Nitric oxide: the missing lusitrope in failing myocardium

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This editorial refers to ‘Differential effects of arginine methylation on diastolic dysfunction and disease progression in patients with chronic systolic heart failure’† by W.H. Wilson Tang et al., on page 2506

During the last decade, the effects of nitric oxide (NO) on contractile function of failing myocardium elicited a lot of interest and research activity. Because of initial reports of NO acutely reducing the extent and velocity of shortening of isolated cardiomyocytes, especially following β-adrenergic stimulation, and because of increased activity of inducible nitric oxide synthase (NOS2) in human cardiomyopathic myocardium, numerous reports and reviews have persistently and erroneously identified NO as deleterious for contractile function of failing myocardium.1 Shortly after these initial reports, a rosier picture of NO’s functional myocardial effects emerged, highlighting NO’s ability to improve left ventricular (LV) diastolic function. In the normal human heart, intracoronary administration of NO donors instantaneously hastens onset of LV relaxation and increases LV diastolic distensibility.2 These effects appear to be especially favourable in patients with dilated cardiomyopathy as they lower LV filling pressures and increase LV preload reserve. The mechanisms involved in these favourable lusitropic effects of NO differ between exogenous NO and NO produced by some of the nitric oxide synthases (NOSs) because of compartmentalization of the NOSs within the cardiomyocyte. Protein kinase G (PKG)-induced troponin I phosphorylation results in myofilamentary desensitization and thereby mediates lusitropic effects of exogenous NO and of NO produced by endothelial nitric oxide synthase (NOS3),2 whereas sarcoplasmic reticular (SR) phospholamban phosphorylation and an increase in calcium sequestration by the SR accounts for lusitropic effects of NO produced by neuronal nitric oxide synthase (NOS1).3

Tang and co-workers from the Cleveland Clinic reappraised the importance of myocardial NO bioavailability for LV diastolic function in patients with ischaemic and non-ischaemic dilated cardiomyopathy.4 In this study, low myocardial NO bioavailability, evident from high plasma levels of monomethylarginine (MMA), asymmetric dimethylarginine (ADMA), and symmetric dimethylarginine (SDMA) were strongly related to diastolic LV dysfunction or to N-terminal pro-B-type natriuretic peptide (NT-proBNP) plasma levels, and weakly to systolic LV dysfunction. Furthermore, plasma levels of ADMA and SDMA were predictors of survival, and plasma levels of MMA and ADMA were significantly lower in patients treated with β-blockers. These observations are intriguing as they relate diastolic LV dysfunction in dilated cardiomyopathy to microvascular inflammation and to lusitropic effects of NO and as they support correction of myocardial NO bioavailability as a treatment of diastolic LV dysfunction.

Microvascular inflammation and diastolic LV dysfunction

Accumulation in circulating blood of methylated L-arginine metabolites results from enhanced vascular production by protein arginine methyletransferases (PRMTs) and/or from reduced vascular breakdown by dimethylarginine dimethylaminohydrolases (DDAH). In numerous diseases such as hypercholesterolaemia, diabetes, renal disease, and heart failure, vascular or microvascular inflammation appears to be the common trigger for upregulation of PRMT, downregulation of DDAH, and resultant accumulation of methylated L-arginine metabolites. Myocardial vascular and microvascular inflammation has also been associated with diastolic LV dysfunction in both clinical and experimental observations. In patients with hypercholesterolaemia, endothelial-dependent changes in coronary blood flow were blunted and associated with lower tissue Doppler mitral annular lengthening velocity.5 Patients with myocarditis induced by parvoviral B19 infection presented with both coronary endothelial and diastolic LV dysfunction.6 A marker of microvascular inflammation is endothelial expression of adhesion molecules such as E-selectin. In a recent
study of endomyocardial biopsies procured in patients with dilated cardiomyopathy, diabetic patients had both elevated microvascular E-selectin expression in the biopsies and reduced diastolic LV distensibility evident from upward displacement of the end-diastolic LV pressure–volume relationship. Finally, in a rabbit toxic cardiomyopathy model, coronary microvascular inflammation following intravenous lipopolysaccharide injection resulted in delayed and slowed relaxation of twitch contractions of papillary muscles isolated from these hearts. This impairment of cardiac muscle relaxation derived from an imbalance between NO-induced relaxation hastening and endothelin-1-induced relaxation slowing. This study directly linked vascular inflammation to diastolic dysfunction of surrounding myocardium via reduced NO bioavailability.

