

## REVIEW

## Nitric oxide: an emerging role in cardioprotection?

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Over a decade of research has shown nitric oxide (NO) to be a ubiquitous modulator of biological phenomena from cell signal to effector and from physiology to pathophysiology. The involvement of NO in cardiovascular biology has contributed significantly to our understanding of complex disease states including atherosclerosis, systemic and pulmonary hypertension, endotoxic shock, pre-eclampsia,<sup>1</sup> cardiomyopathy,<sup>2</sup> and cardiac allograft rejection.<sup>3</sup> However, the emerging role of NO in the maintenance of cell physiology from immunomodulation to calcium signalling has highlighted the importance of this fascinating molecule in cytoskeleton. This dichotomy of effector function is the “double edged sword” of NO in biological systems. However, the balance between cytostatic and cytotoxic effects of NO may lie in the tissue concentration of NO produced, the particular NO synthase (NOS) isoform activated (that is, “high output” or “low output”), and the complex interaction with other free radicals such as superoxide. However, a much greater understanding of the molecular and cellular actions of NO as a physiological regulator has resulted in a body of recent research increasing our understanding of NO, and thus NO releasing agents, in cytoprotection. Current evidence is outlined below.

### The NO dichotomy: physiology versus pathology?

NO is produced by the catalytic action of NOS on the substrate L-arginine. The reaction involves the oxidation of one of the guanidinonitrogens of arginine and the process involves the oxidation of NADPH (nicotinamide adenine dinucleotide phosphate, reduced form) and the reduction of molecular oxygen. Three NOS enzymes have been characterised: type I (ncNOS), type II (iNOS), and type III (ecNOS). ncNOS and ecNOS are calcium dependent and low output enzymes associated with NO production in the picomolar range, whereas iNOS is a calcium independent and high output enzyme associated with NO production in the nanomolar (nmol) range. All three NOS isoforms have been shown to be present in human myocardium<sup>4</sup> and may be activated in response to hypoxia or ischaemia. Laboratory studies of experimental myocardial infarction have shown an increased induction of iNOS, ecNOS, and NO concentrations in the heart, together with increased plasma concentrations of nitrate and nitrite, the oxidation

products of NO.<sup>5–7</sup> The isoform specific amount of NO generated may account, in part, for physiological versus pathological effects of NO in biological systems; low concentrations are associated with cytoskeleton and high concentrations are associated with cytotoxicity.<sup>8</sup>

A further explanation for the dichotomous effects of NO may lie in its complex interaction with reactive oxygen species, which is particularly pertinent in the context of ischaemia-reperfusion. NO can interact in direct equimolar concentrations with superoxide to form the potent oxidant peroxynitrite, which is toxic to cardiac myocytes.<sup>9</sup> The amount of peroxynitrite production therefore depends on the ratio of superoxide to NO. The greater availability of superoxide may therefore favour peroxynitrite production and toxicity. Thus, superoxide may be an important rate limiting factor determining the protective versus toxic effects of NO.<sup>10</sup> Although the interaction of NO with reactive oxygen species is very complex, this simple hypothesis may explain why, even though the majority of animal studies have shown cytoprotective effects<sup>11–14</sup> of NO against ischaemia-reperfusion injury, others have shown cytotoxicity.<sup>15 16</sup>

Emerging evidence suggests that a fundamental explanation for the dichotomous roles of NO may lie at a subcellular level. NO has been shown to modulate mitochondrial function through reversible and irreversible interactions with respiratory chain complexes.<sup>17</sup> Physiological concentrations of NO inhibit cytochrome oxidase (complex IV) in a reversible manner, in competition with oxygen. However, long term exposure can irreversibly inhibit complex I by S-nitrosation of critical thiols in the enzyme complex. The reversible interaction may play an important part in the physiological regulation of mitochondrial function by reducing oxygen consumption without causing adenosine triphosphate (ATP) depletion. This may be beneficial during ischaemia.

