Oxidative Stress and Nerve Function After Cardiopulmonary Bypass in Patients With Diabetes

Robina Matyal, MD, Sruthi Sakamuri, BS, Thomas Huang, BA, Khurram Owais, MD, Samir Parikh, MD, Kamal Khabbaz, MD, Angela Wang, BA, Frank Sellke, MD, and Feroze Mahmood, MD

Departments of Anesthesia, Medicine, and Surgery, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, Massachusetts; and Division of Cardiothoracic Surgery, Alpert Medical School of Brown University, Rhode Island Hospital, Providence, Rhode Island

Background. **Chronic hyperglycemia has been associated with increased oxidative stress in skeletal muscle and sympathetic nerve dysfunction. We investigated the effect of chronic hyperglycemia on the myocardium of patients with uncontrolled diabetes (UD) compared with patients with well-controlled diabetes (CD) and patients without diabetes (ND) after cardioplegic cardiopulmonary bypass (CP/CPB) with acute intraoperative glycemic control.**

Methods. **Atrial tissue and serum were collected from 47 patients (ND = 18 with glycated hemoglobin [HbA1c] of 5.8 ± 0.2; CD = 8 with HbA1c of 6.1 ± 0.1; with UD = 21 with HbA1c = 9.6 ± 0.5) before and after CP/CPB for immunoblotting, protein oxidation assays, immunohistochemical evaluation, and microarray analysis.**

Results. **The uncontrolled group had increased total protein oxidation ($p < 0.05$) and decreased levels of antioxidative enzyme manganese superoxide dismutase (MnSOD) ($p < 0.05$) after CP/CPB compared with the controlled group. Collagen staining revealed increased fibrosis in patients with UD ($p < 0.05$) compared with patients with CD and patients without diabetes. The uncontrolled group also showed a decrease in the neurogenic and angiogenic markers nerve growth factor (NGF) ($p < 0.05$), neurotrophin (NT)-3 ($p < 0.05$), and platelet-derived growth factor (PDGF)-β ($p < 0.05$) compared with the other groups after CP/CPB. Atrial and serum microarray analysis showed increased oxidative stress and sympathetic nerve damage, increased fibrosis, and a decrease in angiogenesis in patients with UD ($p < 0.03$) compared with patients without diabetes.**

Conclusions. **CP/CPB led to higher oxidative stress in patients with UD before surgical intervention, even after normal glucose levels were maintained intraoperatively. Thus, controlled HbA1C in addition to acute intraoperative glucose control may be a more suitable end point for patients with diabetes undergoing cardiac operations.**

(Ann Thorac Surg 2014;98:1635–44)

Chronic hyperglycemia and insulin resistance resulting from type 2 diabetes mellitus (DM) are associated with inhibition of glycolysis, impaired fatty acid metabolism, and inflammation [1, 2]. The resulting myocardial oxidative stress leads to cardiomyocyte dysfunction [2]. Poorly controlled DM is also associated with increased oxidative stress and apoptosis in cardiomyocytes after cardioplegic cardiopulmonary bypass (CP/CPB) [2–4]. This mechanism could possibly explain increased myocardial damage after cardiac operations in patients with DM [5]. Autonomic neuropathy that develops in more than 50% of patients with DM is deemed responsible for higher perioperative and cardiovascular instability [6]. Compared with a normal state, there are fewer sympathetic nerves and reduced neurotransmitter concentrations, particularly neuropeptide Y (NPY), in myocardial cells from patients with diabetes [7, 8]. Consequently, the protective effect of neurotransmitters (decreased apoptosis and increased angiogenesis) in response to myocardial ischemia is attenuated [9, 10].

Increased activity of antioxidant enzymes has been associated with absence of cardiomyopathy in rats with diabetes [11]. Similarly, local infiltration of NPY in chronically ischemic myocardium has been shown to increase nerve growth, enhance angiogenesis, and reduce apoptosis and fibrosis in animal models of metabolic syndrome [9, 12]. Nerve growth factor (*NGF*) gene therapy improves sensory and sympathetic innervation in the hearts of animals with diabetes [13–15]. Separately, use of transgenic antioxidant enzyme expression has been shown to reduce diabetic neuropathy and cardiomyopathy [16]. Further examination can potentially delineate new targets for therapeutic intervention.

The association of chronic hyperglycemia with oxidative stress and sympathetic nerve dysfunction in human skeletal muscle has been demonstrated [17]. A similar association in human myocardial cells has not been

Accepted for publication June 9, 2014.

Presented at the Fiftieth Annual Meeting of The Society of Thoracic Surgeons, Orlando, FL, Jan 25–29, 2014.

