Coronary artery disease, nitric oxide and oxidative stress: the "Yin-Yang" effect – a Chinese concept for a worldwide pandemic

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Summary

Prevention of coronary artery disease (CAD) and reduction of its mortality and morbidity remains a major public health challenge throughout the "Western world". Recent evidence supports the concept that the impairment of endothelial function, a hallmark of insulin resistance states, is an upstream event in the pathophysiology of insulin resistance and its main corollaries: atherosclerosis and myocardial infarction. Atherosclerosis is currently thought to be the consequence of a subtle imbalance between pro- and anti-oxidants that produces favourable conditions for lesion progression towards acute thrombotic complications and clinical events. Over the last decade, a remarkable burst of evidence has accumulated, offering the new perspective that bioavailable nitric oxide (NO) plays a pivotal role throughout the CAD-spectrum, from its genesis to the outcome after acute events.

Vascular NO is a critical modulator of coronary blood flow by inhibiting smooth muscle contraction and platelet aggregation. It also acts in angiogenesis and cytoprotection. Defective endothelial nitric oxide synthase (eNOS) driven NO synthesis causes development of major cardiovascular risk factors (insulin resistance, arterial hypertension and dyslipidaemia) in mice, and characterises CAD-prone insulin-resistant humans. On the other hand, stimulation of inducible nitric oxide synthase (iNOS) and NO overproduction causes metabolic insulin resistance and characterises atherosclerosis, heart failure and cardiogenic shock in humans, suggesting a "Yin-Yang" effect of NO in the cardiovascular homeostasis. Here, we will present a concise overview of the evidence for this novel concept, providing the conceptual framework for developing a potential therapeutic strategy to prevent and treat CAD.

Key words: coronary artery disease; nitric oxide; oxidative stress; Yin-Yang effect; cardiovascular homeostasis

Introduction

Prevention of coronary artery disease (CAD) and reduction of its mortality and morbidity remains one of the greatest public health challenges throughout the Western world. Over the last decade, a remarkable burst of evidence has accumulated, offering the new perspective that nitric oxide (NO) plays a pivotal role in CAD.

Vascular NO is a critical modulator of coronary blood flow through inhibition of smooth muscle contraction and platelet aggregation, and plays an important role in angiogenesis [1]. On one hand, defective endothelial nitric oxide synthase (eNOS) driven NO synthesis causes majors cardiovascular risk factors (insulin resistance, arterial hypertension and dyslipidaemia) in mice [2–4], and characterises CAD-prone insulin-resistant humans [3, 5–9]. Defective intravascular NO can occur through several mechanisms. These mechanisms include impaired eNOS protein expression, uncoupling of NOS activity (leading to enhanced production of superoxide) and/or trapping of NO by reactive oxygen species (ROS), but are not mutually exclusive and may happen simultaneously in humans.

On the other hand, stimulation of inducible nitric oxide synthase (iNOS) and NO overproduction also plays a role in insulin resistance [3, 10, 11] and characterises atherosclerosis [12, 13], heart failure [14] and cardiogenic shock [15], suggesting a Yin-Yang effect of NO in the cardiovascular homeostasis. Imbalance in normal cellular conditions disturbs the physiological regulation of the three isoforms of NOS, which results in profound disturbances leading to endothelial dysfunction, insulin resistance, atherosclerosis, and myocardial infarction. It further represents an underlying fea-

ture of acute and chronic heart failure, as well as cardiogenic shock, suggesting a key role of this fragile balance throughout the CAD-spectrum.

Here, we will review the evidence for this novel concept, and thereby provide a framework for developing potential novel therapeutic strategies to prevent [3] and treat CAD.