**Lusitropic actions of NO**

NO elicits both acute and chronic lusitropic myocardial effects. Acute lusitropic effects of NO affect myocardial relaxation and diastolic distensibility, whereas chronic lusitropic effects of NO mainly influence myocardial diastolic distensibility. Myocardial NO concentration varies during the cardiac cycle probably as a result of myocardial mechanical deformation, which is an important stimulus for NO release from cardiac endothelial cells. With the use of an intramyocardial porphyrinic NO sensor, the myocardial NO concentration profile during a single cardiac cycle was recorded in the LV of a beating rabbit heart. These recordings revealed a striking peak of myocardial NO concentration at end-systole. This end-systolic rise of myocardial NO concentration synchronizes onset of LV relaxation because of abrupt cessation of contraction as a result of PKG-induced troponin I phosphorylation and myofilamentary desensitization. Although never investigated, this NO-induced synchronization of onset of LV relaxation is probably deficient in failing myocardium because of reduced end-systolic mechanical deformation and higher myofilamentary calcium sensitivity.

Apart from this acute lusitropic effect of NO within a single cardiac cycle, NO also instantaneously modulates myocardial relaxation and diastolic distensibility when exogenously provided to the cardiomyocytes either from NO donors or from the coronary endothelium. In isolated cat papillary muscles, ejecting guinea pig hearts, and normal or cardiomyopathic human hearts, administration of NO donors or of substance P, which releases NO from the coronary endothelium, hastened onset of LV relaxation, accelerated LV pressure decline, and increased diastolic LV distensibility. The relaxation effects were more pronounced following pre-treatment with the β-agonist dobutamine because of concerted phosphorylation of troponin I by PKG and protein kinase A. Assuming incomplete diastolic cytosolic calcium removal, the instantaneous increase in diastolic LV distensibility following administration of NO donors or of substance P was similarly ascribed to PKG-induced troponin I phosphorylation, myofilamentary desensitization, and prevention of diastolic cross-bridge cycling. Recently, however, elastic properties of the giant cytoskeletal protein titin have been recognized as an important determinant of myocardial diastolic distensibility. Titin functions as a bidirectional spring controlling both end-systolic recoil and late diastolic distensibility of cardiomyocytes. Titin of rat uterus muscle was shown to have phosphorylation sites for PKG. The presence of such sites in myocardial titin could equally well explain the acute increase in myocardial diastolic distensibility induced by exogenous NO.

In mechanically stressed myocardium, high NO bioavailability also preserves LV diastolic function through chronic effects on calcium handling, cardiomyocyte hypertrophy, and extracellular matrix deposition, as evident, respectively, from transgenic mice with cardiomyocyte-specific NOS1 overexpression subjected to transverse aortic constriction, from transgenic mice with cardiomyocyte-specific NOS3 overexpression subjected to permanent coronary artery ligation, and from numerous experimental rat models chronically treated with NOS enhancers or NOS inhibitors.

**Treatment of diastolic LV dysfunction**

In a pacing-induced heart failure dog model, development of restrictive LV filling corresponded to lower myocardial NO production evident from lower coronary sinus wash-out of NO metabolites. Boosting myocardial NO production through provision of additional L-arginine was therefore presumed possibly to prevent a failing heart from evolving to a restrictive phenotype. In transplant recipients, an intracoronary co-infusion of L-arginine and of substance P indeed resulted in a larger immediate increase in diastolic LV distensibility than a single infusion of substance P. Furthermore, in heart failure patients, oral L-arginine treatment improved both forearm blood flow during exercise and 6-min walk tests. Despite these encouraging acute results, beneficial effects of chronic L-arginine supplementation on heart failure mortality or morbidity have never been reported, probably because of supplemented L-arginine being mainly diverted towards methylated L-arginine derivatives, which block both L-arginine transport into the cell and NO synthesis. The present study by Tang et al., however, suggests a cure for this diversion as they observed less ADMA production in patients on β-blocker treatment. When concomitantly with β-blockers, L-arginine supplementation could therefore more effectively raise myocardial NO bioavailability and improve diastolic LV distensibility. Combining β-blockade with enhancement of NO bioavailability has already been shown to result in favourable effects on diastolic LV dysfunction in diastolic heart failure patients treated with the β-blocker nebivolol, which not only blocks β1-adrenoreceptors but also increases NOS2 activity through β2-adrenoreceptor activation. Chronic use of nebivolol in patients with diastolic heart failure resulted in a significant fall in LV filling pressures at unaltered LV filling volumes, in contrast to atenolol which left LV filling pressures unaltered.

**Conclusions**

The relationship between circulating methylated L-arginine metabolites and diastolic LV dysfunction shown by Tang et al. fills important blanks in our understanding of the progression of diastolic LV dysfunction in dilated cardiomyopathy. Their observations...
establish a cascade of events consisting of microvascular inflammation, L-arginine methylation, low myocardial NO bioavailability, depressed lusitropic action of NO, restrictive LV filling dynamics, and poor prognosis. Future therapeutic efforts to curb this evolution should try to interrupt this cascade of events.

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References

The above article uses a new reference style being piloted by the EHJ that shall soon be used for all articles.