### Novel cellular mechanisms of protection

#### ANTI-APOPTOSIS

Apart from promoting apoptosis NO can also paradoxically inhibit apoptosis. Proposed mechanisms include the suppression of caspase 1 and 3 activity by NO induced S-nitrosation;

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cyclic guanosine monophosphate (GMP) mediated suppression of calcium mediated apoptotic cell death; and induction of the cytoprotective stress proteins heat shock protein 70 and heat shock protein 32 (haemoxygenase).<sup>18</sup>

#### ANTIOXIDANT EFFECTS

NO has been shown to induce the expression of haemoxygenase in vascular and smooth muscle cells. Haemoxygenase and carbon monoxide, the product of the breakdown of haeme by haemoxygenase, can bind the haeme moieties of NOS and soluble guanylate cyclase and thereby inhibit NO production. This may therefore provide a novel adaptive defence mechanism against the oxidative stress associated with sustained production of NO.<sup>19</sup>

#### ANTI-INFLAMMATORY EFFECTS

NO has for a long time been linked to the modulation of the immune response and effects on cell mediated immunity may have a role in cytoprotection. High doses of NO have been shown to modulate the production of interleukin 12 negatively, thus reducing the T helper cell 1 immune response.<sup>20</sup> In addition NO appears to reduce polymorphonuclear leucocyte mediated endothelial dysfunction in myocardial ischaemia-reperfusion, probably through the specific interaction with adhesion molecules.<sup>21</sup>

#### CYCLIC GMP

This important second messenger is produced by the action of NO on soluble guanylate cyclase, which catalyses the conversion of guanosine triphosphate (GTP) to cyclic GMP. Cyclic GMP may exert protective effects by reducing the influx of calcium through L type calcium channels and by stimulating a cyclic GMP sensitive phosphodiesterase with a resultant reduction in concentrations of cyclic AMP. This, together with the known effect of NO in reducing myocyte contractility,<sup>22</sup> would serve to reduce oxygen consumption and energy demand.

#### PRECONDITIONING

Ischaemic preconditioning is a powerful adaptive phenomenon whereby exposure to period(s) of sublethal ischaemia protects against subsequent lethal ischaemia. NO may trigger an early preconditioned state,<sup>23</sup> and an extensive body of evidence has implicated NO as a trigger and mediator of late preconditioning.<sup>24</sup> The mechanism of protection by NO in late preconditioning may involve the mitochondrial potassium ATP dependent channel<sup>25</sup> and the isoform selective activation of specific protein kinase C isoforms.<sup>26</sup>

#### MODULATION OF MITOCHONDRIAL FUNCTION

A reversible suppression of mitochondrial respiration has been shown to explain myocyte adaptation to chronic hypoxia without compromising cell survival or accelerating ATP depletion.<sup>27</sup> Recent studies have shown that NO is indeed a physiological regulator of myocardial

oxygen consumption<sup>28</sup> and that reduced oxygen consumption by this mechanism may contribute to cardioprotection during preconditioning.<sup>29</sup> Mitochondrial dysfunction is a critical component of ischaemia-reperfusion injury, which is characterised by dissipation of the membrane potential, ATP depletion, induction of the mitochondrial permeability transient, and mitochondrial calcium overload.<sup>30</sup> We have data to suggest that NO induced depolarisation of the mitochondrial membrane potential protects cardiomyocytes by reducing mitochondrial calcium overload during hypoxia-reoxygenation injury.<sup>31</sup> This may be a novel explanation linking the NO mediated modulation of mitochondrial energetics to cytoprotection.

#### INHIBITION OF MYOCARDIAL DYSTROPHIN PROTEOLYSIS

Recent exciting data have shown that NO inactivates coxsackieviral protease 2A, which cleaves human and mouse dystrophin in a dose dependent manner.<sup>32</sup> This provides a further novel mechanism for protection by NO against viral myocarditis and may explain why NO concentrations are significantly raised in idiopathic dilated cardiomyopathy.

#### NO and clinical cardioprotection

The known physiological effects of NO in reducing left or right ventricular filling pressure, augmenting collateral coronary flow, and inhibiting platelet aggregation<sup>33</sup> provide a powerful theoretical basis for the routine use of NO in the treatment of acute coronary syndromes, myocardial infarction, and heart failure. Despite this the routine use of NO donors, such as nitrates, for clinical cardioprotection remains a controversial area. The clinical evidence for the use of NO in cardiovascular disease is reviewed.