Nitric oxide and the current application of Han's concept

Nitric oxide and reactive oxygen species

Nitric oxide is produced by one of the three distinct isoforms of the enzyme nitric oxide synthase (NOS – EC 1.14.13.39) during the oxidation of the amino acid substrate L-arginine to L-citrulline [16]. Endothelial NOS (eNOS or NOS III-chromosome 7) is classically found in the vasculature and also in good quantity in the skeletal muscle tissue, targeted by caveolin-1 to plasmalemmal caveolae [17] and at low levels to (cardio-)myocyte mitochondria [18]. Its regulation depends mainly of the calcium-calmodulin interaction, which induces a low, intermittent pattern of synthesis. Its activity is classically increased by exercise and decreased by aging. Neuronal NOS (nNOS or NOS I – chromosome 12), first purified from rat and porcine cerebellum [19–21], is the most frequent isoform found in skeletal muscle tissue, co-localised with the dystrophin-associated protein α_1-syntrophin along the sarcolemma of type 2 fast-twitch fibres [22–24]. Whereas there is no doubt that nNOS plays a crucial role in sympathetic activity, its role in normal cardiac physiology is unclear and still debated [25]. Under pathological conditions however it may contribute to coronary blood flow [26] and could also be a determinant for basal contractility in the mammalian myocardium [27]. Its regulation is close to the previous endothelial NOS. Inducible NOS (iNOS or NOS II – chromosome 17) [17, 24, 28–30] can be induced in macrophages and in many other cells, such as endothelial cells, cardiomyocytes or skeletal myocytes, where it is present at very low concentrations, connected to the membrane through the protein caveolin-3. In contrast to the two other isoforms, calmodulin binds to iNOS with high affinity even at resting Ca^{2+} levels, which produces a high, continuous pattern of synthesis. Inflammation and aging elevate iNOS protein expression, whereas exercise training attenuates its expression. Cofactors required include NADPH, biopterin, flavin adenine dinucleotide and flavin mononucleotide.

Reactive oxygen species (ROS) are highly reactive molecules that include free radicals, such as superoxide (O^{2-}) and hydroxyl ($\cdot OH^-$), as well as compounds such as hydrogen peroxide (H_2O_2). The fate of free radical production is dependent upon the cellular redox status and the activity of several enzymes. In cardiovascular pathophysiology the mitochondrial electron transport chain, the xanthine oxidase, lipo-oxygenase, non-phagocytic NADPH oxidases, NO synthase itself (mainly iNOS), haem-oxygenase and the cytochrome P450 mono-oxygenases seem to produce the most, when not all, ROS [31].

Local conditions: the "Yin-Yang" effect

At *physiological concentrations*, both NO and ROS exert beneficial effects and can function as second messengers. NO produced in low concentration acts as a messenger and cytoprotective factor, via direct interactions with transition metals and other free radicals [32]. ROS may regulate enzymatic function and participate in signal transduction being essential for normal cell proliferation and growth [33].

Alternatively, under *pathophysiological conditions*, when the circumstances allow the formation of substantial amounts of NO and modify the cellular microenvironment (pro-oxidant molecules generated > tissue antioxidant reserve), ROS and NO react avidly. Consequently, the half-life of the bioactive NO is reduced [34] and "reactive nitrogen species", particularly dinitrogen trioxide and peroxynitrite ($ONOO^-$), will be generated, causing significant damage to cellular components (proteins, membranes, nucleic acid), leading to chromosomal alterations, protein nitration, lipid peroxidation, subsequent cellular dysfunction and cellular death.

Inflammation, aging, hyperglycaemia, hyperlipidaemia, imbalance in obligate cofactors of NOS or hypoxia (with subsequent reperfusion) are examples of conditions modifying the cellular NO-microenvironment supporting the generation of reactive nitrogen species and, thus, making up a functional decrease in NO bioavailability [3]. These examples occur as a consequence of various possible mechanisms; for instance, under conditions of reduced availability of L-arginine or tetrahydrobiopterin, the NOS will preferentially produce superoxide anion from oxygen, a mechanism known as NOS "uncoupling" [35–37]. Alternatively, in eNOS deficiency, iNOS (but not nNOS) will try to compensate for this lack of NO, replacing the "lamb" by a "wolf" [38]. This latter example demonstrates an important aspect of NO physiology: the notion that the local amount of NO and its subcellular localisation are crucial in determining the effect. As an example for this novel concept, by tightly regulating the rate at which molecular oxygen enters the respiratory chain, NO controls mitochondrial respiration. Physiologically produced NO acts as protective molecule by making the cell "hibernate": it inhibits

aerobic mitochondrial metabolism, thus reducing oxygen consumption and preventing the onset of apoptosis. In pathological production of both NO and ROS however, reactive nitrogen species bind irreversibly to multiple components of the mitochondrial respiratory chain, terminating cell respiration and precipitating cell necrosis [39].

