Oxidative Stress as the Leading Cause of Acute Myocardial Infarction in Diabetics

Clara Di Filippo1,3, Salvatore Cuzzocrea4, Francesco Rossi1,3, Raffaele Marfella2,3*, and Michele D’Amico1,3*

1Department of Experimental Medicine, 2Department of Geriatrics and Metabolic Diseases and 3“Excellence Centre for Cardiovascular Diseases,” Second University of Naples, Naples, Italy
4Department of Clinical and Experimental Medicine and Pharmacology, University of Messina, and 5IRCCS Centro Neurolesi “Bonino-Pulejo”, Messina, Italy

Keywords: Diabetes — Hyperglycemia — Inflammation — M40403 — Myocardial infarction — Oxidative stress.

ABSTRACT

The risk factors, such as hypertension and metabolic syndrome, tend to promote heart pathology. These risk factors can aggravate concomitant heart insults as well. Diabetes mellitus represents one of the most important risk factors for the development of heart pathology. By itself it represents a source of vascular and heart dysfunction through formation of reactive oxygen species (ROS) and can compromise the recovery from cardiovascular diseases. This review focuses on the evidence that cellular oxidative stress is the leading cause of the worst outcome of myocardial infarction (MI) in diabetics. Hyperglycemia is viewed in this article as the primary mediator of a cascade of heart damaging events, starting from ROS formation and leading to myocardial ischemia, inflammation and death of myocytes. This article also provides insights into why diverse therapeutic interventions, which have in common the ability to reduce oxidative stress and inflammation, can impede or delay the onset of complications of myocardial infarction in diabetic patients.

Address correspondence and reprint requests to: Michele D’Amico, Department of Experimental Medicine, Section of Pharmacology, Via Costantinopoli 16, 80138 Naples, Italy; Tel.: +39 (081) 566-5881; E-mail: michele.damico@unina2.it.
*Share last authorship.
INTRODUCTION

Diabetic patients with a nonfatal myocardial infarction (MI) experience a more complicated course of the disease than non-diabetic patients. They have more frequent postinfarction angina, larger infarcts, and are more likely to develop congestive heart failure (69). The basis for these differences in outcome is still unclear. An unusually high prevalence of glycosuria was found to complicate MI as far back as 1931 (11), thus pioneering hyperglycemia as a risk factor for adverse outcome of acute MI in patients with and without diabetes (48). It is now known that in patients who have just experienced MI, glucose values in excess of 110–144 mg/dL are associated with a threefold increase in mortality and a higher risk of heart failure (74). Consequently, hyperglycemia during MI is considered an important and potentially modifiable risk factor for poor outcome (6,9). Although recommendations are being developed for strict glucose management in all hospitalized patients, glucose measurement is not included in MI risk indexes and current MI guidelines do not suggest specific therapeutic targets for glucose control. This relative lack of guidance concerning the risk management of MI patients with hyperglycemia may reflect the fact that many aspects of the relationship between glucose levels and mortality in MI patients as well as the pathogenetic mechanisms responsible for the adverse outcome have not been adequately defined.

In this review we will evaluate the oxidative stress and inflammation as pathogenetic factors responsible for the damaging effects of hyperglycemia during MI. We will also consider why diverse therapeutic interventions, which have in common the ability to reduce oxidative stress and inflammation, can be useful in the treatment of MI in diabetic patients.

THE DAMAGING MEDIATORS

Oxidative stress is usually associated with increased formation of reactive oxygen species (ROS) that play central roles in cardiac physiology and pathophysiology. ROS are molecules that have one or more unpaired electrons in their outer orbit, a state that greatly increases their reactivity (16). Some of the most destructive free radicals generated in organisms are derived from oxygen (O₂, dioxygen) and are continually generated within cells of aerobic organisms. This molecule that is most critical for sustaining life can also damage cells to the point where most organs and organisms fail. The best known reactive species generated from O₂ include the superoxide anion radical (dioxide or O₂⁻), the hydroxyl radical (OH) and the peroxynitrite anion (ONOO⁻). Because of their high reactivity, these radicals can be devastatingly toxic to other molecules and can cause cellular dysfunction and sometimes cells death (16). Free radical damage is a component of several diseases and a vast amount of evidence implicates oxygen-derived free radicals (especially superoxide and hydroxyl radical) and high-energy oxidant (such as peroxynitrite) as mediators of inflammation, shock, and ischemia/reperfusion injury. Free radicals-mediated reaction can cause structural alterations in DNA (e.g., nicking, base-pair mutations, rearrangements, deletions, insertions and sequence amplification). The endogenous reactions that are likely to contribute to ongoing DNA damage are oxidation, methylation, depurination, and deamination (2,72). NO or, more likely, reactive products
derived from it, such as NO\textsubscript{2}, ONOO, N\textsubscript{2}O\textsubscript{3}, and HNO\textsubscript{2}, are mutagenic agents, with the potential to produce nitration, nitrosation, and deamination reactions on DNA bases (62) (Fig. 1). The chemistry of DNA damage by several ROS has been well characterized \textit{in vitro} (4,20,24,68), although specific information about the changes produced by peroxyl (RO\textsubscript{2} \cdot) alkoxyl (RO\cdot), ozone (O\textsubscript{3}), and several of the reactive nitrogen species (RNS) (e.g., ONOO) is lacking. Different ROS affect DNA in different ways. H\textsubscript{2}O\textsubscript{2} does not react with DNA bases at all (2,9), whereas HO\cdot generates a multiplicity of products from all four DNA bases, and this pattern seems to be a diagnostic “fingerprint” of HO\cdot attack (32). Damage to DNA by ROS/RNS seems to occur naturally, in that low steady-state levels of damaged products have been detected in nuclear DNA from human cells and tissues (2,27, 33,49,57). ROS/RNS can also damage mitochondrial DNA, and such damage has been suggested to be important in several human diseases and in the aging process (43,66).

