Diabetes Upregulation of Cyclooxygenase 2 Contributes to Altered Coronary Reactivity After Cardiac Surgery

Jun Feng, MD, PhD, Kelsey Anderson, Arun K. Singh, MD, Afshin Ehsan, MD, Hunter Mitchell, BS, Yuhong Liu, MD, and Frank W. Sellke, MD

Division of Cardiothoracic Surgery, Department of Surgery, Cardiovascular Research Center, Rhode Island Hospital, Alpert Medical School of Brown University, Providence, Rhode Island

Background. We hypothesized that upregulation of inducible cyclooxygenase 2 (COX-2) contributes to altered coronary arteriolar reactivity early after cardioplegic arrest and cardiopulmonary bypass (CP/CPB) in patients with diabetes mellitus who are undergoing cardiac surgery.

Methods. The right atrial tissue samples of nondiabetes (ND), controlled diabetes (CDM), and uncontrolled diabetes (UDM) patients undergoing cardiac surgery were harvested before and after CP/CPB. Coronary arterioles (80 to 150 μm) were dissected from the harvested atrial tissue samples, cannulated, and pressurized. The changes in diameter were measured with video microscopy. The protein expression and localization of COX-1 and COX-2 were assayed by Western blot and immunohistochemistry.

Results. In the diabetes arterioles, bradykinin-induced relaxation response was inhibited by the selective COX-2 inhibitor NS398 at baseline ($p < 0.05$). This effect was more pronounced in UDM arterioles than CDM ($p < 0.05$). After CP/CPB, bradykinin-induced responses in all groups were inhibited by NS398, but this effect was more pronounced in the UDM patients ($p < 0.05$). The intensities of COX-2 staining of coronary arterioles and COX-2 protein levels in myocardium were higher in diabetes than nondiabetes at baseline ($p < 0.05$). The post-CP/CPB protein levels of the inducible COX-2 were significantly increased compared with pre-CP/CPB values in all groups ($p < 0.05$), whereas this increase was higher with diabetes than with ND ($p < 0.05$). Furthermore, these effects were more profound in UDM than CDM ($p < 0.05$).

Conclusions. Diabetes and CP/CPB are associated with upregulation in COX-2 expression in human coronary vasculature. Upregulation of COX-2 expression may contribute to bradykinin-induced coronary arteriolar relaxation in diabetic patients undergoing cardiac surgery.

(Ann Thorac Surg 2017;104:568–76)

Diabetes mellitus is associated with increased morbidity and mortality in patients after cardiac surgery [1, 2]. That is especially true after heart operations involving ischemic cardioplegic arrest (CP) and cardiopulmonary bypass (CPB) [1–6]. Recently, we have demonstrated that diabetes is associated with increased vascular permeability [4, 6], decreased microvascular function [7–9], increased tissue edema, and prolonged postoperative hospital stay [4, 6].

We have found previously that CP/CPB is associated with cyclooxygenase (COX)-2 overexpression in patients undergoing cardiac surgery [10, 11]. Serotonin-induced constriction partially occurs through activation of COX-2 in patients after CP/CPB and cardiac surgery [10, 11]. Interestingly, a recent study reported that diabetes is associated with an increase in COX-2 expression and prostaglandin-mediated bradykinin-induced dilation in human coronary arterioles [12]. However, it is not known whether CP/CPB may differently affect COX-2 expression and microvascular reactivity between diabetes patients and nondiabetes patients or between patients with well-controlled diabetes (CDM) and uncontrolled diabetes (UDM) after cardiac surgery. Therefore, we hypothesized that diabetes and cardiac surgery may significantly affect COX-2 expression early after CP/CPB. We further hypothesized that COX-2 overexpression induced by diabetes may contribute to the altered coronary arteriolar reactivity early after CP/CPB in patients with diabetes.

Material and Methods

Human Subjects and Tissue Harvesting

Samples of right atrial appendage were harvested from patients undergoing cardiac surgery before and after exposure of the heart to blood CP and short-term reperfusion under conditions of CPB [9, 13–16]. The first sample of atrial appendage was harvested before CP/CPB.

Accepted for publication Nov 7, 2016.

Presented at the Scientific Sessions of the American Heart Association, Orlando, FL, Nov 7–11, 2015.

Address correspondence to Dr Sellke, Division of Cardiothoracic Surgery, Rhode Island Hospital, 2 Dudley St, MOC360, Providence, RI 02905; email: fsellke@lifesapn.org.

0003-4975/$36.00
http://dx.doi.org/10.1016/j.athoracsur.2016.11.025

Abbreviations and Acronyms

CDM = controlled diabetes
COX = cyclooxygenase
CP = cardioplegic arrest
CPB = cardiopulmonary bypass
ND = nondiabetes
NOX = nicotinamide adenine dinucleotide phosphate oxidase
PBS = phosphate-buffered saline
UDM = uncontrolled diabetes

The second sample of atrial appendage was harvested after CP/CPB. The standard pump-prime solution (total 1,700 mL) was combined 1,200 mL Plasma-Lyte A (Baxter Healthcare, Deerfield, IL) and 10 mL heparin (1,000 U/mL), 50 mL $NaHCO_3$ (8.4%), 250 mL albumin (5%), 60 mL mannitol (20%), and 10 mL lidocaine (2%). The final ionic concentrations (mmol/L) were 140 Na^+, 4.6 K^+, 2.8 Mg, 76.6 Cl^-, 21 acetate 10 HCO_3^-, and 18 gluconate. Cardiopulmonary bypass was established with a membrane oxygenator (Medtronic, Minneapolis, MN) with target flow rates of 2.4 to 2.8 $L \cdot min^{-1} \cdot m^{-2}$ for all patients.