The "Yin-Yang" effect and the genesis of insulin resistance

In humans, the incidence of metabolic syndrome, a set of metabolic insulin resistance and traditional major cardiovascular risk factors, has risen dramatically in the Western world [3, 40–43]. Over the past decade, studies in humans have contributed importantly to generating the new concept that a defect in NO bioavailability may play a central role in the pathogenesis of this syndrome [3, 7, 44, 45].

In *lean subjects*, insulin stimulates blood flow and decreases vascular resistance in skeletal muscle [3, 6, 44, 46–49]. Stimulation of muscle blood flow, and subsequent glucose delivery, is mediated by NO, as this effect is abolished by the stereospecific inhibitor of NOS, N^G-monomethyl-L-arginine (L-NMMA), and by inhibition of tetrahydrobiopterin synthesis [6, 50, 51]. By promoting substrate delivery to skeletal muscle tissue, NO-mediated stimulation of muscle blood flow by insulin is thought to play a role in the regulation of muscle glucose uptake.

In *insulin-resistant humans*, endothelial NO bioavailability (decreased NO synthesis and/or increased consumption by ROS) is impaired, which thereby leads to metabolic insulin resistance. The evidence is as follows. Endothelium-dependent vasodilatation is defective in insulin-resistant subjects [44], a defect that is directly related to metabolic insulin resistance [6, 7, 44, 52, 53]. Essential hypertension, an insulin-resistant state, is characterised by defective endothelial NO synthesis, and is associated with eNOS gene polymorphism [54–57]. Furthermore, arterial hypertension could

Figure 1

Metabolic phenotype of mice with complete disruption of the endothelial isoform of NO synthase (eNOS$^{-/-}$ mice). Adapted from Cook et al. [2]
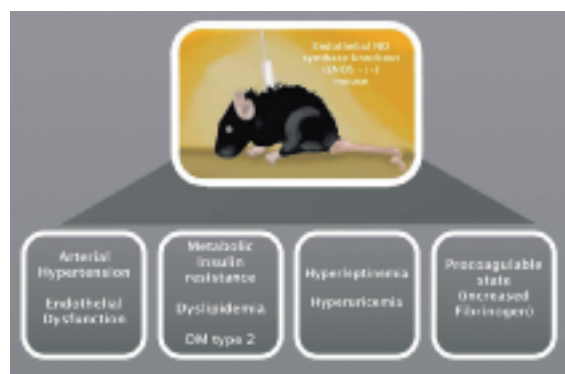

Table 1

NOS Polymorphism and association with insulin resistance and CAD.

Disease	NOS	Polymorphism	Association	Population	Reference
Arterial hypertension	eNOS	E298D mutation in exon 7	Carriers of the mutation showed higher rate of essential hypertension	Japan	55
			Carriers of the mutation showed higher rate of essential hypertension	Japan	56
			Carriers of the mutation showed higher rate of preeclampsia	Japan	80
			Carriers of the mutation showed higher rate of pregnancy-induced hypertension	Scotland	81
			Carriers of the mutation showed higher rate of essential hypertension	Japan	83
	iNOS	NOS2A bi-allelic tetranucleotide repeat	Carriers of the mutation showed higher rate of essential hypertension	Australia	93
	nNOS	Microsatellite polymorphism	No difference between the groups	Japan	85
Diabetes	eNOS	E298D mutation in exon 7	associated with decreased endothelial responses (endothelial-dependent brachial artery blood flow) compare to controls after a diet-challenge of n-3 fatty acid-diet.)	United Kingdom	
	iNOS		Linkage of the human NOS2 gene to IDDM	Denmark	87
			Carriers of the allele had twice the risk of developing retinopathy	India	88
			Carriers of the allele had twice the risk of developing complications such as microalbuminuria, overt nephropathy, retinopathy and clinical neuropathy.	Australia	89
	nNOS	unknown			
Atherosclerosis	eNOS	T786C in the 5'-Flanking region	Independent risk factor for severe artery stenosis	Italy	74

Table 1

Continue.