The roles that ROS or RNS play in the DNA damage have not yet been completely elucidated. DNA damage can be repaired by the action of a series of enzymes (17). DNA damage by ROS/RNS can cause multiple lesions, including single and double strand breaks, apurinic/apyrimidinic sites and modified pyrimidines and purines. Repair of these lesions occurs primarily by base excision repair, although nucleotide excision repair may also be involved. A repair system for the abasic apurinic/apyrimidinic sites produced by spontaneous depurination also exists. Areas of current interest include the role of poly(ADP-ribose) polymerase (PARP) in the rejoining of DNA strand breaks, including those induced by ROS (64,65). Poly(ADP) synthetase (PARS) [also known as PARP or poly(ADP-ribose) transferase] is a protein modifying and nucleotide-polymerizing enzyme that is present abundantly in the nucleus (1,15). The obligatory trigger of PARS activation is the nicks and breaks in the DNA strand, which can be induced by a variety of environmental stimuli and free radical (or oxidant) attacks. The physiological function of PARS and poly(ADP-ribosylation) is still under much debate. In the 1980s, Berger and Okamoto observed rapid depletion of NAD\textsuperscript{+} due to PARS activation, leading to cellular ATP depletion, and functional alterations of the cell, with eventual necrotic-type cell death: this process has been termed “the PARS suicide hypothesis.” Research into the “suicidal” role of PARS gained new momentum in the mid-1990s because of the observations \textit{in vitro} that NO\cdot or peroxynitrite can trigger DNA single-strand breakage and PARS.

\textbf{FIG. 1.} Oxidative stress products that induce DNA damage through DNA strand breaks.
activation (23, 56, 70). NO· and peroxynitrite can also inhibit mitochondrial respiration and exert other cytotoxic effects on their own. Thus, it is likely that a synergistic relationship exists between the PARS-mediated pathways and PARS-independent pathways of cellular metabolic suppression. Furthermore, the observations that NO· and peroxynitrite are important mediators of the cellular damage in various forms of inflammation suggest that the PARS-related suicide pathway might play a role in various pathophysiological conditions in vivo.

THE LINK OF MYOCARDIAL INFARCTION TO DIABETES

Hyperglycemia and Diabetic Stress

It appears that hyperglycemia per se can induce oxidative stress. In fact, a feature common to all cell types that are damaged by hyperglycemia is an increased production of reactive oxygen species (ROS) (5, 21, 51). Particularly, increased production of mitochondrial ROS by hyperglycemia is recognized as a major cause of the clinical complications associated with diabetes (5). An overproduction of superoxide by the mitochondrial electron transport during hyperglycemia has been documented (31, 39). Moreover, new interesting data come from studies of nitrotyrosine and the hyperglycemic oxidative stress in the heart (7). Nitrotyrosine formation has been detected during acute hyperglycemia in working rat hearts during hyperglycemia (8). Acute exposure to high glucose increases iNOS gene expression, paralleled by a simultaneous increase of both NO and O₂⁻ production (8). The interaction of O₂⁻ with NO is very rapid and leads to inactivation of NO and production of the potent oxidant peroxynitrite (8). Furthermore, increased plasma nitrotyrosine levels have been detected in diabetic subjects (8, 30). Frustaci et al. showed a co-localization of nitrotyrosine and ischemic damage within the heart of diabetic patients indicating that the two events are correlated. It has been clearly shown in another study performed on patients with acute coronary syndrome undergoing aortic bypass that diabetic heart specimens from ischemic as compared to non-ischemic area contain higher levels of superoxide anion and of nitrotyrosine (45). Moreover, there is evidence that after myocardial infarction, iNOS expression is associated with increased NO and nitrotyrosine levels, as well as increased cytokine levels and apoptosis in the diabetic myocardium of wild-type mice compared with the iNOS⁻/⁻ mutant mice. Furthermore, increases in NO production and nitrotyrosine levels in the wild-type diabetic mice are associated with increased myocardial injury, suggesting that increased NO production from iNOS enhances peroxynitrite formation contributing to myocardial damage (44). This latter event is due to the fact that iNOS abundance leads to nitrosative stress in the heart muscle with accumulation of S-nitrosylated proteins to hazardous levels (Fig. 2).

