figure).

Pearson correlation analysis of the preoperative NO concentrations of all individuals showed no significant relationships with respect to the preoperative parameters of age ($P = .45$; $r = 0.06$), sex ($P = .52$; $r = –0.05$), calcium channel blocker use ($P = .42$; $r = 0.07$), β-blocker use ($P = .90$; $r = –0.01$), preoperative nitroglycerine use ($P = .08$; $r = 0.15$), and NYHA class ($P = .54$; $r = –0.05$).

Pearson correlation analysis of the postoperative plasma NO concentrations of all individuals also showed no significant relationship with any of the following parameters: age ($P = .26$; $r = 0.09$), sex ($P = .12$; $r = 0.13$), calcium channel blocker use ($P = .61$; $r = –0.04$), β-blocker use ($P = .55$; $r = 0.05$), NYHA class ($P = .95$; $r = 0.006$), aortic cross-clamp time ($P = .34$; $r = 0.08$), CPB time ($P = .67$; $r = 0.03$), and number of grafts ($P = .34$; $r = 0.08$). The only significant correlation found was with preoperative nitroglycerine use ($P = .007$; $r = 0.23$). The linear regression analysis of postoperative NO performed at the same time for all individuals for all of the parameters ($R = 0.314$; $R^2 = 0.099$; $F = 1.463$; $P = .16$) revealed a significant relationship only with respect to preoperative nitroglycerine use ($P = .007$; $t = 2.746$). These findings demonstrate that the NO level is significantly impaired in the brachial artery of patients after CPB, whereas preoperative nitroglycerine use correlates with increased NO production.



Comparison of preoperative and postoperative plasma nitrite levels in group 1 (solid line) and group 2 (dashed line) before and after reactive hyperemia. Data are presented as the mean ± SD. *$P < .05$.

DISCUSSION

We addressed 3 important issues in our study. First, preoperative atorvastatin treatment causes a significant increase in plasma NO levels at baseline and after reactive hyperemia. Second, by 2 hours after CPB, plasma NO levels at baseline and after reactive hyperemia were not significantly different. This result might be related to the depletion of plasma NO levels or to impaired eNOS activity during CPB. Impaired eNOS activity after reactive hyperemia might be related to the release of cytotoxic and vasoactive substances during CPB [Downing 1992]. Third, preoperative nitroglycerine use correlates with higher plasma NO levels after CPB.

Several routes of nitrate formation in plasma exist in humans. The concentration of nitrate in plasma is the consequence of its formation and consumption. Nitrate is an oxidation product of eNOS activity. Rassaf and colleagues revealed that 72% to 90% of circulating plasma NO is derived from eNOS activity. Apart from plasma, nitrates are also found within red blood cells. The reduction in NO after CABG with CPB may be related to the destruction of red blood cells [Dejam 2005; Rassaf 2006]. The release of cytotoxic and vasoactive substances may be the causative mechanism of ischemia during surgery [Downing 1992]. Therefore, we cannot demonstrate the exact mechanism for the depletion of NO during CPB.

Previous studies have revealed that endothelial dysfunction in atherosclerosis can be attributed to decreased endothelial NO production. Our findings of decreased preoperative plasma NO levels after reactive hyperemia in the brachial artery correlate with these findings [Anderson 1995]. In most vascular beds, NO release is stimulated by an increase in shear stress associated with an increase in the flow velocity and thereby plays an important role in vasodilatation [Furchgott 1996]. Shear stress–induced NO-dependent dilation of the brachial artery can be measured noninvasively with high-resolution ultrasound as flow-mediated dilatation [Corretti 2002].

In our study, the brachial-occlusion provocative test was used to determine the capacity of eNOS activity in patients undergoing CABG with CPB. Many reports have indicated that statins improve endothelial function in vessels, and the mechanism is believed to be increased by eNOS expression [Kerst 2004]. The present study has proved that preoperative atorvastatin treatment before CABG operation markedly increased plasma NO levels after reactive hyperemia before surgery. In addition, this finding correlates with previous studies that showed considerable improvement in the blood flow in the forearm after reactive hyperemia [Masumoto 2001; Wassmann 2004].

Furthermore, no significant correlation was found in the present study between preoperative and postoperative NO levels in the group treated with atorvastatin or in the group that was not. After surgery, plasma NO levels were decreased in both groups, and no statistical difference was found. Chello and colleagues [2005] found that plasma NO levels were significantly impaired in patients who underwent coronary surgery with CPB; our study findings correlate with those of that study [Chello 2005].

Our finding in the current study demonstrates that preoperative nitroglycerine use has effects on the plasma NO level after CABG with CPB. Nitroglycerine produces vasodilatation by directly activating guanylate cyclase in vascular smooth-muscle cells by providing an inorganic source of NO [Ignarro 2002]. Tsao and colleagues [1990] showed that preoperative nitroglycerine has cardioprotective effects. In our study, preoperative nitroglycerine use correlated with higher plasma NO levels.

Conclusion

This study demonstrated that the atorvastatin treatment in patients with coronary artery disease increases the levels of plasma NO preoperatively; however, after CABG with CPB, plasma NO levels in the patients who received preoperative atorvastatin therapy were not significantly different from the levels in patients who did not receive such therapy.

Limitations of the Study

A better study design for simultaneous evaluation of flow-mediated dilatation and plasma NO levels is recommended for further studies. A prospective randomized study is ongoing in our clinic to compare plasma NO levels with or without CPB (ie, on-pump versus off-pump surgery). The sample sizes of these studies need to be increased to provide valuable data.

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