Disease	NOS	Polymorphism	Association	Population	Reference
Coronary artery disease (CAD)	eNOS	VNTR in intron 4 (a/b)	Carriers of the mutation showed a higher smoking-dependent risk of CAD	Australia	57
			Carriers of the mutation showed more severe coronary artery narrowing and increasing risk of myocardial infarction after this prospective autopsy serie	Finland	95
		VNTR in intron 4 (a/b), E298D mutation in exon 7 and G10T polymorphism in intron 23	Carriers of the G-allele of G10T polymorphism showed a higher plasma NOx and a higher independent risk of CAD	Korea	94
		E298D mutation in exon 7	Carriers of the mutation showed a higher independent risk of CAD	England	75
			Carriers of the mutation showed a higher independent risk of coronary spasm after intracoronary injection of Ach	Japan	79
			Carriers of the mutation showed a higher independent risk of coronary artery disease	Australia	78
			Carriers of the mutation showed a higher independent risk of myocardial infarction	Japan	77
		T786C in the 5'-Flanking region	Carriers of the mutation showed a higher independent risk of coronary spasm after intracoronary injection of Ach	Japan	82
Others		E298D mutation in exon 7 and 786 T->C	Carriers of either of the mutations showed higher post-PTCA restenosis rate		73
		VNTR in intron 4 (a/b)	Amelioration of coronary blood flow by pravastin in ba genotype but not in bb genotype	Sweden	90
	iNOS		"+" allele is associated with glucose intolerance, obesity and unstable angina pectoris	Australia	91
Micellenaous	eNOS	E298D mutation in exon 7	Carriers of the mutation showed impaired response to L-NMMA. Authors conclude that this mutation is asociated with impaired NO production.	Germany	96
		T786C in the 5'-Flanking region	T786C: C allele is associated with promoter activity that is less than half of the T allele	Japan	82
		VNTR in intron 4 (a/b)	4a/4b associated with altered plasma nitric oxide levels	Australia	97

be related to elevated plasma concentrations of endogenous NOS inhibitors such as asymmetric and symmetric dimethyl arginine [58–60]. Finally, insulin resistance is associated with metabolic abnormalities which could down-regulate eNOS expression, such as oxidized low density lipoprotein (LDL) [61–63].

Whereas these first human studies wove the conceptual framework of this new theory, animal studies allowed major breakthroughs in our current understanding. Consistent with the concept of an important role of NO in the regulation of insulin sensitivity, NOS inhibitors reduce insulin-stimulated muscle glucose uptake in rats in vivo [64]. Moreover, eNOS is expressed in the skeletal muscle [65], and NO donors stimulate glucose transport in isolated rat muscle preparations in vitro [66–68]. Studies in specific gene knockout mice permit further understanding of the specific role played by NOS isoforms and their interrelationship in insulin resistance. *Mice lacking the gene coding for eNOS (eNOS$^{-/-}$)* display a phenotype mimicking the human metabolic syndrome: eNOS$^{-/-}$ mice are hypertensive [2, 4, 69, 70] and are insulin resistant, as evidenced by fasting hyperinsulinaemia and glucose infusion rates during euglycaemic clamp studies that are 30–40% lower than in wild-type mice [2, 4]. In these mice, insulin resistance is related specifically to impaired NO synthesis, because in equally hypertensive 1-kidney/1-clip mice (a model of renovascular hypertension) insulin-stimulated glucose uptake is normal [4]. The eNOS knockout mice provide the first direct evidence that a defect of insulin stimulation of muscle blood flow contributes to insulin resistance. Insulin stimulation of muscle blood flow is about 40% smaller in eNOS$^{-/-}$ than in wild-type mice, and insulin stimulation of muscle blood flow and muscle glucose uptake is strongly related. It is possible that the impairment of insulin stimulation of muscle blood flow in eNOS$^{-/-}$ mice may be related in part to decreased skeletal muscle capillary density. In addition to arterial hypertension and insulin resistance, eNOS$^{-/-}$ mice show dyslipidaemia, and increased plasma concentrations of leptin, uric acid and fibrinogen, intrinsic components of the human syndrome X [2] (figure 1).