The observation that the increased apoptosis of myocytes, endothelial cells, and fibroblasts in heart biopsies from patients with diabetes (30), as well as in hearts from STZ-induced diabetic rats (25), is selectively associated with the levels of nitrotyrosine found in those cells (8) suggests that the oxidative damage and apoptosis of myocardial cells are parts of the mechanism by which hyperglycemia exerts its damaging action in diabetes. Even if the exact link of hyperglycemia with the increased apoptotic myocardial cell death remains to be established, the demonstration that cytokine-induced apoptosis is
mediated by iNOS induction and peroxynitrite formation in primary cultures of neonatal rat myocytes (3) suggests a central role for proinflammatory cytokines in myocardial damage (Fig. 2).

The Inflammatory Component

Hyperglycemia-dependent myocardial oxidative damage could be amplified by consequent inflammatory process. Recent studies have shown that hyperglycemic stress during MI is associated with increased levels of some inflammatory markers, including C-reactive protein and interleukin-18. Patients with acute MI have also an enhanced expression of natural killers (CD16/CD56) associated with the reduced expression of some T-cells (CD152) known to limit the immune process in patients. These findings are in agreement with animal studies showing increased levels of proinflammatory cytokines (tumor necrosis factor-α, IL-6, IL-18) and peroxynitrite (an index of oxidative stress) in the myocardium of hyperglycemic mice. They are strictly correlated with the blood glucose levels (43) and lead eventually to myocardial apoptosis and greater infarct size. Finally, analysis of ventricular biopsy specimens from type II diabetic patients with acute
coronary syndrome and undergoing coronary bypass surgery showed reduced (in comparison with non-diabetic ischemic patients) expression of some important angiogenic factors, such as hypoxia-inducible factor-1α (HIF-1α) and vascular endothelial growth factor (VEGF) (43). The results of these studies suggest that hyperglycemia may, by amplifying both oxidative stress and inflammatory responses to myocardial ischemia and by impairing angiogenic factors, affect the prognosis of patients with myocardial infarction. A mechanism involving cytoplasmic mediators, nuclear transcription factors as well as intracellular protein degradation seems to play a central role in the enhancement of hyperglycemia in diabetic patients with MI. There is emerging evidence that the major pathway for nonlysosomal intracellular protein degradation in eucaryotic cells, the ubiquitin-proteasome system links myocardial ischemia, oxidative stress, and inflammation with diabetes. This system controls inflammation in the initial and subsequent stages of myocardial ischemia (37,55); it is upregulated by diabetes in rat muscles (40) and in human atherosclerotic plaques (42). Our ongoing research leads to the hypothesis that by increasing ubiquitin-proteasome activity hyperglycemic oxidative stress may enhance the inflammatory potential of ischemic damage, thus worsening the outcome of MI in diabetics (Fig. 3). On the other hand, the ability of proteasome inhibitors to decrease the inflammatory response triggered by ischemia has been well documented (53,55).

Utility of Knowing the Scenario

The classic antioxidant therapy with vitamins has not been adequately proven to be beneficial in the therapy of such diabetic complications as MI (14). Today some of the older drugs, used in the treatment of hypertension or diabetes, are thought to have additional beneficial effects because of their antioxidant activity or actions on different molecular triggers of stress. These drugs include statins, ACE inhibitors, AT-1 blockers, calcium channel blockers and thiazolidinediones. The latter activate the nuclear peroxisome proliferator-activated receptor gamma, a nuclear receptor for ligand-dependent transcription factors. This activation leads to inhibition of the inducible nitric oxide synthase and
consequently reduction of peroxynitrite generation (14). ACE-inhibitors prevent stress by reducing hyperglycemia-induced excessive generation of angiotensin II, AT-1 blockers block the effects angiotensin II at AT-1 type receptors, while calcium channel blockers, in addition to blocking L-type Ca^{2+} channels, inhibit the peroxidation of cell membrane lipids. Finally, statins possess the ability to increase the bioavailability of NO and decrease the superoxide production by interfering with NADPH activity and by stimulating eNOS expression (14). There are also molecular defenses against free radicals and cardiovascular alterations caused by them. These are molecules that directly neutralize free radicals as well as cellular mechanisms that expel or destroy these reactive species and other inflammatory mediators. Organisms developed an antioxidative defense system that consists of direct free radical scavengers, metal chelators and enzymes that metabolize the radicals to non-harmful products. These enzymes include heme oxygenase-1 (HO-1), that has been proven beneficial in cardiovascular pathologies, such as MI (19), as well as molecules that mimic the endogenous antioxidant pathways, e.g., superoxide dismutase (SOD) (10,13,50).