#### MYOCARDIAL INFARCTION

In a small number of myocardial infarction trials predating the thrombolytic era intravenous nitrates were thought to have beneficial effects on infarct size and mortality. Subsequent meta-analysis of these trials indicated up to a 49% reduction in mortality with the use of prolonged intravenous glyceryl trinitrate.<sup>34</sup> However, the later and larger megatrials such as ISIS-4 (fourth international study of infarct survival)<sup>35</sup> and GISSI-3 (Gruppo Italiano per lo studio della sopravvivenza nell'infarto miocardico)<sup>36</sup> could not confirm a beneficial effect on mortality in contradiction to previously published data. In GISSI-3 the only positive elements were a significant 10% reduction of the combined rate of death and left ventricular dysfunction in patients over the age of 70 years, and in women a more pronounced effect of lisinopril on mortality when nitrates were coprescribed. One major criticism of the ISIS-4 and GISSI-3 megatrials is the widespread use of open label oral nitrates in the placebo and control groups (62% and 57%, respectively), which may have diluted the true effects of nitrates in both studies.<sup>37</sup> Of course the use of intravenous nitrates was not

addressed in these studies and no study has specifically assessed the potential benefit of nitrate treatment before myocardial infarction. The effects of the NO donor intravenous linsidomine followed by oral molsidomine within 48 hours of myocardial infarction were studied in the ESPRIM (European study of prevention of infarct with molsidomine) trial.<sup>38</sup> No reduction in mortality was seen but, as in the ISIS-4 and GISSI-3 studies, nitrates were frequently used in the placebo group.

The delay often associated with reperfusion therapy in patients with ongoing myocardial infarction provides a clear indication for the use of adjunctive cardioprotective agents. The known cytoprotective actions of NO during ischaemia-reperfusion and favourable effects on diastolic dysfunction,<sup>39</sup> often associated with reperfusion injury, provide a rationale for the use of NO releasing agents in this context.

#### ANGINA

Despite clear demonstrable effects in relieving symptoms and electrocardiographic changes of ischaemia, nitrates have not been shown to influence mortality in stable or unstable angina. The observation that preinfarction angina may be a correlate of preconditioning in humans has been shown in several studies but remains controversial. In one of the largest studies analysing patients in the TIMI 4 (thrombolysis in myocardial infarction (phase 4)) study<sup>40</sup> the presence of preinfarction angina reduced the combined end points of inhospital death, severe congestive heart failure or shock from 12% in patients with no preceding angina to 4%. As expected the use of oral antianginal medication was higher in the angina group before myocardial infarction. In particular nitrate usage was 34% in the angina group compared with 14% in the no angina group, and it is interesting to speculate as to the contribution of antecedent nitrate treatment to cardioprotection.

#### ANGIOPLASTY

The ACCORD (angioplastie coronaire, corvasal et diltiazem) study<sup>41</sup> investigated the effect of direct NO donors linsidomine and molsidomine on angiographic restenosis after coronary balloon angioplasty. Pretreatment with the NO donor was associated with a modest improvement in immediate angiographic result compared with diltiazem, which was maintained at six months.

#### HEART FAILURE

The beneficial haemodynamic effects of nitrates in preload reduction inducing a reduction in pulmonary and left ventricular end diastolic pressure are helpful in heart failure. A recent study of the use of intravenous nitrates in acute severe pulmonary oedema showed a significant reduction in seven day mortality.<sup>42</sup> No study has assessed the sole use of nitrates in chronic heart failure but the V-HeFT (vasodilator-heart failure) trials I and II<sup>43</sup> showed that the combination of isosorbide dinitrate and hydralazine produced a 38% reduction in mortality at one year compared

with placebo or prazosin. In addition favourable effects of NO on diastolic relaxation suggest a beneficial effect in diastolic heart failure.<sup>44</sup>

#### CARDIAC SURGERY

Ischaemia-reperfusion injury is an important phenomenon during cardiac surgery and the pharmacological manipulation of cardioplegic solutions is a powerful clinical tool in the preservation of myocardial integrity. Two studies have addressed the cardioprotective role of NO during cardiac surgery by supplementation of blood cardioplegia with L-arginine<sup>45</sup> and SPM-5185, a cysteine containing NO donor.<sup>46</sup> These agents respectively produced both reduced infarct size and improved postischaemic contractile performance. If NO can precondition the myocardium then NO releasing agents may also have a protective role before routine cardiac surgery.

#### NO donors

The development of a number of NO donors has allowed the investigation and application of biologically active NO in experimental research and therapeutic trials. However, commercially available compounds vary considerably in the rate, amount of NO release, and the possibility that some agents also release other radicals.