Extrapolation of these findings in mice to humans was not straightforward since eNOS gene deficiency had not been reported in humans thus far. There was evidence, however, that cardiovascular disease states such as hypertension, coronary artery disease, and myocardial infarction, were associated with functional [71, 72] eNOS gene polymorphism [54, 55, 57, 73–97] (table 1) and impaired NO synthesis [57, 78]. We therefore considered a revisited hypothesis of the so-called "two hit" model of gene dysfunction advanced by Knudson [98] postulating that, in our mice, an environmental factor could trigger a pathological phenotype in genetically altered mice (partially eNOS deficient mice – eNOS$^{+/-}$). When fed a normal diet, eNOS$^{+/-}$ are normotensive and have normal insulin sensitivity. However, when fed a high-fat diet, eNOS$^{+/-}$ mice develop exaggerated arterial hypertension, and insulin resistance, as evidenced by a 40% lower insulin-stimulated glucose uptake than control mice [2, 10], a defect which may be related, at least in part, to impaired insulin stimulation of muscle blood flow and substrate delivery. *Mice lacking the inducible isoform of the NO synthase* provide evidence that large amounts of NO produced by iNOS induction may have detrimental effects on insulin sensitivity. Under normal conditions, rodent and human tissues express very low levels of iNOS, and there is no evidence that iNOS plays a role in the regulation of glucose of glucose metabolism. However, in several pathological states cytokines such as tumour necrosis factor-α and interleukin-6 are augmented. In mice, high-fat diet induces iNOS expression in skeletal muscle and fat tissue together with insulin resistance, and iNOS knockout mice are protected from high-fat diet-induced insulin resistance [11, 99]. Alternatively, mice over-expressing iNOS display arterial hypertension and insulin resistance.

In summary, there is substantial evidence showing that the small amounts of NO produced by eNOS regulate both vascular and metabolic homeostasis. A defective eNOS-driven NO synthesis causes insulin resistance in experimental animals, and characterises insulin-resistant states in humans. On the other hand, there is also large body of evidence that the large amounts of NO produced by iNOS induction may have detrimental effects on insulin-stimulated glucose uptake, suggesting a Yin-Yang effect of NO in the regulation of metabolic homeostasis (figure 2). As demonstrated by heterozygous eNOS mice, we speculate that in humans the different mechanisms interplay through "gene-gene" and/or "gene-environment" interaction, raising the possibility of the "two hits" law. A speculative example of this theory is illustrated by the 1173 C-> T iNOS promoter polymorphism, which is highly expressed in African populations and is thought to be a natural protection against severe malaria [100]. When challenged with "foreign" environments, such as a "Western" diet, it could on the other hand promote insulin resistance in the black population.

The "Yin" and the "Yang" of atherosclerosis

Far from only regulating insulin sensitivity and most of the "major cardiovascular risk factors" endothelium-derived NO is also the most potent vasodilator known and is a critical modulator of blood flow, platelet aggregation, oxidative modification of LDL-cholesterol, proliferation of vascular smooth muscle cell, and leucocyte adherence [2, 4, 101–106]. Consistently, it makes sense to believe that a defect in endothelium-derived NO plays a role in atherosclerosis, not only by favouring the "medium", but also by more directly affecting the component of the vascular endothelium as well as its function.

Accordingly, there is a growing body of evidence suggesting that alterations in the *synthesis* of NO may promote atherosclerosis. Long-term inhibition of endothelial NOS by administration of L-NAME to rats does induce coronary inflammation and subsequent arteriosclerosis [107, 108]. Similarly, ApoE-deficient mice, an atherosclerosis prone model of mice, were treated with L-NAME for 8 weeks and had a significant increase in the atherosclerotic plaque/surface area in the aorta [109]. Knockout mice demonstrated that both neuronal [110] and endothelial NO synthase have intrinsic vasculoprotective effects [111, 112]. Consistent with this new aspect, eNOS gene polymorphisms have been demonstrated in patients with atherosclerosis (see table 1) and asymmetric dimethyl arginine (ADMA), a competitive endogenous inhibitor of NOS, is increased in atherosclerosis plaques and could contribute to atherosclerosis in patients with chronic renal failure [113]. Alternatively, stimulation of eNOS by statins slows down the progression of atherosclerosis and has even been associated with plaque regression, one so-called "pleiotropic" effect of statins [114–116].