Cardiac enzyme HO-1 is induced by several stimulants such as hemolysis, inflammatory cytokines, oxidative stress, heat shock, heavy metals, endotoxin (22,28,29,41,61,71). It catalyzes the oxidation of heme to biliverdin, carbon monoxide, and free iron (52), which are powerful cytoprotective agents. HO-1 has potent antiinflammatory (75,76) and cardioprotective (17,35) properties. Recently, in cardiac tissue following experimental acute myocardial infarction HO-1 has been shown to reduce the release of inflammatory mediators, including cytokines and chemokines, and to protect hearts of rats with experimental streptozocin (STZ)-induced diabetes, or of normoglycemic animals, from the damage caused by the neutrophilic leukocytes infiltrated into the tissue (19). Noteworthy, these leukocytes are contributing to the expansion of the infarcted area in patients with MI due to their ability to release superoxide anions (58). Diabetes seems to impair the expression and the activity of the endogenous HO-1 leading to an imbalance in the protection provided by this antioxidant (19).

In various pathological situations the activity of native superoxide dismutase (SOD) enzymes emphasized the importance of O_2^- in the disease and, thus, the therapeutic potential of exogenous SOD enzymes (26,38,73). However, the native SOD enzyme has not been evaluated in hyperglycemia-induced cardiovascular alterations. Thus, the role of superoxide in this condition is not yet defined. There are drawbacks or issues associated with the use of native enzymes as therapeutic agents (e.g., solution instability, immunogenicity of non-human enzymes, bell-shaped dose-response curves, high susceptibility to proteolytic digestion) or as pharmacological tools (e.g., they do not penetrate cells or cross the blood-brain barrier, limiting the dismutation of superoxide only to the extracellular space or compartments). To overcome the limitations associated with native enzyme therapy a series of SOD mimetics that catalytically remove O_2^- have been developed. M40403 (Fig. 4), synthetic manganese-containing bicsyclohexylpyridine superoxide dismutase mimetic (18), is a prototypic example of a stable, low molecular weight, non-peptidic molecule possessing the function and catalytic rate of a native SOD enzyme, but with the advantage of being a much smaller molecule (63). An important property of these SOD mimetics is that they catalytically remove superoxide at a high rate without interacting with other biologically important reactive species including nitric oxide, peroxynitrite, hydrogen peroxide, oxygen or hydroxyl radicals (59,60). This property is not
shared by other classes of SOD mimetics or scavengers, including several metalloporphyrins such as tetrakis-(N-ethyl-2-pyridyl) porphyrin and tetrakis-(benzoic acid)-porphyrin, that interact with other reactive species such as nitric oxide and peroxynitrite that clearly play an important role in inflammation (54). The cardioprotective activity of M40403 has been demonstrated in isolated working rat hearts perfused with a solution containing high glucose concentration by studying its effects on: (i) QT interval prolongation (ii), coronary perfusion pressure increase (iii), lipid peroxidation, (iv) superoxide dismutase (SOD) activity, (v) nitration of tyrosine residues, (vi) poly (ADP-ribose) polymerase (PARP) activation, and (vii) DNA damage. In all these experimental procedures M40403 was cardioprotective (18), in addition to having beneficial effects in models of systemic inflammation as well as in the models of shock and non-diabetic MI (12,46). These effects of M40403 should be beneficial in the therapy of MI, since patients with MI show decreased activities of glutathione peroxidase, catalase and SOD, which are initiators of the scavenging of lipid peroxide and superoxide radicals (36). These effects are particularly important for diabetic myocardium, which has greater propensity for oxidative stress that leads to the development of heart failure (67).

CONCLUSION

Since oxidative stress is the likely leading cause of acute myocardial infarction in diabetics, better approaches can be developed to the prevention or treatment of cardiovascular complications in general and to MI in diabetics in particular. By targeting specific endogenous antioxidant pathways or by using focused radical scavengers the perspectives for the treatment of MI in diabetics can be substantially improved.

REFERENCES


70. Szabó C, Zingarelli B, O’Connor M, Salzman AL. DNA strand breakage, activation of poly (ADP-ribose) synthetase, and cellular energy depletion are involved in the cytotoxicity of macrophages and smooth muscle cells exposed to peroxynitrite. *Proc Natl Acad Sci USA* 1996;93:1753–1758.


