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Erythrocytes From Patients With Type 2 Diabetes Induce Endothelial Dysfunction Via Arginase I

Zhichao Zhou, MD, PhD,[a,*] Ali Mahdi, BSc,[a,*] Yahor Tratsiakovich, MD, PhD,[a] Szabolcs Zahorán, MSc,[b] Oskar Kövamees, MD, PhD,[a] Filip Nordin, BSc,[a] Arturo Eduardo Uribe Gonzalez, MD,[c] Michael Alvarsson, MD, PhD,[d] Claes-Göran Östenson, MD, PhD,[d] Daniel C Andersson, MD, PhD,[c,e] Ulf Hedin, MD, PhD,[f] Edit Hermesz, PhD,[b] Jon O Lundberg, MD, PhD,[c] Jiangning Yang, MD, PhD,[a] John Pernow, MD, PhD[a,e]

ABSTRACT

BACKGROUND Cardiovascular complications are major clinical problems in type 2 diabetes mellitus (T2DM). The authors previously demonstrated a crucial role of red blood cells (RBCs) in control of cardiac function through arginase-dependent regulation of nitric oxide export from RBCs. There is alteration of RBC function, as well as an increase in arginase activity, in T2DM.

OBJECTIVES The authors hypothesized that RBCs from patients with T2DM induce endothelial dysfunction by up-regulation of arginase.

METHODS RBCs were isolated from patients with T2DM and age-matched healthy subjects and were incubated with rat aortas or human internal mammary arteries from nondiabetic patients for vascular reactivity and biochemical studies.

RESULTS Arginase activity and arginase I protein expression were elevated in RBCs from patients with T2DM (T2DM RBCs) through an effect induced by reactive oxygen species (ROS). Co-incubation of arterial segments with T2DM RBCs, but not RBCs from age-matched healthy subjects, significantly impaired endothelial function but not smooth muscle cell function in both healthy rat aortas and human internal mammary arteries. Endothelial dysfunction induced by T2DM RBCs was prevented by inhibition of arginase and ROS both at the RBC and vascular levels. T2DM RBCs induced increased vascular arginase I expression and activity through an ROS-dependent mechanism.

CONCLUSIONS This study demonstrates a novel mechanism behind endothelial dysfunction in T2DM that is induced by RBC arginase I and ROS. Targeting arginase I in RBCs may serve as a novel therapeutic tool for the treatment of endothelial dysfunction in T2DM. (J Am Coll Cardiol 2018;72:769–80) © 2018 by the American College of Cardiology Foundation.

Type 2 diabetes mellitus (T2DM) is an important risk factor for cardiovascular diseases, including atherosclerosis and ischemic heart disease. Endothelial dysfunction plays a major role in the etiology of diabetes-induced macrovascular and microvascular complications. This encompasses an imbalance between vasodilators and anti-inflammatory molecules including nitric oxide (NO),

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From the [a]Division of Cardiology, Department of Medicine, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden; [b]Department of Biochemistry and Molecular Biology, Faculty of Science and Informatics, University of Szeged, Szeged, Hungary; [c]Department of Physiology and Pharmacology, Karolinska Institutet, Stockholm, Sweden; [d]Division of Endocrinology and Diabetology, Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden; [e]Heart and Vascular Theme, Karolinska University Hospital, Karolinska Institutet, Stockholm, Sweden; and the [f]Division of Vascular Surgery, Department of Molecular Medicine and Surgery, Karolinska Institutet, Stockholm, Sweden. *Dr. Zhou and Mr. Mahdi contributed equally to this work. Dr. Pernow has received grants to support this work from the Swedish Research Council, the Swedish Heart and Lung Foundation, the Stockholm County Council (ALF), the Karolinska Institutet/Stockholm County Council Strategic Cardiovascular Program, the Söderberg Foundation, the Novo Nordisk Foundation, and the Diabetes Research and Wellness Foundation. Dr. Zhou has received grants from the Olausson Fund of Thorax, Karolinska University Hospital and the Karolinska Institutet Fund for Young Scientist. All other authors have reported that they have no relationships relevant to the contents of this paper to disclose.