A reduction in NO *bioavailability* (NO trapping) is another important mechanism for endothelial dysfunction and atherogenesis. Free radical oxygen species such as superoxide anion can rapidly react with and inactivate nitric oxide, enhancing *per se* proatherogenic mechanisms (leucocyte adherence, impaired vasorelaxation, platelet aggregation) [117]. Evidence for this theory lies in the fact that end-product of reactive nitrogen species, such as 3-nitrotyrosine, has been detected in atherosclerotic plaques in vivo [118–120], and that antioxidant compounds such as, vitamin C, vitamin E, coenzyme Q, diphenyl-

phenylenediamine, butylated hydroxytoluene, probucol, or its analogues and taurin [121] can reverse endothelial function and/or the development of atherosclerosis in animal models [122].

Finally and as demonstrated for insulin sensitivity, iNOS stimulation is associated with oxidative stress in the vessel wall and iNOS knockout mice are protected from atherosclerosis. After 6 months of high-fat feeding, apoE/iNOS-double knockout mice have an aortic lesion area reduced by 40% compared with apoE-knockout mice, which is associated with lower plasma levels of lipoperoxides, a marker for oxidative stress [123].

In summary, the small amounts of NO produced by eNOS are stimulated by endothelial cell surface receptors (for example, by acetylcholine) or by physical phenomena, such as shear stress, and act as a potent protective shield against oxidative stress. Defective eNOS-driven NO synthesis causes atherosclerosis in experimental animals, and characterises CAD-prone humans. Conversely, iNOS induction is found very precociously in the atherosclerostic plaque and iNOS-driven NO overproduction induces nitrative stresses thus participating in the development of atherosclerosis and also suggesting a "Yin-Yang" effect of NO in atherogenesis.

Outcome after myocardial infarction: is NO a factor?

Whereas the balance between NO and ROS constitutes a key factor in the development of atherosclerosis, it may also be of primary importance in the outcome of its subsequent complications, namely myocardial infarction and heart failure. The outcome after myocardial infarction depends of three major determinants: infarct size, arrhythmia and development of cardiogenic shock. In models of cardiac ischaemia-reperfusion, mice with eNOS knockout suffer significantly larger infarcts than wild-types littermates [124], whereas its overexpression [125, 126] or its stimulation by statins lead to significant attenuation in myocardial reperfusion injury [127–129]. Inversely, iNOS knockout mice show absence of late ischaemic preconditioning effect, a powerful endogenous cardioprotective mechanism corresponding to the clinical finding of an improved outcome in human patients experiencing angina before myocardial infarction [130]. When wild-type mice were preconditioned, the size of the infarcts was decreased by 67% compared with sham-preconditioned controls, whereas the infarct size remained unchanged in iNOS knockout mice.

In the setting of coronary artery occlusion, *infarct size* depends on occlusion time and the area at risk, is inversely proportional to collateral supply (arteriogenesis) [131] and susceptibility (preconditioning, apoptosis) of the cardiomyocyte to ischaemia and reperfusion injury. There is a growing body of evidence showing that NO is important for each of these factors when area at risk and time are kept constant. Arteriogenesis, a process for adapting the pre-existing circuit of vessels into functional collateral conduits, is tightly regulated by nitric oxide a topic recently reviewed in details [34]. Consistently, HMG-CoA reductase has been consistently shown to promote collateral growth in response to ischaemia [132, 133], which is abolished in eNOS knockout mice [134]. As previously discussed, the role of NO in cellular death is tightly regulated by its local quantity. NO can act as a cytoprotective molecule by inducing cell "hibernation" or can be directly cytotoxic, precipitating cell apoptosis [135] and necrosis. Albeit complex, it is suggested once more that NO produced by iNOS is involved in directly causing cell death under pathological situations, such as ischaemia-reperfusion, whereas eNOS-driven NO is responsible for the cytoprotective effects. Inducible nitric oxide synthase (iNOS) is expressed in the myocardium after myocardial infarction. Increased NO production from iNOS expression not only increase infarct size, but also causes myocardial dysfunction (stunning) and results in higher mortality after myocardial infarction, as demonstrated by the protective effect of iNOS deficiency [136].