Cold blood CP (4° to 8°C) consisted of a 4:1 mixture of oxygenated blood with a hyperkalemic crystalloid CP solution (CAPS, Lanham, MD). An initial 600 to 1,000 mL (introduction) of cold-blood (4° to 8°C) hyperkalemic (K^+ 25 mmol/L) CP solution was delivered antegrade into the aortic root, followed by 200 to 500 mL (maintenance) of cold, low-potassium CP solution (K^+ 12.5 mmol/L) every 15 to 20 minutes. Sections of atrial samples were immediately frozen in liquid nitrogen (immunoblotting), fixed in 10% formalin for 24 hours, followed by paraffinization and sectioning into 5-μm slices (immunofluorescent staining), or placed in cold (5° to 10°C) Krebs-Henseleit buffer (microvascular studies). All procedures were approved by the Institutional Review Board of Rhode Island Hospital, Alpert Medical School of Brown University, and informed consent was obtained from all enrolled patients.

The patients were then grouped as follows: (1) patients with a normal hemoglobin A1c and no history or treatment for diabetes (ND group); (2) patients with a history of diabetes with a hemoglobin A1c of 7 or less were considered controlled (CDM group); and (3) patients with a hemoglobin A1c of 8.5 or higher were considered poorly controlled (UDM group).

MICOVESSEL REACTIVITY. Coronary arterioles (80 to 150 μm in internal diameter) were dissected from harvested right atrial appendage tissue-samples before and after CP/CPB. Microvessel studies were performed by in vitro organ bath video microscopy, as described previously [7–9, 13-16]. After a 60-minute stabilization period in the organ chamber, the microvessels were preconstricted with the thromboxane A2 analog U46619 25% to 40% of the baseline diameter. The dose-dependent relaxation was measured in response to the application of the vasodilators endothelium-dependent bradykinin, 10^{-10} to 10^{-5} M. Six vessels each from pre- and post-CP/CPB were pretreated with the selective COX-2 inhibitor NS398, 10^{-5}M, before the perfusion with bradykinin.

IMMUNOBLOT. Protein purification of atrial tissue samples and the method for Western blot have been previously described in detail [6, 9]. Membranes were incubated with individual rabbit polyclonal primary antibodies COX-1, COX-2, and nicotinamide adenine dinucleotide phosphate oxidase (NOX)-1 (ABCAM, Cambridge, MA). The membranes were then incubated with horseradish peroxidase-conjugated secondary antiimmunoglobulin. Peroxidase activity was visualized with enhanced chemiluminescence (Thermo Fisher Scientific, Waltham, MA), and the images were captured with a digital camera system (G Box; Syngene, Cambridge, UK). The imaging bands were quantified with densitometry using ImageJ software (National Institute of Health, Bethesda, MD).

IMMUNOPEROXIDASE STAINING OF COX-1 AND COX-2. Atrial tissue sections from the three groups were deparaffinized in xylene, rehydrated in graded ethanol and phosphate-buffered saline (PBS) solution. The sections were incubated in 3% H_2O_2 for 20 minutes. The section was then incubated with anti-COX-1 and COX-2 antibody, 1 μg/mL, for 60 minutes at room temperature and washed with PBS. The sections were then incubated with biotinylated secondary antibody (Vector Laboratories, Burlingame, CA). The chromogen 3,3′-diaminobenzidine was then used. Tissue was visualized using a Nikon E800 microscope system (Nikon, Melville, NY). Six image photos per slide were taken at the same magnification resolution (×20) for optical density (intensity) analysis (Spot RT3; Diagnostic Instruments, Sterling Heights, MI) [6, 9, 15, 16].

IMMUNOFLUORESCENT STAINING OF COX-2, NOX-2, AND NOX-4. Atrial tissue sections were deparaffinized in xylene, rehydrated in graded ethanol and PBS, and antigen-unmasked with sodium citrate (10 mmol/L, pH = 6.0) followed by PBS wash and blocking with 2% bovine serum albumin in PBS at room temperature for 2 hours [6–9, 11, 12]. After the PBS wash, overnight incubation with anti-COX-2, NOX-2, and NOX-4 (ABCAM) was performed at 4°C. Antimouse, smooth muscle actin (Sigma-Aldrich, St Louis, MO) was used to detect microvascular smooth muscle. Sections were then washed in PBS and incubated with the appropriate Alexa Fluor secondary antibody (Thermo Fisher Scientific) and mounted using fluorescent mounting medium (Vector Laboratories). Tissue was visualized using a Nikon E800 epifluorescent microscope system (Nikon). Six image photos per slide were taken at the same magnification resolution (×20 Plan Fluor [Nikon] objective) for optical density analysis (Spot RT3; Diagnostic Instruments).

PROTEIN OXIDATION. Total protein oxidation in atrial tissue samples (n = 6 per group) was measured according to the manufacturer's recommendation (OxyBlot; Chemicon International, Temecula, CA) [7].

CHEMICALS. Bradykinin, U46619, and NS398 were obtained from Sigma-Aldrich.