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ABBREVIATIONS AND ACRONYMS

ABH = 2(S)-amino-6-boronohexanoic acid

EC = endothelial cell

EDR = endothelium-dependent relaxation

EIR = endothelium-independent relaxation

eNOS = endothelial nitric oxide synthase

GK = Goto-Kakizaki

H_2O_2 = hydrogen peroxide

Healthy RBC = red blood cell from healthy subjects

KH = Krebs-Henseleit

NAC = N -acetyl-cysteine

NADPH = nicotinamide adenine dinucleotide phosphate

NO = nitric oxide

NOS = nitric oxide synthase

NOX = nicotinamide adenine dinucleotide phosphate oxidase

RBC = red blood cell

ROS = reactive oxygen species

T2DM = type 2 diabetes mellitus

T2DM RBC = red blood cell from patients with type 2 diabetes mellitus

vasoconstrictors, and proinflammatory molecules including reactive oxygen species (ROS) (1). The pathogenesis of endothelial dysfunction in T2DM is complex and multifactorial, and this may explain why glucose-lowering therapy has not convincingly reduced mortality among patients at high risk for cardiovascular events (2). Therefore, there is an unmet need to identify key disease mechanisms behind vascular complications in T2DM to develop novel therapies that specifically target such complications.

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Impaired NO bioavailability occurs early and contributes to progression and prognosis of cardiovascular complications in T2DM. NO is produced from L-arginine by endothelial NO synthase (eNOS), which competes with arginase for their common substrate, L-arginine (3). The expression and activity of the 2 isoforms arginase I and II are increased in cardiovascular diseases, including T2DM triggered by several factors including glucose and ROS (3). The increased arginase activity is an important cause of endothelial dysfunction in T2DM as a result of the competition with eNOS for L-arginine and excessive ROS formation stemming from uncoupled eNOS, nicotinamide adenine dinucleotide phosphate (NADPH) oxidase (NOX), or mitochondrial complexes (3). Accordingly, arginase inhibition markedly improves endothelial function in patients with T2DM, a finding suggesting that arginase may serve as a potential therapeutic target for improvement of vascular function (4,5).

Red blood cells (RBCs) may play a fundamental role in cardiovascular homeostasis, by contributing to vascular function and integrity independently from their function as oxygen transporters (6). RBCs undergo functional alterations in T2DM, including reduced NO bioavailability (7) or enhanced oxidative stress (8), which subsequently may affect vascular function. We demonstrated a crucial role of RBCs in control of cardiac function through arginase-dependent regulation of export of NO-like bioactivity from RBCs, thus suggesting a direct interaction of RBCs with cardiovascular function (9). However, key mechanisms behind the interaction of RBCs with the vasculature and their importance in cardiovascular diseases are unclear and elusive. Previous studies suggested that arginase is up-regulated in RBCs from patients with T2DM (10,11). The functional implications of this finding are unknown but may imply a possible causative role of RBC arginase for endothelial dysfunction in T2DM.

Therefore, the present study was designed to test the hypothesis that RBCs from patients with T2DM induce endothelial dysfunction and that this effect is mediated by up-regulation of arginase and ROS formation. We demonstrate a detrimental effect of RBCs from patients with T2DM on endothelial function through up-regulation of arginase at both RBC and vascular levels. In addition, we show that RBCs increase endothelial cell (EC) arginase activity and expression through an ROS-dependent mechanism. These results demonstrate a novel role of RBCs in the development of endothelial dysfunction in T2DM.

METHODS

All experimental protocols regarding human materials were conducted according to the Declaration of Helsinki and were approved by the regional ethical review board in Stockholm. All subjects were informed of the purpose and gave their oral and written consent. All protocols regarding animal studies were approved by the regional ethical committee and conformed to the Guide for Care and Use of Laboratory Animals published by the U.S. National Institutes of Health (NIH publication no. 85-23, revised 1996). An expanded methods section is available in the Online Appendix.

RBC-TISSUE CO-INCUBATION AND FUNCTIONAL EXPERIMENTS. RBCs were isolated from patients and rats with T2DM. Subsequently, RBCs were diluted with Krebs-Henseleit (KH) or serum-free culture medium to a hematocrit of 45% or 10%, and were incubated with aortic rings isolated from rats or internal mammary arteries (IMAs) from nondiabetic patients in cell culture incubator at 37°C with 5% carbon dioxide for 18 h or 1 h. Control arteries were incubated with isolated RBCs from healthy controls, with KH buffer, or with supernatant from RBCs that underwent 18 h incubation. Following incubation, the vessels were thoroughly washed and mounted in wire myograph for determination of endothelium-dependent relaxation (EDR) and endothelium-independent relaxations (EIR) by cumulatively increasing concentrations (10^{-9} to 10^{-5} M) of acetylcholine and sodium nitroprusside, respectively. Additional RBCs and RBC-incubated vessels were subjected to molecular analysis. The same protocols were repeated in the presence of various inhibitors