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EDITORIAL COMMENT

sGC Stimulation Saves the Diabetic Heart

Red Blood Cells to the Rescue*

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Type 2 diabetes mellitus (T2DM) is a major chronic noncommunicable disease with a worldwide prevalence of approximately 9%. Patients with T2D have a 2- to 4-fold increased risk of cardiovascular events and death compared to nondiabetic patients of similar age and demographics.[1] Moreover, the risk of major adverse coronary events in T2DM patients without a history of coronary artery disease is equal to that of patients with coronary artery disease. The risk of a first myocardial infarction (MI) within 10 years of developing T2DM is >20%, whereas the risk of recurrent MI in T2DM patients exceeds 40%. It should be noted that MI is the primary cause of death in T2DM patients.

A key component contributing to cardiovascular complications in T2D is the altered vascular homeostasis characterized by reduced bioavailability of nitric oxide (NO) and increased oxidative stress.[1] Healthy red blood cells (RBCs) release NO metabolites and "NO bioactivity" to exert cardioprotection during ischemia-reperfusion (I-R) injury ex vivo.[2] Cell-specific deletion of endothelial nitric oxide synthase (eNOS) in RBCs exacerbates infarct size and acute MI-induced left ventricular dysfunction after ischemia in mice.[3] In line with the protective role of RBC eNOS in mice, RBCs from patients with T2D with dysregulated eNOS aggravate myocardial I-R injury.[2] Postischemic myocardial recovery is ameliorated by inhibition of arginase or eNOS in RBCs; these pharmacologic interventions result in reduction in oxidative stress and suggest that eNOS is uncoupled in RBCs from T2D patients. These observations taken together provide strong evidence for a protective role of RBC eNOS in the setting of acute MI.

Soluble guanylate cyclase (sGC) has been termed the "NO receptor" and is present in RBCs.[4] It comprises small (β) and large (α) subunits, and a heme prosthetic group that acts as a NO sensor. NO binding to sGC increases its activity to 100-fold and more over baseline. The heme iron in sGC can be found in the oxidized (ferric) or in the reduced (ferrous) NO-responsive form.[4] sGC activity can be increased by 2 distinct classes of compounds: agents that release NO (NO-donors) and agents that work without producing NO. This latter class is further subdivided to sGC activators and sGC stimulators. The difference between the 2 is that sGC activators activate the oxidized, heme-free form of sGC by occupying the heme cavity of the enzyme, whereas stimulators bind to an allosteric site in the heme moiety and increase sGC responsiveness to NO. Riociguat was the first-in-class sGC stimulator to be approved for human use in patients with pulmonary arterial hypertension and chronic thromboembolic pulmonary hypertension. Given the importance of this pathway in the heart and blood vessels, several clinical trials for a variety of cardiovascular diseases such as heart failure (both heart failure with preserved ejection fraction and heart failure with reduced ejection fraction), hypertension, diabetic nephropathy, chronic kidney disease, and peripheral arterial disease have been completed or are currently in progress.[5]

In this issue of *JACC: Basic to Translational Science*, Jiao et al[6] report on the importance of sGC

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stimulation in RBCs to limit cardiac injury and improve cardiac function following I-R injury. Initially, they show that postischemic recovery of the left ventricular diastolic pressure (LVDP) was hampered and infarct size increased in isolated rat hearts perfused with RBCs from patients with T2D compared to hearts perfused with RBCs from healthy individuals. Incubation of RBCs isolated from T2D patients with the sGC stimulator CYR715 enhanced recovery of LVDP and limited infarct size, providing evidence that enhancing sGC signaling improves the function of diabetic RBCs and protects the ischemic myocardium. In contrast to the sGC stimulator, diethylamine NONOate failed to improve the function of RBCs; this is perhaps caused by insufficient dosing or degradation of NO by reactive oxygen species in RBCs from patients with T2D. RBCs per se were not required and the protective effect could be observed by using the supernatant from RBCs stimulated with CYR715, indicating that erythrocytes release soluble factor(s) upon sGC stimulation to limit cardiac damage.

Although previous studies have shown that sGC activators and stimulators exert cardioprotective effects in several models,[7] direct exposure of the isolated hearts to CYR715 in the present study did not improve LVDP recovery, suggesting that cardiac cells do not express functional sGC. Because the sGC activator BAY58-2667 limits infarct size in the same preparation,[7] one might speculate that sGC in cardiomyocytes exists in its apo (hemeless) form. The occurrence of NO/cyclic guanosine monophosphate (cGMP) signaling in cardiac cells has been a matter of debate; in a recent study in mice with cardiomyocyte-specific expression of a fluorescence resonance energy transfer-based cGMP indicator, Menges et al[8] demonstrated that cGMP formed in cardiac fibroblasts enters cardiomyocytes via gap junctions to inhibit phosphodiesterase 3 enabling cAMP signaling. Using MK-571, a nonselective compound that inhibits the multidrug resistance protein 4/5 and the organic anion transporter that imports cGMP into cells, Jiao et al[6] propose that cGMP released from RBCs following sGC stimulation enters the cardiomyocytes to activate downstream signaling. They also observed that incubating the heart with cGMP improved LVDP recovery, lending credence to their hypothesis that cGMP is the soluble factor produced by RBCs that is responsible for cardioprotection in their experimental setting.

However, it should be pointed out that when cGMP was measured in the supernatant of CYR715-stimulated RBCs, it was found to be in the pmol/L range, whereas Jiao et al[6] used 100 μmol/L of exogenously added cGMP in the isolated heart perfusate to demonstrate cardioprotection. Cyclic nucleotides are well known to have very limited membrane permeability, hence researchers have traditionally used modified analogs (eg, 8-Br and 8-(4-Chlorophenylthio) (8-pCPT) pCPT analogs) to facilitate cell entry. Although transport of cGMP between cells does occur, it requires gap junctions.[8] In the case of RBCs and RBC supernatant, it is unclear how cGMP would cross the cardiomyocyte plasma membrane. One could hypothesize that cGMP is transported through exosomes or other small membrane vesicles released by RBCs or that RBCs release soluble factors that enhance the uptake of cGMP or improve its membrane permeability. Alternatively, cGMP might bind to cardiomyocyte receptors to elicit signaling responses, as is the case for nociceptors.

Overall, the findings of the study by Jiao et al[6] propose a novel paracrine model for cGMP signaling in the cardiovascular system and provide evidence that supports cellular targeting of sGC stimulation as a means to reduce infarct size or to limit diabetes-associated cardiovascular complications in the heart and circulation.

We would like to end with a word of caution, because several therapeutic approaches to treat acute MI and myocardial reperfusion injury that have yielded positive results in preclinical models have failed to translate in the clinic. There are multiple reasons that can account for failure of developing therapeutic agents to treat acute MI in humans. These include differences in the biology between animals and humans and flaws in the animal models. The improvement in MI-to-hospital and door-to-balloon times significantly reduces ischemic duration and therefore reperfusion injury and myocardial infarct size. In addition, patients also receive effective antiplatelet medication (ie, $P2Y_{12}$ inhibitors) that reduces myocardial I-R injury and overshadows the effects of any cardioprotective drug that is tested. Long-standing vascular disease and multiple risk factors that exist in humans and are not relevant for otherwise healthy animals used in most preclinical studies add another layer of complexity and lead to failure in translation of drugs that were successful in the animal laboratory.

In spite of the numerous failures thus far to advance drug candidates to everyday clinical practice of treating acute MI, improved experimental design, and better understanding of molecular mechanisms

and pathways will increase the odds of having a new MI-limiting agent available for human use.

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