During and after myocardial infarction, the leading cause of death for patients reaching the hospital alive is due to development of cardiogenic shock [137]. The odd paradigm of this syndrome is that it paradoxically happens when average LV ejection fraction (EF) is only moderately depressed (30–40%). It is often associated with a systemic inflammatory response syndrome (SIRS), which responds minimally to conventional "pressors" and when overcome only leads to mild heart failure [138]. However, new evidence suggests, that the genesis of this paradigm can be explained by an important expression of iNOS, with susbsequent formation of substantial amounts of NO. Contrary to the fact that at *physiological levels* NO acts as a positive inotrope, at higher concentrations, such as the ones found in myocardial infarction or congestive heart failure, NO appears to have a more pronounced negative inotropic effect, depressing myocardial contractility [139–142]. Accordingly, inhibition of NO synthase by competitive inhibitors, as well as knocking out the iNOS gene, appear to have favourable anti-stunning, effects and better survival rate in animal models [136, 143–145]. Accordingly, intervention studies in humans with the nonspecific NOS inhibitor N^G-monomethyl-L-arginine (L-NMMA) demonstrate an improved survival with a 2-fold reduction in 30-day mortality [146–149].

In summary, myocardial infarction is associated with a state of severe oxidative stress, which together with production of substantial amounts of NO by stimulation of iNOS, leads to sustained vasoplegia and formation of reactive nitrogen species. Animal studies show that eNOS-driven NO seems to be cytoprotective (by promoting the collateral supply and decreasing the cellular susceptibility to apoptosis), as well as positive inotrope, whereas iNOS stimulation appears deleterious. Mice overexpressing iNOS in heart develop a higher incidence of sudden death than littermates [150], while on the contrary iNOS knockout mice suffer smaller myocardial infarction. In humans, this imbalance between bioavailable NO and ROS generation is thought to contribute, or to directly cause cardiogenic shock when the global endogenous antioxidant protection is swamped.

The future of NO in coronary artery disease

Figure 2 represents a concise synopsis of our current views. The last two decades have witnessed a burst in understanding the physiological and pathophysiological role of NO/ROS-equilibrium throughout the CAD-spectrum, which clearly suggests that modulation of NO bioavailability constitutes a remarkable opportunity to develop new tools for fighting CAD.

Today's Han's clinical challenge should be to determine when to give a selective inhibitor when there is a suspicion of iNOS overactivity and when to decide to give an appropriate NO-donor. Hopefully, NO-"modulators" and new NO-donors (such as nitroaspirins) [3, 151] would offer this dual activity and may represent the future keystone for the treatment of the CAD-spectrum.

Figure 2

Scheme by which pathological nitric oxide homeostasis can cause metabolic syndrome X. ADMA indicates, asymmetric dimethyl arginine (or similar endogenous methylated arginines). Redrawn and adapted from Cook et al. [3]
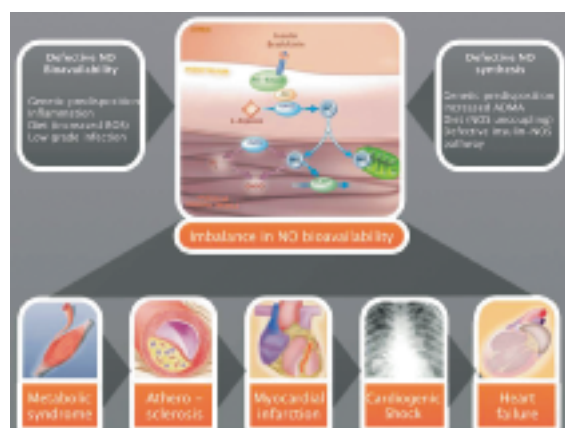
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