Address correspondence to Dr Khabbaz, Beth Israel Deaconess Medical Center, Harvard Medical School, Boston, MA 02215; e-mail: kkhabbaz@bidmc.harvard.edu.

0003-4975/$36.00
http://dx.doi.org/10.1016/j.athoracsur.2014.06.041

Abbreviations and Acronyms

ADIPOR1 = adiponectin receptor 1
CABG = coronary artery bypass grafting
CD = controlled diabetics
CP/CPB = cardioplegia/cardiopulmonary bypass
CPT1-M = carnitine palmitoyltransferase 1-mitochondrion
DM = diabetes mellitus
ET-1 = endothelin-1
HbA1c = glycated hemoglobin
MnSOD = manganese superoxide dismutase
NF-κβ = nuclear factor kappa beta
NGF = nerve growth factor
NOS1 = nitric oxide synthase 1
NOX4 = NADPH oxidase 4
NT-3 = neurotrophin-3
OXA1L = oxidase (cytochrome c) assembly 1-like
PARK7 = Parkinson disease (autosomal recessive, early onset) 7
PDGF-β = platelet derived growth factor-β
PGP9.5 = protein gene product 9.5
SMAD5 = mothers against decapentaplegic homolog 5
UD = uncontrolled diabetics

systematically investigated. A correlation would also offer an opportunity to modify myocardial oxidative stress with tighter long-term blood glucose control in patients with diabetes. Therefore, we hypothesized that compared to uncontrolled diabetics (UD) and controlled diabetics (CD), patients without diabetes are better protected against oxidative stress, thus reducing nerve and angiostatic damage. We sought to demonstrate the relationship between chronic hyperglycemia and enhanced oxidative stress in the myocardia of patients with UD undergoing cardiac operations with CP/CPB.

Patients and Methods

Human Participants and Tissue Harvesting

After institutional review board approval and written informed consent, patients undergoing elective coronary artery bypass grafting with CP/CPB were enrolled in the study. Patients were categorized as non-diabetics, with a glycated hemoglobin (HbA1c) value of less than 6.0 and no clinical diagnosis of diabetes; as CD with an HbA1c value ranging from 6.1 to 6.5 and a clinical diagnosis of diabetes; or as UD with an HbA1c value greater than or equal to 6.6. Exclusion criteria consisted of pulmonary disease, valvular disease, cancer, and refusal to participate.

Initial right atrial tissue was harvested after the induction of general anesthesia and median sternotomy, before 600 to 800 mL of cold blood (8°C–48°C) hyperkalemic (15 mmol/L K^+) cardioplegic solution was delivered antegradely into the aortic root. After exposure to cold blood cardioplegia, CPB, reperfusion at the completion of the procedure, and removal of the aortic cross-clamp, a second right atrial tissue sample was collected. The venous cannula was kept in place by a loose suture to prevent ischemia from physical pressure at the site of tissue harvest. Tissue samples (50–100 mg each) were snap frozen in liquid nitrogen for microarray and immunoblot analysis. Tissues for immunohistochemical analysis were fixed in 10% buffered formalin for 24 hours, embedded in paraffin, and then sectioned into 5-mm slices.

Blood was collected through a radial arterial line in the holding area. Ten milliliters of blood was collected and

Table 1. Patient Characteristics

Characteristic	Patients Without Diabetes[a]	Patients With Controlled Diabetes[a]	Patients With Uncontrolled Diabetes[a]	*p* Values No Diabetes Versus Uncontrolled Diabetes
Male/Female, n	16/2	4/4	13/8	0.067
Age (y)	69.7 ± 2.3	66 ± 3.2	63.6 ± 1.4	0.031
HbA1c, %	5.8 ± 0.2	6.1 ± 0.1	9.6 ± 0.5	<0.001
Cross-clamp time, min	59.8 ± 5.4	57.1 ± 8.5	62.4 ± 6.8	0.876
Duration of CPB, min	83.3 ± 6.2	74.1 ± 10.1	86.8 ± 6.9	0.876
Patient blood glucose level, mg/dL, before CPB	143.0 ± 7.3	153.4 ± 19.8	174.4 ± 9.1	0.005
Patient blood glucose level, mg/dL, after CPB	125.3 ± 3.7	125.6 ± 10.6	136.1 ± 8.0	0.279
Diabetes control, n				
Diet	18	3	1	. . .
Oral therapy	0	4	9	. . .
Insulin	0	2	11	. . .
Preoperative aspirin	18	8	21	1.000
Statin drugs	18	8	20	1.000
β-blockers	16	8	21	0.640
Atrial fibrillation	8	1	4	0.181

[a] Data expressed as mean ± standard error of the mean.

CPB = cardiopulmonary bypass; HbA1c = glycated hemoglobin.