- **Glyceryl trinitrate** as an organic nitrate requires the biotransformation of nitrate to release NO. This is a multistep enzymatic process, involving the formation of S-nitrosothiols, dependent on the availability of glutathione.
- **SIN-1** is the hepatic metabolite of molsidomine, which is available in systemic form (linsidomine). SIN-1 releases nitrate, nitrite, and superoxide anions during the liberation of NO.
- **SNP**, a nitroprusside dianion consisting of a complex of ferrous ion with five cyanide ions, releases NO by interaction with a thiol reducing agent in the presence of light.
- **SNAP** is a nitrosothiol that directly releases NO slowly without prior biotransformation. The long half life leads to prolonged action and stable release pharmacokinetics.
- **DETA-NO** is a diazenium diolate with a 20 hour half life. The prolonged duration of action opens up a therapeutic potential for this compound.
- **NO aspirins** are a new class of NO donors attached to an aspirin moiety originally designed for gastric mucosal protection. Examples are NCX 4016 and NCX 4215.<sup>47</sup>

#### Drugs with NO modulating properties

##### ADENOSINE

This purine nucleotide produces NO via the activation of constitutive NOS (cNOS). Apart from its vasodilatory and atrioventricular nodal blocking actions it is thought to be an important trigger of preconditioning in animals and patients.<sup>48</sup>

## SILDENAFIL

Originally developed as an antihypertensive, the cyclic GMP type V phosphodiesterase inhibitor sildenafil (Viagra; Pfizer) has revolutionised the treatment of erectile impotence. Sildenafil has been shown to enhance flow mediated vasodilation in patients with chronic heart failure,<sup>49</sup> and the development of new and more cardiospecific cyclic GMP phosphodiesterase inhibitors may well herald a new era of cardioprotective agents for use in clinical cardiology.

## STATINS

Substantial interest has focused on the non-cholesterol lowering or pleiotropic effects of this group of drugs. The statins have favourable effects on endothelial function and NO may be implicated in the mechanism. Pravastatin sodium has been shown to cause endothelial dependent vasorelaxation of aortic rings by activating cNOS and releasing NO in a dose dependent manner.<sup>50</sup> The mechanism of cNOS activation by statins is unknown.

## NICORANDIL

Nicorandil is a selective potassium dependent ATP channel blocker but also has an NO releasing moiety. It has been implicated in the preconditioning phenomenon and shown to be protective in unstable angina.<sup>51</sup> It is possible that the NO producing effect of this drug contributes to its cardioprotective properties.

## ANGIOTENSIN CONVERTING ENZYME INHIBITORS

Angiotensin converting enzyme (ACE) inhibitors prevent the breakdown of bradykinin that in turn increases NO production by the activation of cNOS. ACE inhibitors appear to have a number of protective effects independent of ACE inhibition. Recent evidence suggests that this might be explained by an NO mediated improvement in endothelial function.<sup>52</sup>

## NEVIBOLOL

Nevibolol is a new cardioselective  $\beta$  blocker with NO modulating properties<sup>53</sup> licensed for treating hypertension. However, these combined effects may prove useful in the treatment of coronary disease and heart failure.

## AMLODIPINE

This calcium blocker was found unexpectedly to release NO from coronary microvessels through a kinin mediated mechanism.<sup>54</sup> Amlodipine also releases NO from coronary microvessels in human failing hearts, which may have provided a novel mechanism for its cardioprotective effects in a recent heart failure trial.<sup>55</sup>

Evidence for the NO mediated effects of ACE inhibitors, amlodipine, and nevigolol is speculative and controversial. Further evidence is needed to explore the potential NO modulating properties of these drugs.

## Conclusion

NO will remain one of nature's fascinating molecules and provide cardiovascular scientists with a tool to understand further the workings

of complex physiological systems. A greater understanding of the molecular basis of NO mediated actions in cellular homeostasis will allow us to harness its actions as a potentially powerful cardioprotective agent. What is first needed is the development of more refined pharmacological NO donors together with clinical trials based on emerging biological principles. This may lead to the use of NO type agents as adjunctive pharmacotherapy for use in the future management of acute coronary syndromes, before high risk percutaneous transluminal coronary angioplasty, surgical cardioplegia, and heart failure.

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## Implementing the National Service Framework for Coronary Heart Disease

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